SYNTHESIS, BIOLOGICAL ACTIVITY AND QSAR STUDIES OF SOME HETEROCYCLIC COMPOUNDS

A

THESIS

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FOR

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IN

PHARMACEUTICAL CHEMISTRY

BY

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Statement Under O.Ph.D. 7 of Saurashtra University

The work included in the thesis is my own work under the supervision of **Prof. Kishor S. Jain** and **Prof. Anamik K. Shah** and leads to good contribution in the field of Pharmaceutical Chemistry and is supported by recent references.

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This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by **Mr. Jitender Bariwal** has been the result of work carried out under our supervision and is a good contribution in field of Pharmaceutical Chemistry of "Heterocyclic Compounds" with a special emphasis on synthetic, biological and QSAR studies.

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Dedicated to
my Beloved Parents

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M. Pharm.

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Abbreviations used

^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	^{13}Carbon Nuclear Magnetic Resonance
ABC	ATP Binding Cassette
Ach	Acetylcholine
ACTH	Adrenocorticotropic Hormone
ADR	Adriamycin
AIDS	Acquired Immune Deficiency Syndrome
AMP	Adenosine Monophosphate
APA	Acid Pump Antagonists
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
BCRP	Breast Cancer Resistance Protein
cAMP	Cyclic Adenosine Monophosphate
CCK2	Cholecystokinin-2
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CNS	Central Nervous System
COX	Cyclooxygenase
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexyl carbodiimide
DHP	Dihydropyridine
DHPM	Dihydropyrimidine
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DU	Duodenal Ulcers
ECL	Enterochromaffin-like
EGFR	Epidermal Growth Factor Receptor
FAR	Fluorescence Activity Ratio
GERD	Gastro Esophageal Reflux Disease
GSH	Glutathione
GU	Gastric Ulcers
H. pylori	*Helicobacter pylori*

HA	Histamine
HIV	Human Immunodeficiency Virus
IC50	Concentration for inhibiting growth of 50% organisms
IL	Ionic Liquid
IR	Infrared
KSP	Kinesin Spindle Protein
LD50	Lethal Dose 50
MAOS	Microwave Assisted Organic Synthesis
m-CPBA	*m*-Chloroperbenzoic acid
MCR	Multi-Component Reaction
MDC	Methylene dichloride
MDR	Multi Drug Resistance
MWI	Microwave Irradiation
NCE	New Chemical Entities
NDDR	New Drug Discovery Research
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
OECD	Organization for Economic Co-operation and Development
p.o.	Per oral
Pgp	Glycoprotein
PMSB's	Pyridinylmethylsulfinylbenzimidazoles
PPI's	Proton Pump Inhibitors
PTC	Phase Transfer Catalysis
QSAR	Quantitative Structure Activity Relationships
QSPKR	Quantitative Structural Pharmacokinetic Relationship
RF	Reversal Factor
RT	Room Temperature
SAR	Structure Activity Relationship
SEM	Standard Error of Mean
TEBA.Cl	Triethyl Benzylammonium Chloride
T-LESR	Transient Lower Esophageal Relaxation
TMD	Transmembrane Domain
TMSCl	Trimethylsilyl chloride

UV	Ultra Violet
VEGFR	Vascular Endothelial Growth Factor Receptor
WCR	World Cancer Report
WHO	World Health Organization
Z-E	Zollinger-Ellison Syndrome

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Preface

Heterocyclic chemistry is vastly expending because of the enormous amount of research work being done in this area. The majority of known molecules are heterocycles and heterocycles dominate the field of biochemistry, medicinal chemistry, dyestuff, photographic sciences and are of increasing importance in many other areas including polymers, adhesives and molecular engineering.

Among the heterocyclic compounds, pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acid to their current use in the chemotherapy of AIDS. During the last four decades, several pyrimidines have been developed as chemotherapeutic agents and have found wide clinical applications as anticancer, antiviral and anti-AIDS, antitubercular, sedative/hypnotic/antiepileptic, cardiac agents, as well as analgetics, diuretics, antibiotics and metabolic electrolytes *etc*.[1]

Thus, the focus of the present work is to synthesize some novel pyrimidine, condensed pyrimidine and dihydropyrimidine derivatives and to evaluate them for their antiulcer and multidrug reverting activities.

This thesis has been divided into following three parts.

Part-I of this work deals with the *"Synthesis, Pharmacological Evaluation and QSAR of some Pyrimidylmethylsulfinylbenzimidazoles as potential reversible Proton Pump Inhibitors (PPI's)"*.

In 19th Century, light diet consisting of food not stimulating gastric acid secretion was recommended for treating peptic ulcer related disorders. Since then a number of strategies have been designed to control these disorders related to the hypersecretion of acid.[2,3] These therapeutic strategies extend from simple conventional antacids to the use of more complex and effective proton pump inhibitors (PPI's).[4] Associated effects of conventional antacids like constipation or diarrhea limit their patient compliance and are today mainly used for fast symptomatic relief. Muscarinic antagonists like pirenzepine inhibit gastric acid secretion as well as decrease gastric motility, but clinical use of these drugs is now limited because of the availability of more effective anti-secretory medications. A new

era in the treatment of acid-peptic disorders dawned with the launch of H_2-receptor antagonist, cimetidine in 1976. This class of drugs however, has a short duration of action. Peptic ulcers caused by *H. pylori* can be treated by combination of antibiotics and anti-secretory medications. However, complex drug regimen and associated side effects limit their usefulness. Launch of Omeprazole in 1988, introduced a conceptually new approach of inhibition of proton pump in the management of acid related disorders. PPI's proved to be superior to any of the previously used drugs, including H_2-anatagonists.[5,6]

Today, almost two decades after introduction of first PPI, the apparent drawbacks of irreversible proton pump inhibitors, mainly because of their prolonged acid suppression are becoming a cause of concern.[7] Hence, researchers worldwide have been attracted towards designing reversible, shorter and rapid acting acid pump antagonists (APA's). Thus, APA's are the conceivable future drugs for the treatment of acid-peptic disorders.

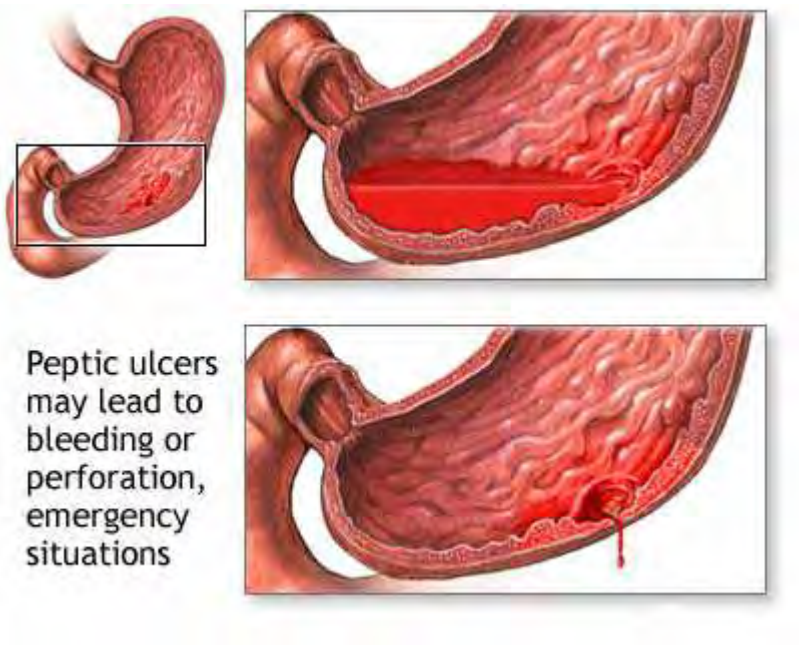


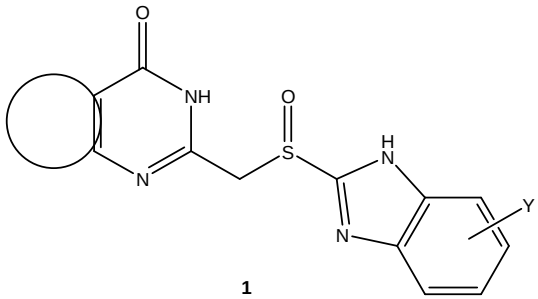
Figure-1. Peptic ulcer

The mechanism of action of existing PPI's of the pyridylmethylsulfinylbenzimidazole (PMSB) class, at the H^+/K^+ATPase enzyme or the Proton Pump, involves the acid induced transformation of the drug molecule to the sulfenamide intermediate, which irreversibly binds through a sulfide linkage to the Cystine-813 and Cystine-822 of the pump, leading to its irreversible inhibition and many observed drawbacks of these agents.

This entire cascade of reaction is initiated at the basic 'N' atom of the basic pyridine ring of the PMSB.[8]

In the present work, the basic pyridine ring of these compounds has been replaced with less basic pyrimidine ring, so the binding of these types of compounds is not so strong as PMSB and the target compounds can hopefully be even potential reversible PPI's.

Thus, a series of condensed pyrimidylmethylsulfinylbenzimidazoles **1** have been synthesized through the reaction of appropriate condensed 2-chloromethylpyrimidin-4(3*H*)-one and 2-mercaptobenzimidazoles followed by the selective mild S-oxidation of the thioether linkage of the intermediates.



1

In all 35 new target compounds have been synthesized and evaluated for antiulcer activity by the Shay's rat pylorus ligation model[9] and results compared with omeprazole as the standard, mainly keeping in mind and different observations or biological effects *viz.* pH of the gastric juice secreted, secreted acidity of the gastric juice secreted, volume of gastric secretion and ulcer score. Some of the compounds have exhibited anti-acid secretory and antiulcer activities comparable to the standard drug, omeprazole.

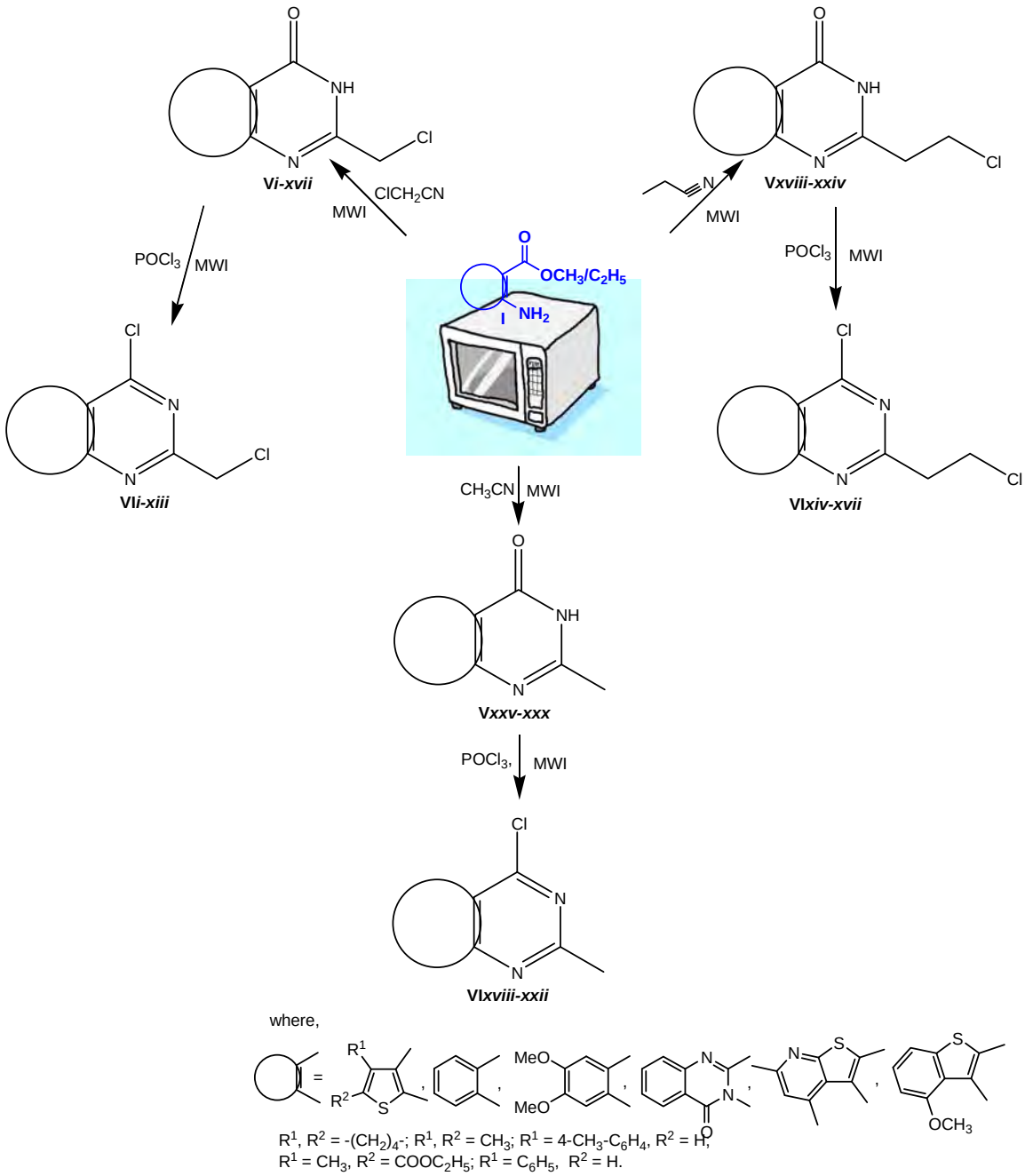
A meaningful QSAR has been worked out to determine the optimal physico-chemical characteristics and properties as well as the structural feature of these molecules, for achieving optimal activity.

Part-II of this work deals with "*The Novel Microwave Assisted Green Chemical Synthesis of Condensed 2-Substitutedpyrimidin-4(3H)-ones Under Solventfree Conditions, their MWI Assisted Facile and Rapid Chlorination and their Multidrug Reverting Activity*".

In this part, rapid cyclocondensation of various nitriles with *o*-aminoesters of thiophene, benzene, dimethoxybenzene, 4,6-dimethylthieno[2,3-*b*]pyridine, 4-methoxybenzo[*b*]-thiophene and quinazolin-4-one, in the presence of catalytic amount of conc. HCl, under MWI, was carried out to afford the compound library of their corresponding condensed 2-substituted pyrimidine-4(3*H*)-ones (scheme-1).

This type of condensation under MWI, using R-CN as the -C-N-fragment of the pyrimidine ring, is hitherto not reported in literature and is therefore novel and has great applicability for the rapid parallel synthesis of such derivatives, especially to buildup molecular libraries for New Drug Discovery Research (N.D.D.R.).

Though there are many reported methods for chlorination of heterocycles conventionally, there are only a few reports on MWI assisted chlorination of heterocycles especially, pyrimidines. To the best of our knowledge there are just two reports[10,11] on the chlorination of 4-hydroxypyrimidines to 4-chloropyrimidines under MWI. So it was decided to use MWI assisted methodology for the conversion of condensed-4-hydroxypyrimidines to condensed-4-chloropyrimidines, which is one pot, solvent free, facile, eco-friendly and highly productive as well (Scheme-1). The 30 newly synthesized condensed 4-hydroxy-2-substituted pyrimidines and their 22, 4-chloro derivatives (in all 52 compounds) have been characterized using spectroscopic techniques. All the newly synthesized compounds have been evaluated for their multidrug reverting activity, as well as, antiproliferative activity.



Scheme 1

Part III of this work deals with the *"Synthesis, Characterization and Anticancer Activity of some Aza-analogue of DP-7"*.

3,5-Dibenzoyl-1,4-dihydropyridine (DP-7) is a potent multidrug reverting agent that inhibits efflux of drug from cell wall by inhibiting activity of ATP binding cassettes (ABC).[12,13] A dihydropyrimidine (DHPM) derivative, (aza analogue) namely, monestrol inhibits the Eg5 protein, which is responsible for the separation of daughter chromosomes during cell division and controls the growth of tumor cells.[14,15]

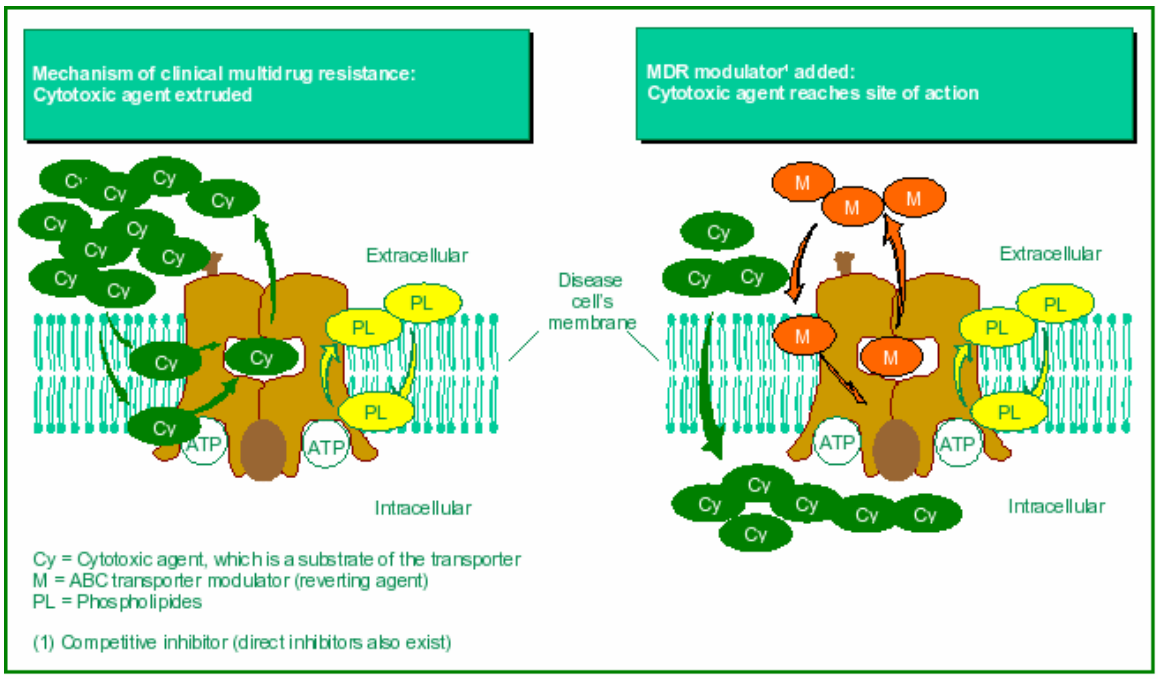
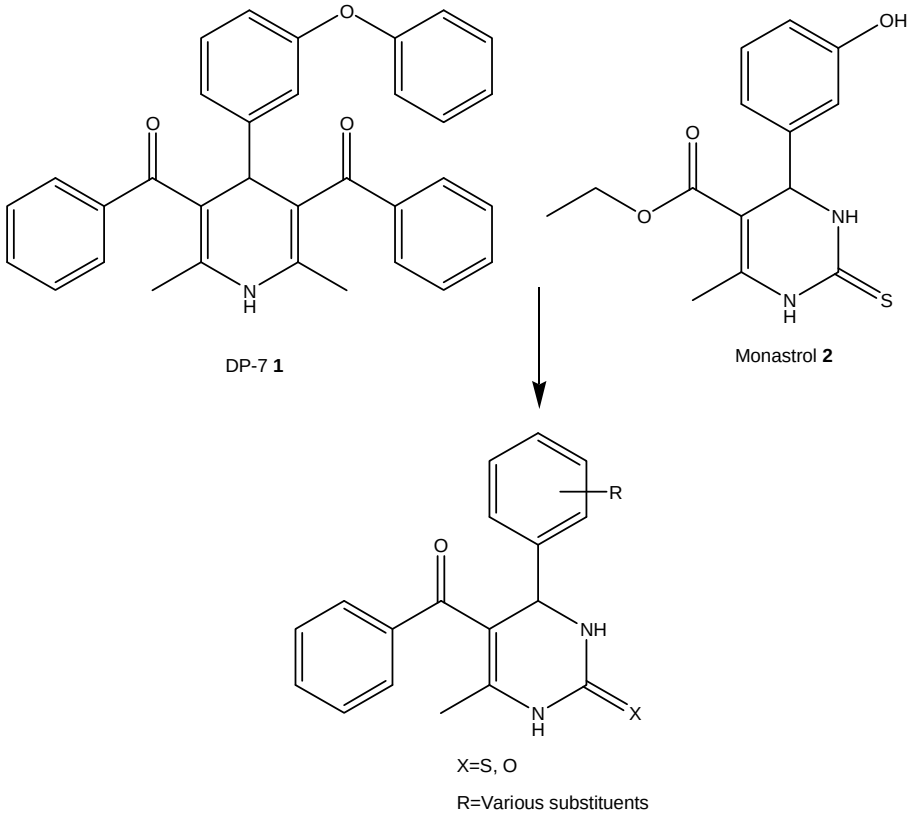


Figure-2. Role of MDR protein in making cancer cells resistant to chemotherapeutic agents and role of MDR protein inhibitor to revert the cell resistance.

In the present work, it was thought to hybridize these two potent molecules to get the duel action in cancer chemotherapy by synthesizing various thio and oxo analogues, bearing variety of substituents at 4th position of the DHPM ring (Scheme-2). The 30 newly synthesized compounds were screened for antiproliferative effects in *mdr1*-gene transfected mouse lymphoma cellline (l5178 y). Some compounds exhibited potent antiproliferative activity.



Scheme-2

Thus, in all 117 new target compounds have been synthesized, characterized and biologically evaluated in the work presented in this thesis.

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PART-I

Synthesis, Pharmacological Evaluation and QSAR studies of some Pyrimidylmethylsulfinyl Benzimidazoles as Potential Reversible Proton Pump Inhibitors (PPI's)

1. Recent Advances in Proton Pump Inhibitors and Management of Acid-Peptic Disorders: A Review

1. Recent advances in proton pump inhibitors and management of acid-peptic disorders: A Review

1.1 Introduction

'Hurry, Worry & Curry' are the causes of many disorders in today's world of globalization. Of these acid-peptic ulcers and diseases have assumed a distinctly high proportion. The pathophysiology of acid-peptic disease is attributed to the imbalance between aggressive factors (like acid, pepsin, *H. pylori* infection) and local mucosal defenses (like secretion of bicarbonate, mucus and prostaglandin's). Although treatment is often directed at reduction of aggressive factors, it can also be directed at strengthening mucosal defenses of stomach and duodenum[1].

The inhibition of gastric acid secretion is a key therapeutic target for the ulcer diseases (*viz.* peptic, duodenal ulcers or that through *H. pylori* infection), Gastro Esophageal Reflux Disease (GERD), Zollinger-Ellison Syndrome (Z-E) and Gastritis. Currently this is achieved by blocking the acid secretary effect of histamine (HA) through the use of H_2-receptor antagonists or the irreversible H^+/K^+-ATPase inhibitors, popularly referred as Proton Pump Inhibitors (PPIs). The incidence of ulcer diseases shows global variation and their treatment should be designed to alleviate the symptoms, while keeping the risk of adverse effects minimum. In western countries duodenal ulcers are more common, whereas in eastern countries gastric ulcers predominate. These differences are attributed to factors like diet and genetic make up. As a result the therapeutic strategies also differ from east to west. In western countries, the conventional therapy for duodenal and gastric ulcer is eradication of *H. pylori*. Whereas, in Japan unlike the west, H_2-antagonists are commonly used for maintenance therapy along with the PPI's[2].

The discovery of the gastric acid was the first step to understand the role of the stomach in digestion and the diseases associated with hyper secretion of acid[3,4]. The drug discovery process linked with the gastric acid secretion involving H_2-receptor antagonists and PPIs is summarized in table-1, which indicates the gradual change in the focus in the treatment of gastric acid secretion disorders[2].

In this review we have summarized various disorders related with increased gastric acid secretion and therapeutic strategies thereof, to control them. Further, more emphasis has

been laid on the role of PPI's in particular for the treatment of gastric acid disorders. The medicinal chemistry aspects of this class of compounds are also discussed.

Table 1: Some landmarks in therapy of acid-peptic disorders in past 35 years[2]

Year	Company/Discoverer	Event/Discovery
1972	James Black *et al*[5].	Discovery of H_2-receptor and H_2-receptor antagonists
1973	A. Ganser & J.Forte[6]	Discovery of H^+/K^+-ATPase (The Proton Pump)
1976	SmithKline & French[7]	Cimetidine launched (H_2-receptor antagonist)
1982	Allen & Hanburys Ltd[8]	Ranitidine launched (H_2-receptor antagonist)
1988	AstraZeneca[9]	Omeprazole launched (PPI)
1995	Takeda-Abbott[10]	Lansoprazole launched (PPI)
1997	Eisai Co.(licensed to Janssen)[11]	Rabeprazole launched (PPI)
2001	AstraZeneca[12]	Esomeprazole launched (PPI)

1.1.1 Mechanism of Gastric Acid Secretion

Stomach is a primary site of digestion. Presence of food stimulates release of acids and enzymes in stomach. The chemo- and mechanosensitive receptors present in stomach are triggered by presence of food to produce specific responses.[2] The acid secreting parietal cell is the principle cell in gastric glands. The physiological regulation of acid secretion by the parietal cells is an important factor behind the rationale of use of various agents to reduce gastric acidity. Three major pathways activating parietal acid secretion includes; 1) neuronal stimulation *via* the vagus nerve, 2) paracrine stimulation by local release of histamine from enterochromaffin-like (ECL) cells. 3) endocrine stimulation *via* gastrin released from antral G cells. In neuronal pathway, acetylcholine (Ach) released by vagal nerve directly stimulates gastric acid secretion through muscarinic M_3 receptors located on the basolateral membrane of parietal cells. The CNS is considered to be the chief contributor for initiating gastric acid secretion in response to the anticipation of food. Ach indirectly stimulates release of histamine from enterchromaffine-like (ECL) cells in the fundus and gastrin from the G cells in the gastric antrum. ECL cells, the sole source pf gastric histamine involved in acid secretion, are present in close proximity to parietal cells. Histamine released from ECL cells activates parietal cells in paracrine fashion by binding to H_2 receptors. Gastrin is primarily present in antral G cells. Release of gastrin is

under regulation of central neural activation, local distension and chemical composition of gastric content. Gastric stimulates parietal cells by binding with gastrin receptors. Gastrin also exerts its action in an indirect manner by causing the release of histamine from ECL cells.[1] Binding to respective G-protein coupled receptors by Ach, gastrin and histamine results in activation of second-messenger systems.[2] Vagal stimulation and the action of gastrin (from duodenal and antral G cells), stimulate release of histamine from paracrine-ECL cells or mast cells. Thus, increased levels of both intracellular Ca^{2+} by gastrin/Ach and cyclic AMP by histamine, finally cause acid secretion.[13] The final step in acid secretion is mediated by H^+/K^+-ATPase, also called as gastric proton pump.[14] Activation of either the cAMP or Ca^{2+} dependent pathway or both, causes stimulation of H^+/K^+-ATPase on parietal cells[15] (Figure 1).

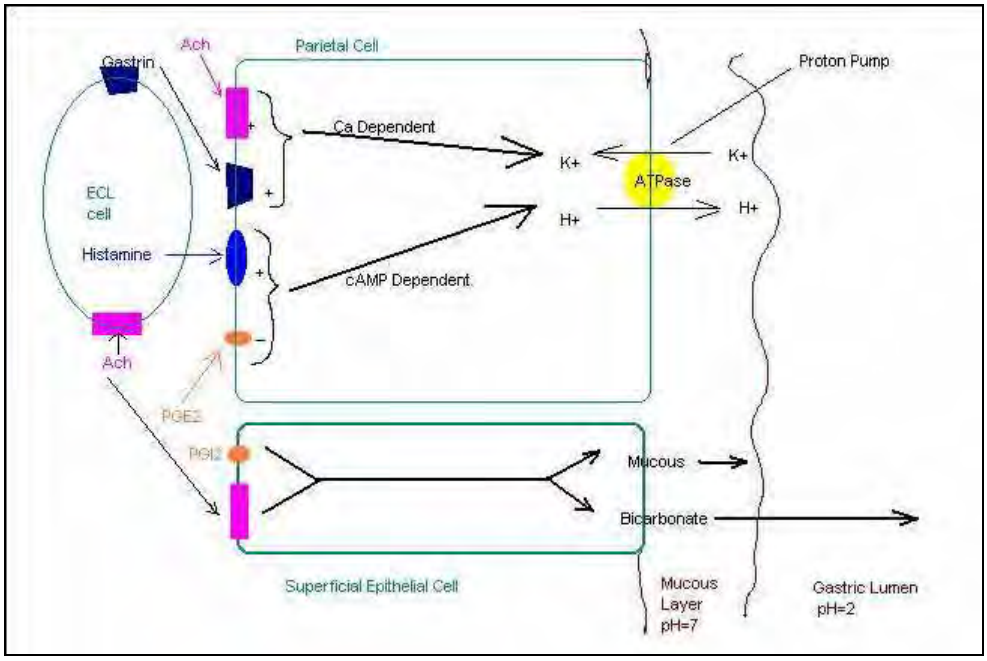


Figure 1: Mechanism of Gastric Acid Secretion[16]

1.1.2. Disorders Associated with Elevated Secretion of Gastric Acid

a) Peptic Ulcers:- Neuropeptide Y, corticotrophin-releasing factor, bombesin, calcitonin, neurotension, interlukin 1, along with somatostatin, prostaglandins, bicarbonates and mucin act as mucosal defense factors. Imbalance between these mucosal defense factors and aggressive factors (acid and pepsin) is involved in peptic ulcers[2] (Figure 2). Their rational treatment is aimed at restoring this balance. In case of duodenal

ulcers (DU) there is increase in basal acid secretion. In gastric ulcers (GU), however, there is weakening of mucosal that can lead to injury in spite of low acid secretion. Differences between DU and GU are summarized in table 2. *H. pylori* and nonsteroidal anti-inflammatory drugs (NSAIDs) play important role in ulcer induction.[1] Particularly NSAID's inhibit production of prostaglandins arachidonic acid by inhibiting enzyme cyclooxygenase (COX). Chronic NSAID users are at 2-4% risk of developing a symptomatic ulcer, gastrointestinal bleeding or associated perforation. In ulcer patients, NSAID's increase the risk of probable complications four folds. Further, these complications may remain undetected because of reduction in pain, thereby worsening the condition. Co-administration of Misoprostol, the synthetic are superior to H_2-receptor antagonist in promoting healing and preventing recurrence of both GU and DU.[1]

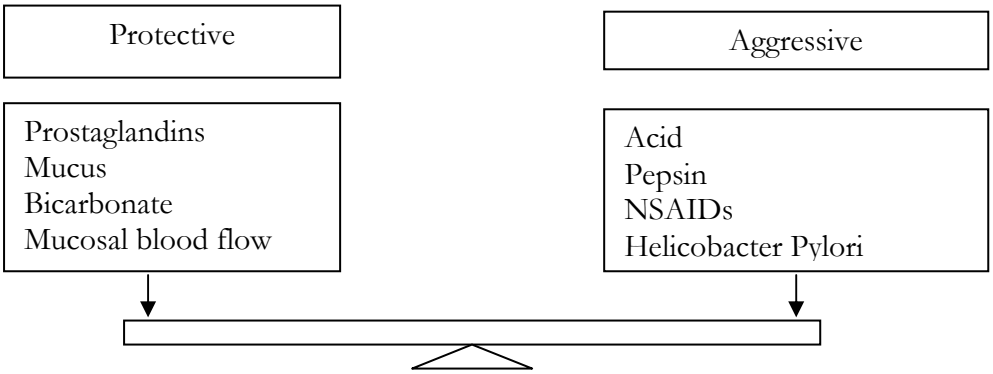


Figure 2: Factors involved in maintaining acid balance

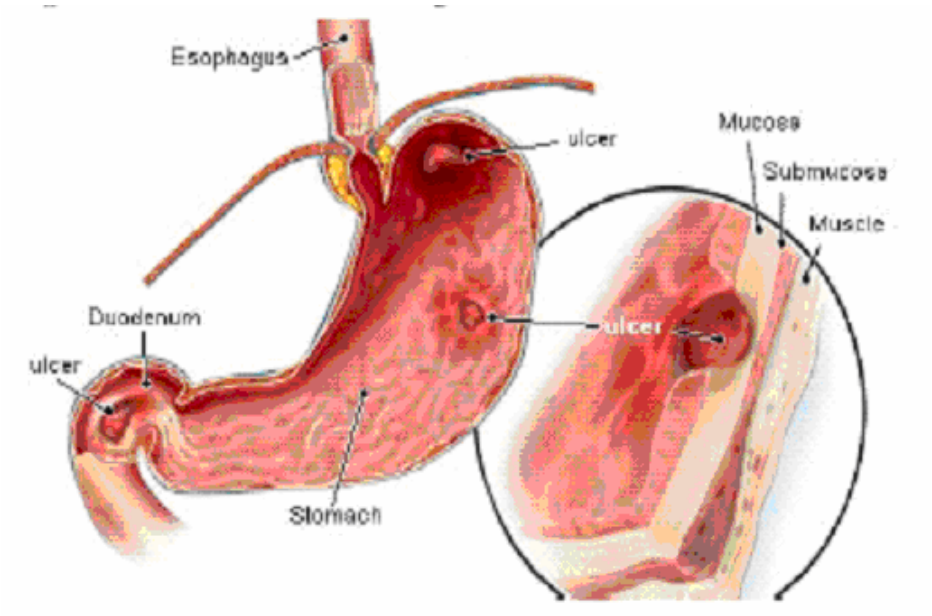


Figure 3: Peptic Ulcer[17]

Table 2: Distinguishing Features of Two Major Forms of Peptic ulcers.[18]

Sr. No.	Feature	Dudodenal Ulcer	Gastric Ulcer
1	*Incidence*	Four times common than astric ulcers. Usual age 25-50 years. More common in males than in females (4:1)	Less common than duodenal ulcers. Usually beyond 6th decade. More common in males than in females (3.5:1)
2	*Etiology*	Most commonly as a result of *H. pylori* infection. Other factors-hyper secretion of acid-pepsin, association with alcoholic cirrhosis, tobacco, hyperparathyroidism, chronic pancreatitis, blood group O, genetic factors	Gastric colonization with *H. pylori* asymptomatic but higher chances of development of duodenal ulcers. Disruption of mucus barrier most important factor. Association with gastritis, bile reflux, drugs, alcohol, tobacco.
3	*Pathogenesis*	Mucosal digestion from hyperacidity most significant factor. Protective gastric mucus barrier may be damaged	Usually normal-to-low acid levels: hyperacidity if present is due to high serum gastrin Damage to mucus barrier significant factor.
4	*Pathologic changes*	Most common in the first part of duodenum. Often solitary, 1-2.5 cm in size, round to oval, punched out	Most common along the lesser curvature and pyloric antrum. Grossly similar to duodenal ulcers
5	*Complication*	Commonly hemorrhage, perforation, sometimes obstruction, malignant transformation never occurs	Perforation, hemorrhage and at times obstruction, malignant transformation less than 1% cases.
6	*Clinical features*	Pain food relief pattern. Night pain common. No vomiting. Melaena more common than heamatemesis, No loss of weight. No particular choice of diet. Marked seasonal ariation. Occurs more commonly in people at greater stress	Food pain pattern. No night pain. Vomiting common. Haematemesis more common. Significant loss of weight Patients choose bland diet devoid of fried food, curries *etc.* No seasonal variation. More often in labouring groups

b) Zollinger-Ellison (Z-E) Syndrome:- In this disease, a non-beta cell tumor of the pancreatic islets may produce gastrin in a quantity sufficient to stimulate the secretion of gastric acid to life-threatening levels. This can lead to severe gastroduodenal ulcerations and other consequences of the uncontrolled hyerchlorhdria. The therapy is aimed at reducing gastric acid secretion. In this the proton pump inhibitors being surely the drugs of choice.[2] Gastric ECL-cells carcinoids are rare events that have been described in association with Z-E syndrome.[19]

c) *Helicobacter Pylori* (*H. pylori*) Infection:- Around 40% of patients over 40 years age and with peptic ulcer disease, are infected with *H. pylori* infection. *H. pylori is* a gram-negative rod shaped bacteria and has clearly been associated with gastritis, peptic ulcers, gastric adenocarcinoma and gastric ∃-cell lymphoma. Upto 80-90% of ulcers may be associated with *H. pylori* infection of stomach. This infection may lead to impaired production of somatostatin by D cells. This results into increased gastric acid secretion along with impaired duodenal bicarbonate production.[1] *H. pylori* infection is now proven to be a risk factor for gastric cancer and the organism was classified as group I carcinogen by WHO[20]. *H. pylori* infection causes inflammation of the antral gastric mucosa. Bacterial products and inflammatory cytokines may produce changes in the endocrine function[21]. It has now become a standard care procedure eradicate the infection in patients with gastric and duodenal ulcers. This strategy is almost successful in eliminating the risk of ulcer recurrence.[1]

Figure 4: *Helicobacter pylori*[22]

d) Gastro Esophageal Reflux Disease (GERD):- It is a disorder of defense mechanism at the esophageal junction, caused by regurgitation of the gastric contents, especially of gastric acid. GERD is associated with decreased gastric emptying and/or increased incidence of transient lower esophageal relaxation (T-LESR).[23] Smoking and obesity increase the incidence of GERD symptoms like heartburn, belching and bloating. GERD is not life threatening, but can cause significant discomfort and increased risk Barrett's esophagus.[2] Relationship between GERD symptoms and incidence of esophageal adenocarcinoma has also been suggested. It has also been linked to tracheopulmonary symptoms like laryngitis and asthma. Besides disturbed gastrointestinal motility, injurious effects of the acid-peptic refluxate on the esophageal epithelium are also responsible for GERD symptoms. Hence along with prokinetic drugs, suppression of gastric acid is the current pharmacotherapeutic approach for its treatment.[1] *H. pylori* infection does not

necessarily correlate with GERD, although a reduction in acid secretion reduces chances of reflux.[23]

e) Stress-related Ulcers:- These are the ulcers of stomach and duodenum that usually occur as a result of severe systemic or CNS illness or trauma. Both acid and mucosal ischemia is involved in the etiology of stress ulcers. Similarly, stress due to physiological factors like septicemia, intracranial lesions, alcohol intake, and smoking can also appreciably contribute to ulcer induction. Intravenous H_2-receptor antagonist and intravenous PPI's are preferred agents for its treatment.[1]

f) Nonulcer Dyspepsia:- It refers to ulcer-like symptoms in patients who are without overt gastroduodenal ulceration. Though pathogenesis of this syndrome remains unclear, it may occur because of gastritis or use of NSAID's. Empirical treatment with acid suppressive agents is used routinely.[1]

1.1.3. Complications Arising from the Disorders Associated with Elevated Secretion of Gastric Acid[18]

1.1.3.1. Obstruction: Development of fibrous scar at or near the pylorus results in pyloric stenosis.

1.1.3.2. Haemorrhage: Minor bleeding by erosion of small blood vessels in the base of an ulcer occurs in all the ulcers and can be detected by testing the stool for occult blood.

1.1.3.3. Malignant Transformation: The dictum '*cancers ulcerate but rarely cancerate'* holds true for most peptic ulcers. A chronic duodenal ulcer never turns malignant, while less than 1% of chronic gastric ulcers may transform into carcinoma.

1.1.3.4. Perforation: Perforation occurs more commonly in chronic duodenal ulcers than chronic gastric ulcers. Following sequel may result.

i) On perforation the contents escape into the lesser sac or into the peritoneal cavity, causing acute peritonitis.

ii) Air escapes from the stomach and lies between the liver and the diaphragm giving the characteristic radiological appearance of air under the diaphragm.

iii) Perforation may extend further to involve adjacent organs (liver and pancreas).

1.2. Therapeutic Strategies

Though, acid secretion is a physiologically important process of the stomach as;

1. Gastric acid induces pepsinogen activation to initiate digestive process and

2. It kills bacteria and other microbes ensuring a stable intragastric environment. However, under certain circumstances secretion of large excess of gastric acid and pepsinogen injure the gastro duodenal mucosa and cause serious and fatal ulcerations.[15] Hence, there is a need of good gastric acid secretion inhibitor.

The secretion of gastric acid occurs at the level of parietal cells of oxyntic glands in the gastric mucosa, producing 2-3 liters of gastric juice per day (HCl of *pH* 1).[24] Based on the understanding of the mechanisms contributing to ulcer development and particularly to gastric acid secretion, variety of therapeutic strategies exist, including suppressing the aggressive factors with use of antacids, specific antagonists of muscarinic -M_1 receptors, gastrin receptors, histamine-H_2 receptors, proton pump inhibitors (PPIs), eradication of *H. pylori* and agonists of prostaglandins/somatostatin receptors[1,15]. These overall strategies are discussed below in terms of specific therapeutic agents.

1.2.1. Antacids

Naturally occurring carbonates, potash, bismuth were used as antacids more than century ago. Since then, they have been developed and are widely used.[25] Antacids are compared quantitatively in terms of their acid neutralizing capacity, defined as the quantity of 1N HCl (expressed in milli equivalents), that can be brought to *pH* 3.5 in 15 min. Antacids neutralize HCl to form water and carbon dioxide. Hydroxides of aluminum and magnesium are the most common constituents of antacid preparations. Sodium bicarbonate, calcium carbonate are also used, as are other carbonates, silicates and phosphates. Some antacid preparations combine $Al(OH)_3$ and $NaHCO_3$ to achieve both the rapid effect of carbonate and sustained effect of $Al(OH)_3$. Simethicone, a surfactant that may decrease foaming and thus, esophageal reflex, is therefore included in many antacid preparations. Common side effects include alkalosis, belching, nausea, abdominal distension, flatulence, diarrhea, and constipation[1].

1.2.2. Muscarinic Antagonists

The secretion of acid, mucus and pepsinogen in the gastric mucosal is stimulated *via* muscarinic receptors. Over expression of M_3 receptors in DU patients is proved by autoradiographic techniques; thus blockade of this receptor subtype will reduce the pain by decreasing the duodenal motility and provide an effective anti-secretory therapy[26]. Based on its high affinity to block the muscarinic receptors on the intramural ganglia of stomach wall, pirenzepine **1** was developed as an anti-secretory drug, which was followed by telenzepine **2**, a more potent derivative with improved healing rates.[27] Parasympathetic side effects of these agents include dry mouth, blurred vision and constipation. These side effects along with their incomplete inhibition of gastric acid secretion limit their clinical utility[28].

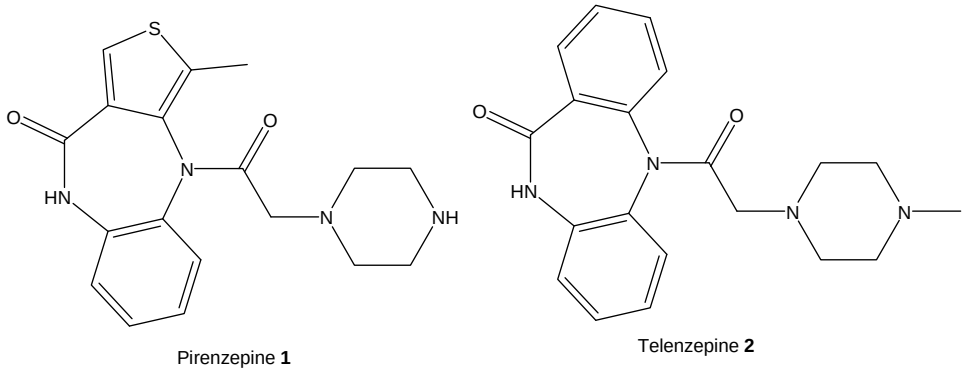


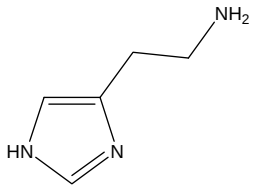
Figure 2: Structures of Muscarinic Antagonists.

1.2.3. H_2 Receptor Antagonists

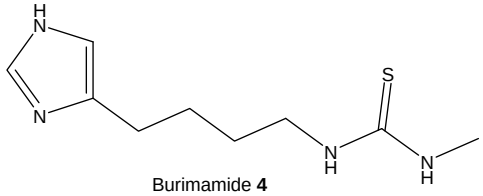
H_2 receptor antagonists completely inhibit the interaction of histamine **3** with H_2 receptors, thereby reducing both volume and H^+ ion concentration of the gastric juice. They are selective and have little or no effect on H_1 receptors. They also inhibit acid secretion elicited by gastrin, muscarinic agonists, food, sham feeding, fundic distension, as well as, other pharmacological agents. They also inhibit basal and nocturnal acid secretion. This effect contributes in a major way to their clinical efficacy[1].

Black *et al.*,[5] identified H_2-receptor and prototype H_2-receptor antagonist, burimamide **4**. The potency of burimamide at inhibiting gastric acids secretion far exceeded than that produced by anticholinergic drugs and was devoid of side effects. However, it had poor bioavailability. It was subsequently replaced by metiamide **5**, which also because of its side effects like agranulocytosis, was withdrawn from the clinical trials.[29,30] Cimetidine[7] **6**

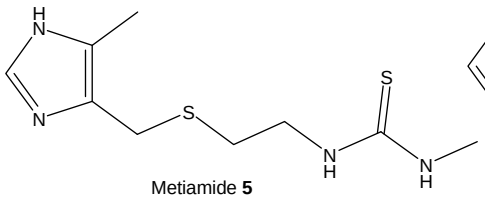
was the third H_2 receptor antagonist to be tested in humans and was similar to metiamide in its pharmacological profile, but did not cause agranulocytosis. Discovery of this molecule reduced the necessity of surgical procedures for peptic acid diseases. Further, ranitidine[8] **7** was introduced as more potent drug in 1981 with a much superior safety profile.[2] Third and most potent antagonist was famotidine[31] **8** available for clinical use, being 20-50 times more potent than cimetidine and 6-10 times more potent than ranitidine.[32] nizatidine[33] **9** and roxatidine[34] **10** followed famotidine. Each of these drugs are rapidly absorbed and eliminated after oral administration.[35] H_2 receptor antagonists are histamine congeners that contain a bulky cysteamine side chain in place of ethylamine moiety of histamine. Earlier representatives of these groups such as burimamide and cimetidine retained the imidazole ring of histamine. This ring was further replaced by furan as in ranitidine, by thiazole as in famotidine and nizatidine and piperazine and benzene as in roxatidine.[1] This helped to avoid unwanted cytochrome P450 interactions.[36]



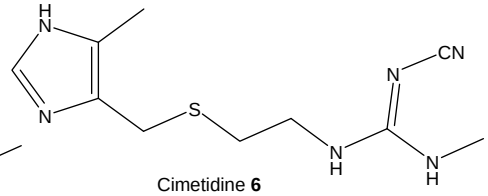
Histamine **3**



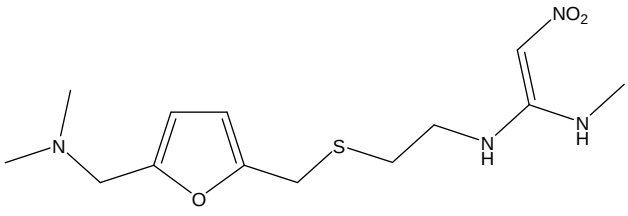
Burimamide **4**



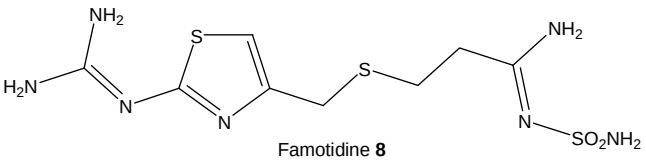
Metiamide **5**



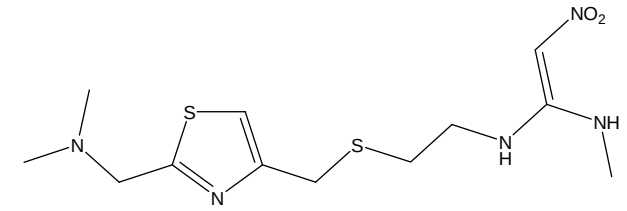
Cimetidine **6**



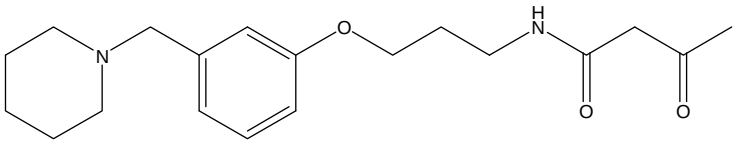
Ranitidine **7**



Famotidine **8**



Nizatidine **9**



Roxatidine **10**

Figure 6: Structures of H_2-receptor antagonists.

H_2 receptor antagonists are generally extremely safe drugs with incidence of adverse effect of cimetidine less than 3%. Adverse effects include dizziness, nausea, skin-rashes, somnolence, confusion, impotence, gynecomastia, hematological effects and altered function of immune system. Rarely they may cause bone marrow depression, hepatitis, and anaphylaxis.[1] Cimetidine selectively showed anti-androgen properties in a small number of patients.[37]

1.2.4. Eradication of *H. Pylori* Infections:

H. pylori is a gram-negative rod shaped bacilli that colonizes in the mucus on the luminal surface of gastric epithelium. *H. pylori* infection causes inflammatory gastritis and is a putative contributor to peptic ulcer disease, gastric lymphoma and adenocarcinoma.[1] Infection may not always be causative as ulcers may recur in patients who have undergone successful eradication treatment.[38] Double or triple antimicrobial therapies, in combination with antisecretory drugs, are being used successfully to treat peptic ulcers. Bismuth compounds are also been included in regimen probably due to their cytoprotective action. Triple therapy with metronidazole, a bismuth compound and either tetracycline or amoxycilline for two weeks is recommended to treat *H. pylori* infections. However, therapeutic limitations of this triple therapy include complex regimen and related nausea, diarrhea and dizziness.[1]

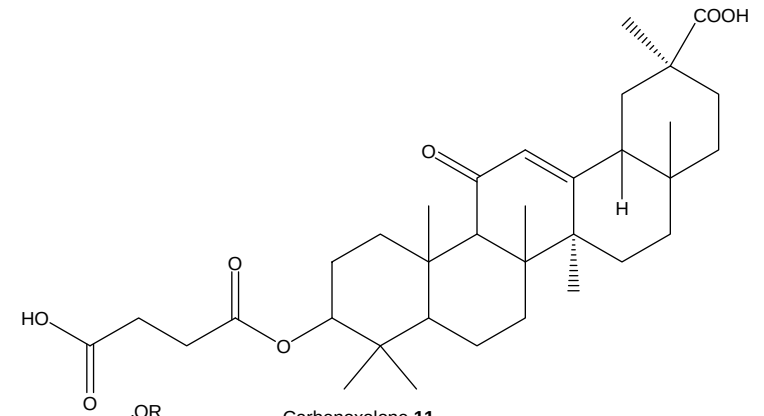
1.2.5. Other Agents Used

Carbenoxolone **11**, an olendane derivative of glycyrrhizic acid, a compound found naturally in licorice is also useful in the treatment of peptic ulcer. Mechanism of action is

not clear, but appears to alter the composition and quantity of mucus. It is not approved for use in U.S., but is being used in Europe since 1962 for the treatment of peptic ulcer. Being a steroid analog, it exhibits substantial mineralocorticoid activity like hypertension, hypokalemia, fluid retention[1] *etc.*

Sucralfated polysaccharides inhibit pepsin mediated protein hydrolysis. The octasulfate of sucrose was observed to inhibit peptic hydrolysis *in vitro*. Reaction of sucrose octasulfate with $Al(OH)_3$ forms a viscous substance, sucralfate **12**. A variety of mechanisms have been proposed to account for the cytoprotective and healing effects of sucralfate, including stimulation of prostaglandin synthesis, absorption of pepsin and stimulation of local production of epidermal growth factor.[39]

Prostaglandins PGE_2 **13** and PGI_2 **14** are synthesized by gastric mucosa and stimulate the secretion of mucus and bicarbonate. Because the administration of prostaglandins protects the gastric mucosa of animals against various ulcerogenic insults, a number of slowly metabolized prostaglandin analogs have been developed and tested in human beings. Example includes misoprostol **15**, which is currently approved for prevention of gastric ulcers. Side effects of misoprostol include diarrhea, abdominal cramps and abortifacient in pregnant women.[40]



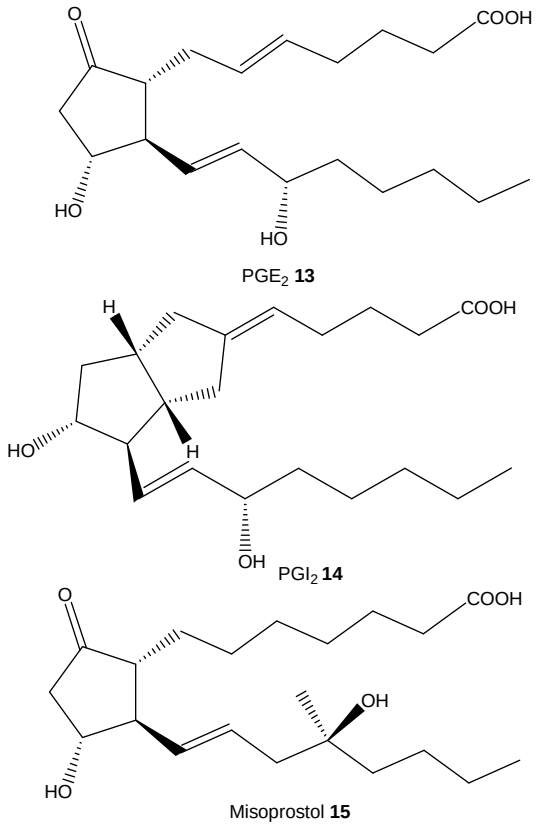


Figure 7: Structures of the other classes of drugs used in treatment of peptic ulcers.

1.2.6. Proton Pump Inhibitors (PPIs)

Proton pump is the ultimate mediator of gastric acid secretion by parietal cells. With the identification of H^+/K^+-ATPase as the primary gastric proton pump, it was proposed that activation of H^+ secretion occurred by incorporation of H^+/K^+-ATPase rich tubulovesicles into the apical plasma membrane and that the pumps were re-sequestered back into the cytoplasmic compartment on return to the resting state.[41] Inhibition of the protons pumping H^+/K^+-ATPase as a means of controlling gastric pH has attracted considerable attention in recent years with the discovery of benzimidazole sulfoxide class of anti-secretory agents. In 1973, Ruwart *et al.*,[42] identified timoprazole **16** as one of the first well-defined inhibitor of gastric proton pump. Timoprazole was followed by more potent picoprazole **17** (1976) and omeprazole[43] **18** (1979). Chemically, the basic structure consists of substituted benzimidazole ring & a substituted pyridine ring connected to each other by a methylsulfinyl chain. Clinically used PPIs include Omeprazole **18**, Lansoprazole **19**, Rabeprzole **20,** Pantoprazole **21** and Esomeprazole **22**.

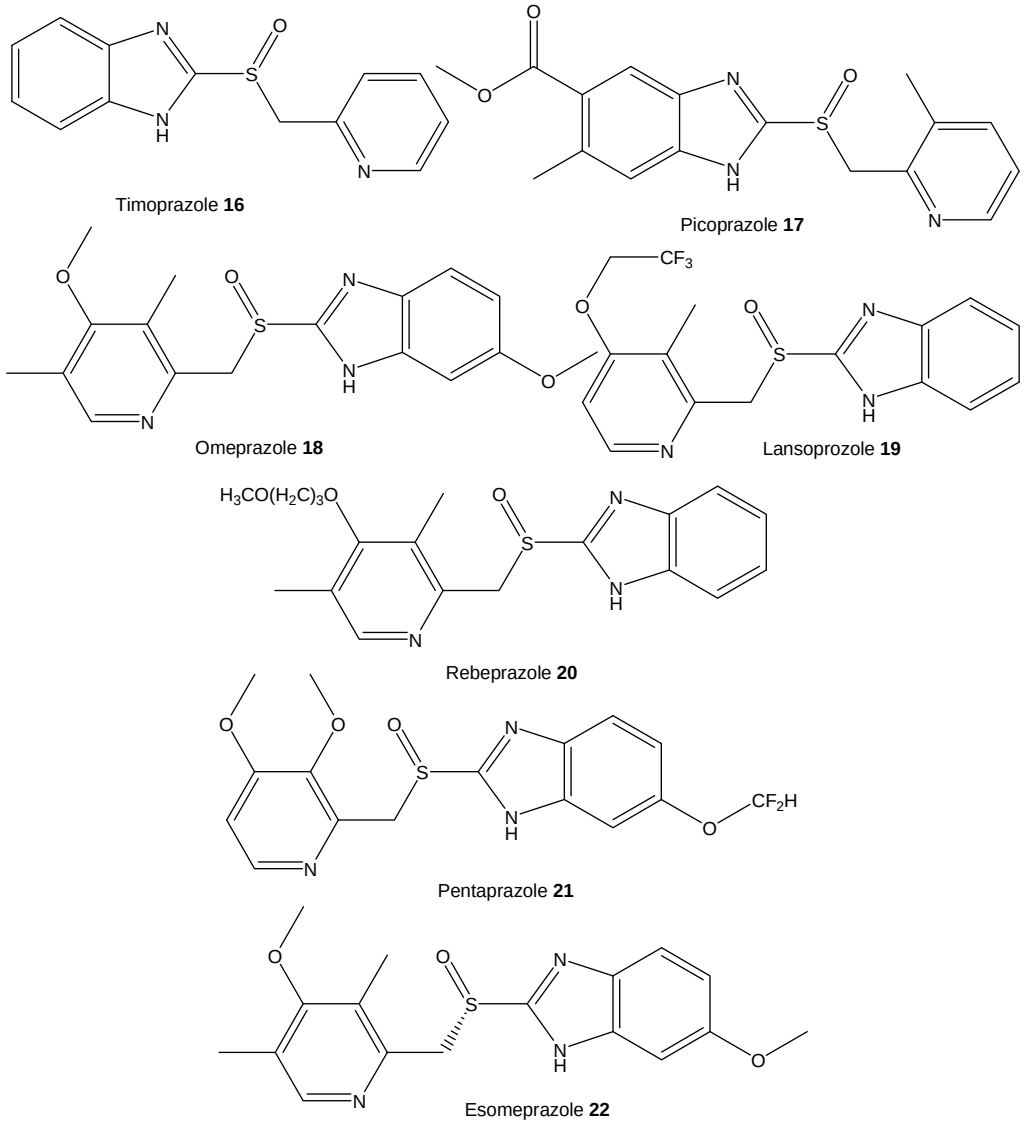


Figure 6: Structures of Proton Pump Inhibitors.

These compounds have proved to be effective in clinic for the treatment of acid related gastrointestinal disorders. They bind to the gastric proton pump on the parietal cell membrane, inhibiting the release of hydrogen ions from the parietal cells into the lumen of the gastric glands and hence stomach.[44] Some of the adverse effects of PPIs include nausea, diarrhea, dizziness[45], hypergastrinemia[46], enteric infections[2] *etc*. It has been demonstrated that irreversible inhibition of H^+/K^+-ATPase occurs following acid activation of these compounds within the acidic compartments in the parietal cells and covalent binding of the reactive intermediate to one or more critical thiol groups on the enzymes present in apical membrane[47] as in Figure 9. Acid secretion is therefore blocked at the final step of its production independent of the different kind of its stimulation.[48]

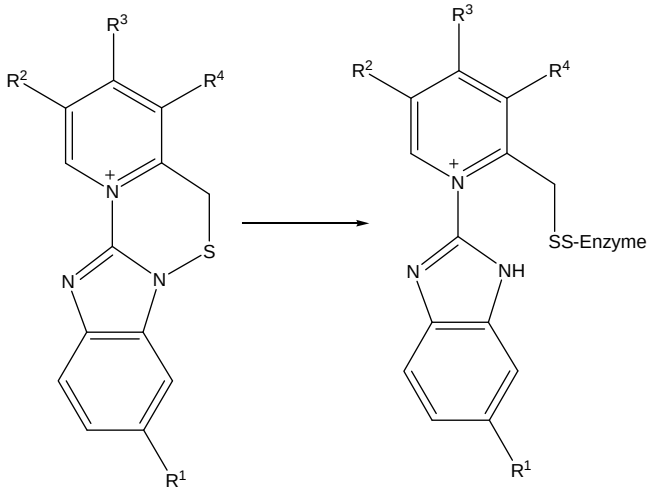


Figure 9: Covalent binding of sulfenamide with thiol group of proton pump.

1.3. Structure of the Proton Pump

The gastric H^+/K^+-ATPase is a member of the P2-type ATPase family and undergoes a cycle of phosphorylation and dephosphorylation coupled to the outward and inward transport of hydrogen and potassium ions, respectively, in the secretory canaliculus of the parietal cells. Conformations of the enzyme that bind ions for outward transport are defined as E1, whereas those that bind luminal ions for inward transport are termed E2. Ion binding to E1 activates phosphorylation from MgATP to form the intermediate E1-P, which then converts to E2-P in the acid transporting step. In the gastric H^+/K^+-ATPase as well as the Na^+/K^+-ATPases, K^+ binding to E2-P stimulates dephosphorylation to give the occluded form $E2{\bullet}K^+_{occ}$ followed by conversion to $E1{\bullet}K^+$ and release of K^+ to the cytoplasm. The gastric H^+/K^+-ATPase sustains a 10-fold inward potassium gradient (150 K^+ in, 15 mM K^+ out) and a transmembrane outward hydrogen ion gradient of greater than 1 million fold to generate a luminal pH of 0.8. This is the largest ion gradient generated by a P2-type ATPase. The exported ions are presumed to be hydronium rather than protons partly because of the ability of Na^+ to act as a competent surrogate for H^+ at pH 8. Hence, there is a functional similarity to the Na^+/K^+-ATPase at this pH. The primary structure of the gastric H^+/K^+-ATPase (HK R1) shows significant homology to the Na^+/K^+-ATPase (62%) and the sr Ca-ATPase 1 (29%). The ion binding sites of the H^+/K^+-ATPase are homologous to these, and other, P2-type ATPases in that they have only carboxylate side chains as the counter charge species.

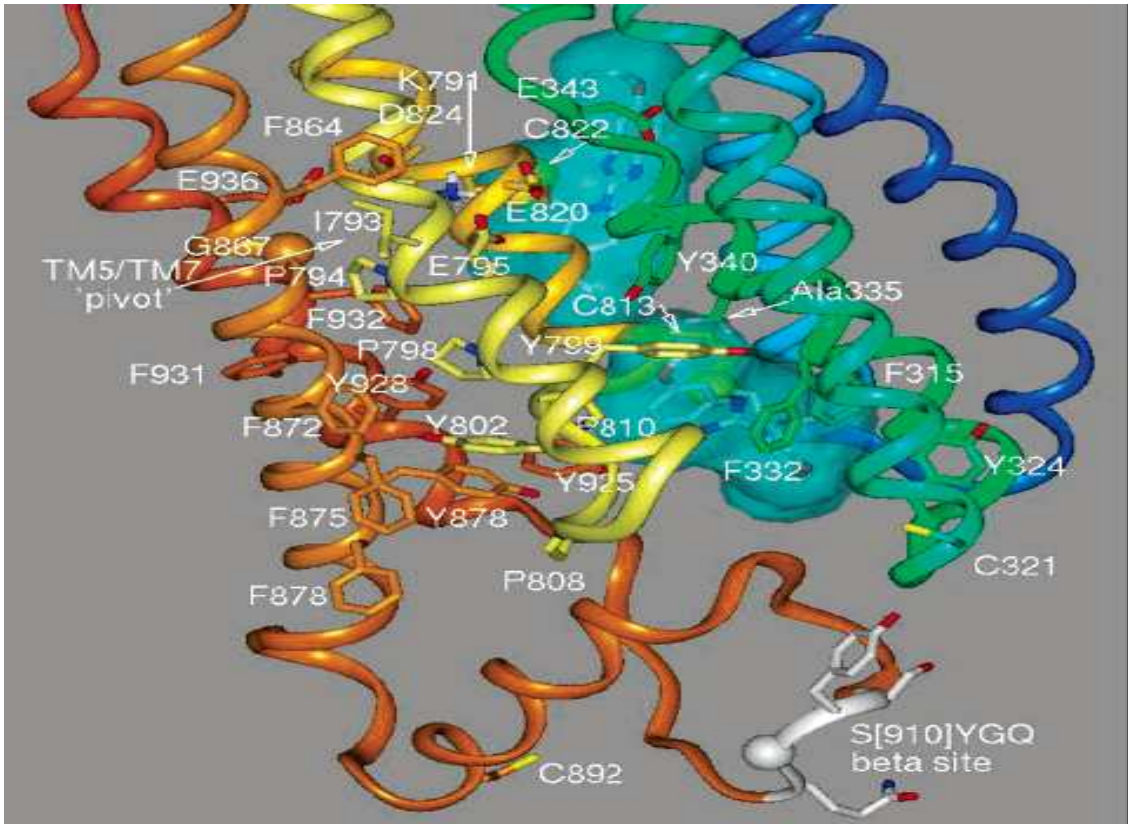
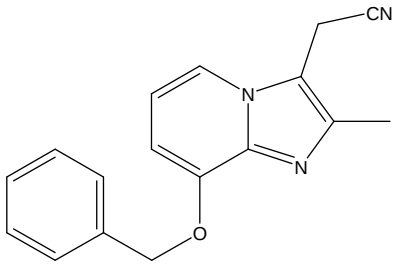


Figure 10: Membrane domain of the H,K-ATPase E2-P model with pantoprazole, bound at Cys813 and Cys822 (stick with Connolly surfaces in cloud). A known site of *a* subunit interaction (*36*, *37*), S[910]YGQ, is highlighted (white ribbon) in the TM7/TM8 loop.Cys813, Cys892, and Cys321 are labeled (*38*) by various proton pump inhibitors (all at Cys813, omeprazole at Cys892, and lansoprazole at Cys321) and are solvent-accessible in the model. Labeling at the latter two sites is not correlated with inhibition (*3*). The crossover point ("pivot") between TM5 near Ile793 and TM7 near Gly867 (gold sphere) is apparently conserved in the P2-type ATPases. An extensive array of aromatic side chains (in stick form) replaces non-aromatic sr Ca-ATPase residues and affects the spacing between helices. TM9 and TM10 are omitted for clarity. Reprinted with permission from *Biochemistry* **2005**, *44*, 5267. Copyright 2005 American Chemical Society.

It is known that all PPIs bind to cysteine 813, resulting in covalent inhibition of the enzyme *via* formation of this disulfide that stabilizes the enzyme in the E2 conformation (Figure 10). The acid pump antagonists, APAs such as SCH28080 **23** (Figure 11), represent a second class of inhibitor now under development. These are reversible, K^+ competitive inhibitors with a substituted 1,2-R-imidazopyridine core structure, that also bind to the E2 form of the ATPase.[49]



SCH 28080 **23**

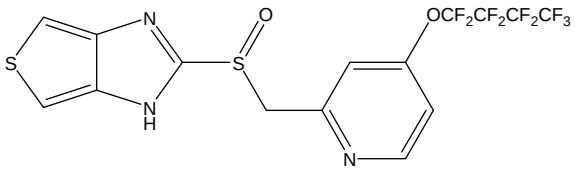
Figure 11: Acid-Pump Antagonist.

1.4. Classification of PPI's

1.4.1. Irreversible Gastric PPI's:- Three main structural features of this class of compounds are, the substituted pyridine ring; the substituted benzimidazole ring and the methylsulfinyl linking group. Irreversible PPIs lacking one or more of these features are rare. They are further classified according to their chemical structure as follows-

1.4.1.1. Pyridinylmethylsulfinyl Benzimidazoles:- The same chemical features are retained by clinically used PPIs, differing only in the substituents present on the benzimidazole and pyridine ring. Examples of this class include Omeprazole **18**, Lansoprazole **19**, Rabeprzole **20,** Pantoprazole **21** and Esomeprazole **22.**

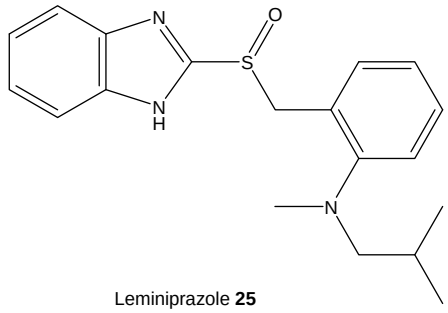
1.4.1.2. Pyridylmethylsulfinyl Thienoimidazoles:- In this class, the benzene ring of imidazole is replaced by thiophene, keeping other structural features same. Examples include saviprazole **24.**



Saviprazole **24**

Figure 12: Structures of thienoimidazoles as irreversible gastric proton pump inhibitors.

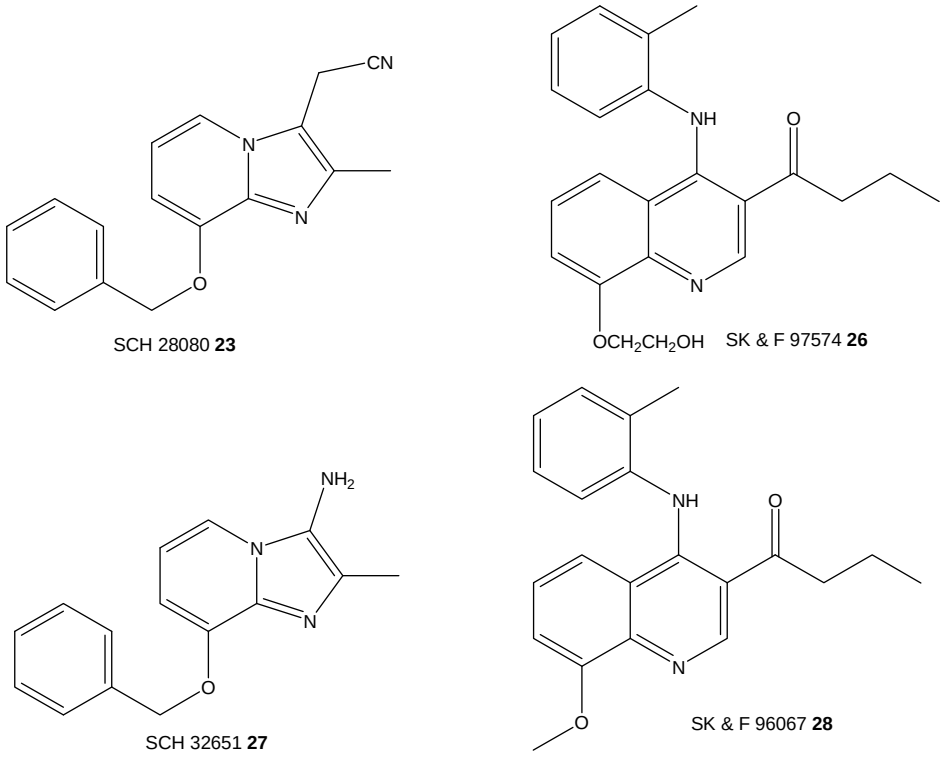
1.4.1.3. Aminobenzylsulfinyl Benzimidazoles:- Here, pyridine ring is replaced by substituted aminobenzyl ring. Examples include Leminoprazole **25**



Leminiprazole **25**

Figure 13: Structure of 2-[(2-aminobenzyl)sulfinyl]-1*H*-benzimidazoles as irreversible gastric proton pump inhibitors.

1.4.2. Reversible Gastric PPIs:- To overcome the drawbacks associated with the use of irreversible PPIs, research has been directed towards discovery of reversible inhibitors. Examples include SCH28080 **23**, SK& F 97574 **26**, SCH 32651 **27** & SKF 96067 **28.**



SCH 28080 **23**

SK & F 97574 **26**

SCH 32651 **27**

SK & F 96067 **28**

Figure 14: Structures of some reversible gastric proton pump inhibitors.

1.5. Irreversible Proton Pump Inhibitors

1.5.1. Introduction

In early 1970's, anti-secretory activity of the analogs of the pyridylthioacetamide (CMN) **29** was studied. This led to the discovery of a class of extremely efficacious inhibitors of gastric acid secretion, with a novel mode of action, of which the pyridylmethyl benzimidazole sulfoxide, timoprazole **16**, is the archetypal structure. Meanwhile H^+/K^+-ATPase enzyme was also discovered by other research group that enabled the demonstration that compounds related to timoprazole were non-competitive inhibitors of the enzyme. This led to the synthesis of picoprazole **17** and omeprazole **18**, new drugs for the treatment of peptic-ulcer and related diseases. This work also helped in generating and understanding the way in which the enzyme operates.[50]

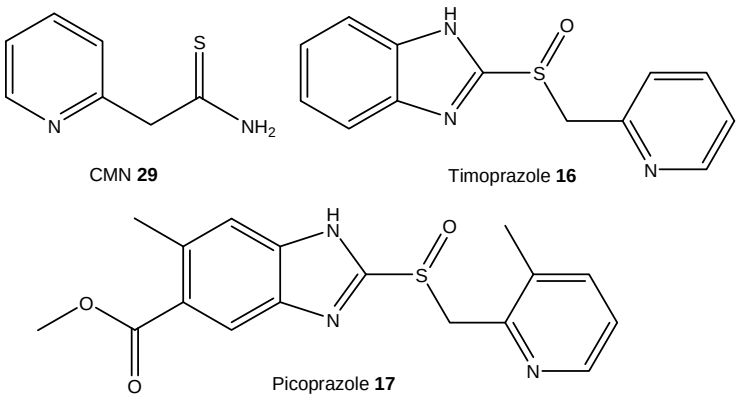


Figure 15: Structure of some initial PPIs

1.5.2. Mechanism of Action

On studying the mechanism of action of these inhibitors of the H^+/K^+-ATPase, several salient features of their action became apparent like; a) the weak basicity of the compounds (pKa≈4), allowing them to accumulate in the acid space adjacent to their site of action (*i.e.*, secretory canaliculus of the parietal cells); b) the sulfoxides themselves have no intrinsic activity, but under the influence of acid undergo a chemical rearrangement to an active species; iii) the active species is thiophillic in nature and covalently binds to thiol functions like cysteinyl residues generating disulfide bridges to the enzymes, thereby causing its inactivation.[50]

The reaction mechanism proposed for the acid transformation of pyridinylmethylsulfinyl benzimidazoles **30** to the sulfenamide **30c** isomers is outlined in figure 16. The reaction is reversible and goes *via* a spiro intermediate, **30a** and the sulfenic acid **30b**. The reversibility was firmly proved by kinetic measurements in both directions for example starting from **30** and **30c**. The formation of the spiro intermediate **30a** *via* Smile's rearrangement[50] is a rate limiting step supported by kinetic measurements. The rate constant obtained for omeprazole analogs is strongly dependent on substituents in the pyridine ring, indicating that a positive charge is created in the pyridine nitrogen atom in the rate-limiting step. The spiro intermediate **30a** is dihyrobenzimidazole with a pronounced tendency to undergo aromatization, thus forming the sulfenic acid **30b** by a C-S bond cleavage. The subsequent formation of the sulfenamide **30c** is in accordance with known reaction between sulfenic acids and amines. This sulfenamide **30c** represents the active enzyme inhibitor and binds covalently to sulfhydryl groups of cysteines of proton pump.[15] Likewise, the reaction of **30c** with β-mercaptoethanol or the cysteine 813 residue of H^+/K^+-ATPase to form adducts **30d** and **30e**, respectively is now easily understood, since sulfenamides or sulfenic acid derivatives in general are known to react with mercaptanes to form disulfides. The adduct **30d** may then react with endogenous thiols or a free thiol group of the enzyme and may react with second molecule of β-mercaptoethanol (or enzyme) in base catalyzed Smiles' reaction to form a sulfide **36g**, probably *via* the unstable mercaptan **30f**, resulting form S-S bond cleavage during simultaneous formation of disulfide of the β-mercaptoethanol. This sulfide **30g**, corresponds to original sulfoxide. Sulfides of this type are known to be oxidized by liver to parent sulfoxides, which raises the intriguing possibility of catalytic drug action in which cycling occurs as shown in Figure.14 for pyridinylmethylsulfinyl benzimidazoles **30** (PMSB's)**.** The recovery of enzymes activity requires *de novo* synthesis of enzyme which is consistent with the long duration of action of drug.[50,51]

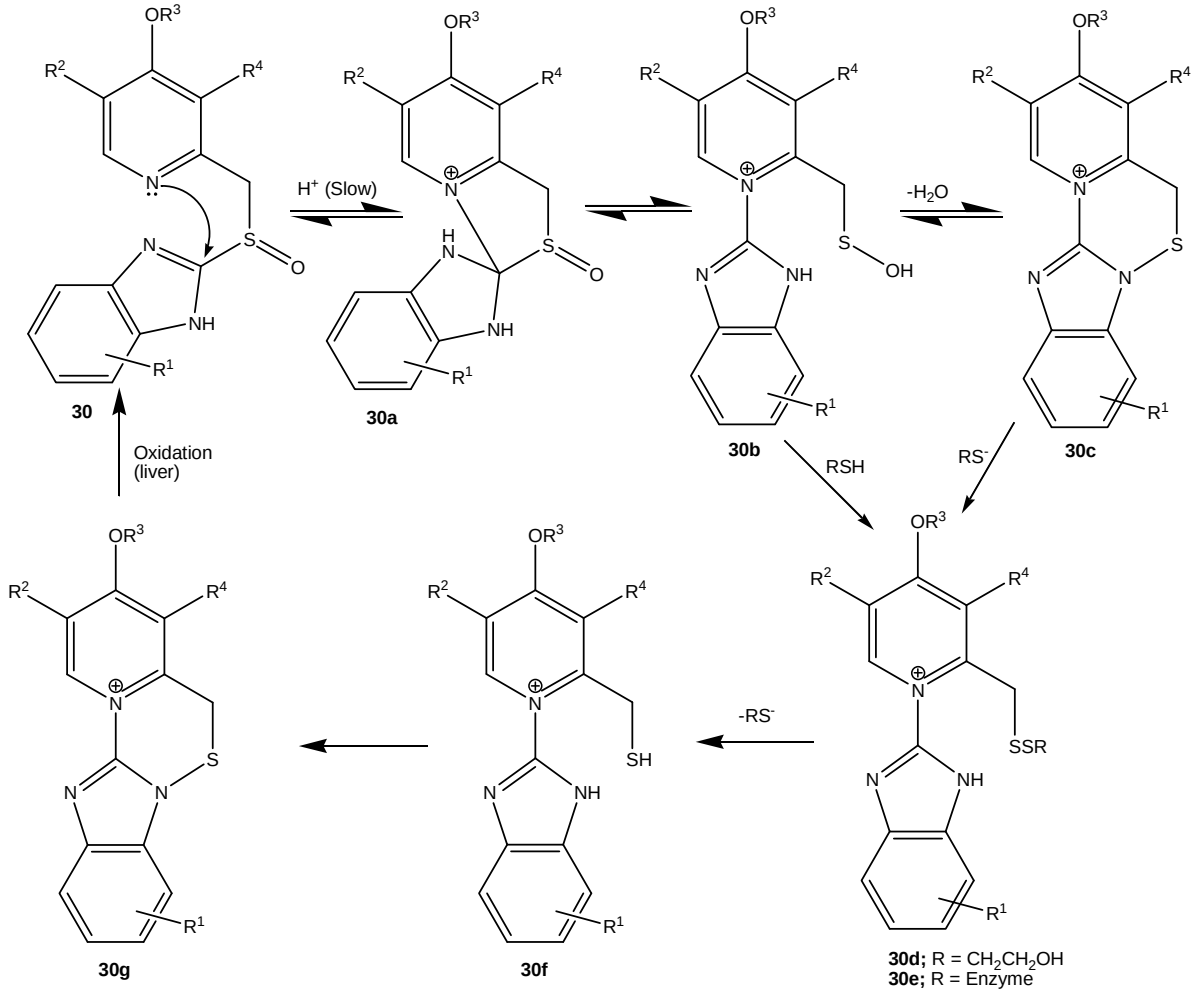
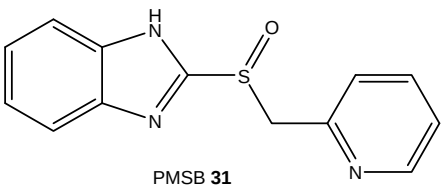


Figure 16: Reaction mechanism proposed for the acid transformation of pyridinyl-methylsulfinyl benzimidazoles **30** (PMSB's) to sulfenamide.

The introduction of methyl group in the 6th position of the pyridine ring of the omeprazole analogs results in compounds stable in acid solutions. This supports the suggested mechanism. Also, the space filling models show that 6-methyl group will experience a strong steric interference with the imidazole ring, which prevents the formation of spiro intermediate **30a.**[51]

1.5.3. Structure Activity Relationships



PMSB **31**

Figure 17: General structure of classical irreversible PPIs

The pyridinylmethylsulfinyl benzimidazole (prototype) **31** (PSMB) can be considered to possess three structural elements: the pyridine ring, the benzimidazole ring system and the linking chain. Replacement of $SOCH_2$ of the linking chain, by a variety of other groups like $-SCH_2$, $-SO_2CH_2$, $-SCH_2CH_2$ and various carbon and oxygen containing chains leads to loss of activity *in vitro*. Extending the length of chain by $-SOCH_2CH_2$ give rise to inactive acid stable compound. In pyridine ring system, degree of nucleophilicity (rather than basicity) of nitrogen atom reflects the ease of spiro intermediate formation. For example, substitution in 6`-positon of the ring results in loss of activity as disfavoring steric interaction. When significant steric effects are absent, a pKa value of ≥ 4 is probably optimal for activity. Weak bases like timoprazole and $4\text{-}CO_2CH_3$ derivatives show greatly reduced activity, as 4-methyl compound is several times less active than 4-alkoxy analogs. In case of omeprazole (pKa = 4), the 4-methyl substitution has little effect on pKa, as it is bent out of plane by the two flanking methyl groups. The substitution in benzimidazole ring does not change the activity to a great extent. Introduction of electron withdrawing substituents like $5\text{-}NO_2$, 5-MeSO, $5\text{-}CF_3$ leading to decreased enzyme inhibition.[50]

With respect to sulfinyl group, gastric proton pump inhibitors exist as a racemic mixture of both enantiomers. Although chirality is lost in corresponding pyridinium sulfenamide formation, it is unclear whether one enantiomer is more susceptible towards acid activation than the other. Both enantiomers of lansoprazole inhibit dbcAMP-induced amino pyridine uptake in isolated canine parietal cells, as well as, H^+/K^+-ATPase activity in canine gastric microsomes with equal activity.[15]

1.5.4. Drawbacks of Irreversible Proton Pump Inhibitors

Extreme acid suppression some times leads to achlorohydria at recommended doses and that may produce enteric infections like typhoid, cholera and dysentery. Significant drug interactions can lead to decreased absorption of some drugs like griseofulvin, ketoconazole, vit.B_{12}, iron salts, *etc.* Unpredictable action and variation in individual responsiveness can cause hypergastrinemia, gastric polips and carcinoma.[52] Other side effects include abdominal pain, diarrhea, nausea and headache. Acute interstitial nephritis progressing to acute renal failure has also been reported to be associated with the use of PPIs.[53]

1.5.5. Pharmacological Properties

Anti-secretory effect of PPIs seems to depend on the presence of *H. pylori* infection because eradication of *H. pylori* has negative consequences on the efficacy of anti-secretory drugs.[54,55] Acid secretion can be restored only through endogenous synthesis of H^+/K^+-ATPase, which has a half-life of production of approximately 50 hours.[56] Rabeprazole shows faster rate of inhibition and a shorter duration of action.[57] Esomeprazole has least bioavailability, whereas, lansoprazole being the most bioavialable.[58] The PPIs are clearly more potent than H_2-receptor antagonist with clinically doses being at 15 times lower than those of H_2 receptor antagonists in the treatment of duodenal ulcers.[59]

Further, Becker *et al.*,[60] evaluated a unique pathway for gastro-protective activity of PPIs demonstrating that both omeprazole and lansoprazole protect human gastric epithelial and endothelial cells against oxidative stress. The antioxidant defense protein heme oxygenease (HO-1) is a target of PPIs in both endothelial and gastric epithelial cells. HO-1 induction might account for the gastroprotective effects of PPIs independently of acid inhibition. Concentration dependent hydroxy radical scavenging activity of PPIs has also been reported suggesting their possible anti-inflammatory activity.[61] As lansoprazole and rabeprazole increased plasma adrenocorticotropic hormone (ACTH) and cortisol levels, they are under study for the treatment of psychiatric disorders involving dysregulation of appetite.[62]

The currently available PPIs have similar pharmacological properties, which are detailed in table 3.

Table 3. Pharmacological properties of the different proton pump inhibitors

Generic name	Half -life (h)	Peak effect (h)	Duration of effect (h)	pKa	Bioavail ability (%)	Metabolism	Excretion (%)
Omeprazole[63]	0.7	2	24-72	~4	30-40	Extensively hepatic	U=77 F=23
Pantoprazole[64]	1	2.5	24-72	~4	77	Extensively hepatic	U=71 F=18
Lansoprazole[65]	2	1.7	>24	~4	80	Extensively hepatic	U=35 F=65
Rabeprazole[66]	1	2-5	24	~5	52	Extensively hepatic	U=90 F=10
Esomeprazole[67]	1.3	1.5	24-27	~4	64	Extensively hepatic	U=80 F=20

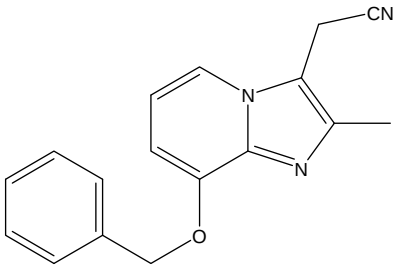
U=urine; pKa=dissociation constant; F=faeces

1.6. Reversible Proton Pump Inhibitor's

1.6.1. Introduction

Prolonged suppression of gastric acid secretion produced by both H_2 receptor antagonists and PPIs produce extended periods of hypergastrinemia, which has been associated with the formation of precancerous changes in human gastric mucosa and gastric carcinoids in long term animal studies. However, research efforts are currently targeted at obtaining reversible proton pump inhibitors often referred as Acid Pump Antagonists (APAs). Several research groups have progressed APAs into development though currently none is marketed.[2]

The imidazopyridine based compound SCH28080 **23** was the prototype of this class.[68] Antisecretory effect of this compound is mediated through gastric proton pump and this has been further demonstrated by its ability to antagonize the binding of omeprazole.[69]



SCH 28080 **23**

Figure 18: Prototype Acid Pump Antagonist

1.6.2. Mechanism of Action

Omeprazole **18** and SCH 28080 **23** differ in inhibition kinetics for their proton pump inhibitory activity. In contrast to omeprazole, SCH 28080 **23** is a competitive inhibitor of high affinity luminal K^+ site of the gastric proton pump. In contrast to Na^+/K^+-ATPase, it is highly selective to H^+/K^+-ATPase activity. SCH 28080 is a protonable weak base (pKa = 5.6), hence like omeprazole it accumulates in the acidic compartments of the parietal cells in its protonated form.[70] SCH 28080 is chemically stable and after protonation, is itself active and does not need an acid induced transformation, as required by omeprazole and its congeners.[71]

1.6.3. Structure-Activity Relationships

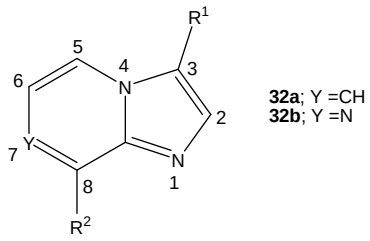


Figure 17: General structure of reversible PPIs to describe SAR

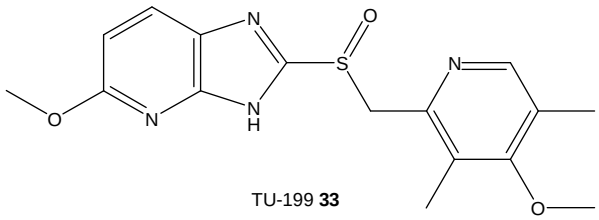
Taking eighty-one derivatives of imidazo[1,2-*a*]pyridine derivatives of **32a** and **32b** related to SCH 28080 **23** were synthesized and studied based on which following observations were made:- 1) a small alkyl group at C-2 (methyl or ethyl) favored activity; 2) cyano methyl or amino group at C-3 was a requirement for maintaining both anti-secretory and cytoprotective activity; 3) activity at 8-position was maximized with benzyloxy, 3-thienylmethoxy or phenylmethylamino substituion; 4) replacement of C-7 by N leads to retention of activity. Surprisingly little work has been reported on these

reversible inhibitors of H^+/K^+-ATPase. Although, highly efficacious drugs could emerge from research on APAs.[50]

1.7. Reports on the Continuing Research and Development on Different PPIs

1.7.1. Irreversible Inhibitors; Related to Omeprazole

K. Uchiyama, *et al.*,[72] have reported the synthesis of (+/-) 5-methoxy-2-[(4-methoxy-3,5-dimethyl-pyridin-2-yl)methylsulfinyl]-1*H*-imidazo[4,5-*b*]pyridine, (TU-199) **33** and its effect on histamine, carbachol and tetragastrin stimulated gastric acid secretion. They have claimed it to be having more potent and long lasting effect on gastric acid secretion via inhibition of H^+/K^+-ATPase than omeprazole.

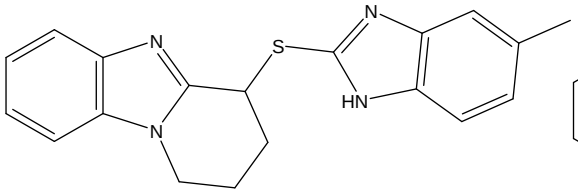


TU-199 **33**

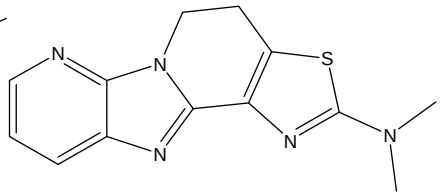
1.7.1.1. Changes Made on/in Benzimidazole Nucleus:

Changes have been made on the benzimidazole nucleus without loss of activity. Following are some reports:

Woo *et al*[73]., have reported the biological evaluation of 2-[3-(2,3-dihydro-1*H*-pyrolo [1,2-*a*]benzimidazolyl)sulfinyl]-5-methyl-1*H*-benzimidazoles, (YJA20379-4) 3**4** which had marked inhibitory effect on H^+/K^+-ATPase. YJA20379-4 also exhibited anti-*H. pylori* activity 3 times higher than omeprazole along with the enhancement of mucosal defense, thus, indicating a wide spectrum of anti-ulcer activities**.** In another related work, Kim, *et al.*,[74] modified, **34,** by fusing imidazopyridines with thiazolopyridines to get YJA-20379-2. **35.** This compound not only suppressed H^+/K^+-ATPase activity, but also had significant reinforcing activity on the defensive factors.

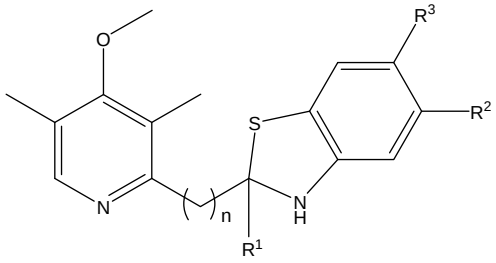


YJA20379-4 **34**



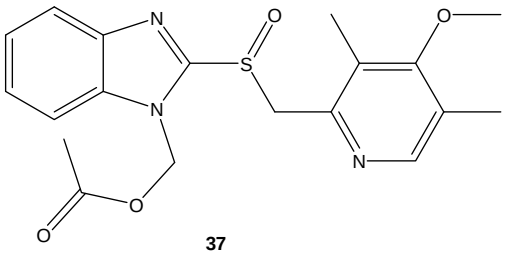
YJA20379-2 **35**

Yoon, *et al.*,[75] have replaced the conventional *benzimidazole* ring system with the bioisosteric benzothiazolidine ring system. They have reported the synthesis of derivatives of 2-[(3,5-dimethyl-4-methoxypyridylalkyl]-benzothiazolidine **36** which were found to be more potent *in vitro* inhibitors of H^+/K^+-ATPase. The methylsufinyl linkage has also been replaced by methylene linkages.



36

N-alkylation/acylation of the benzimidazole ring nitrogen leads to the biolabile *N*-substituted benzimidazole derivatives (prodrugs) of timoprazole. The parent *N*-H compound is liberated either by *in vivo* esterase hydrolysis or requires an acidic environment. *N*-(acyloxy)alkyl-substituted benzimidazoles showed improved chemical stability of which **37** proved twice potent as omeprazole. Similarly **38** was found to be twice active as timoprazole.[76]



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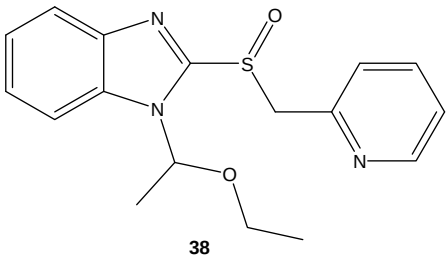
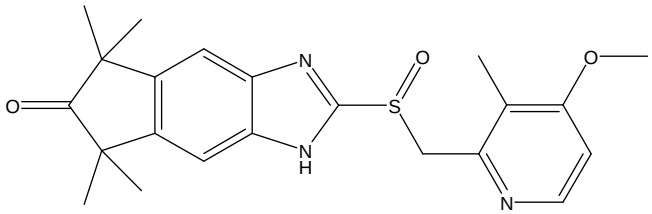


38

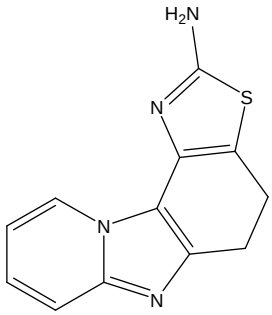
Fusion of one more ring on the benzimidazole nucleus has been shown to be beneficial. Sigrist-Nelson *et al*[77]., have reported the synthesis and evaluation of 5,7-dihydro-2{[(4-methoxy-3-methyl-2-pyridyl)methyl]sulfinyl}-5,5,7,7-tetramethylindeno[5,6-*d*]imidazol-6-(1*H*)-one (Ro 18-5364) **39** as an extremely effective inhibiting agent. Ro 18-5364 produced almost complete inhibition of the H^+/K^+-ATPase activity, as well as, associated

proton translocation. The activity of the inhibitor appeared to be independent of its stereochemistry. However, sulfide analog of Ro 18-5364 was devoid of any significant inhibitory activity.



RO 18-5364 **39**

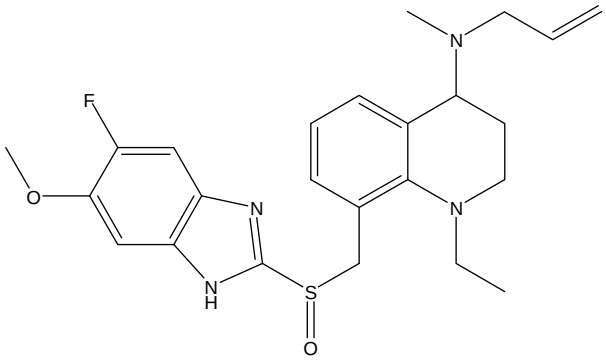
Yoon, *et al.*,[78] have synthesized imidazopyridines fused with benzothiazole moiety **40**. These novel compounds not only showed potent inhibitory activity against H^+/K^+-ATPase but also showed significant cell protective activity.



40

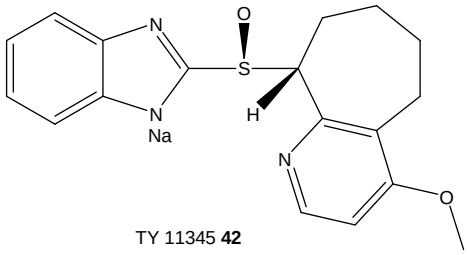
1.7.1.2. Changes made on the Pyridine Nucleus:

The pyridine ring has been annulated to one more ring or its bioisosteric replacement is done or has been replaced by an aromatic carbocycle, without loss of potency. Uchida *et al*[79]., have quinoline analogs of PMSBs. A series of some 4-substituted 8-[(2-benzimidazolyl)sulfinylmethyl]-1,2,3,4-tetrahydroquinolines, has exhibited H^+/K^+-ATPase inhibitory and anti-secretory activities against histamine induced gastric acid secretion. Of these, 4-(*N*-allyl-*N*-methylamino)-1-ethyl-8-[(5-fluoro-6-methoxy-2-benzimidazolyl)sulfinylmethyl]-1-ethyl-1,2,3,4-tetrahydroquinoline **41** was found to have potent anti-ulcer activity. Further, many of the derivatives showed cytoprotective activities. Notably, the methyl sulfinyl side chain is not attached to the pyridine nucleus but to the benzene ring.



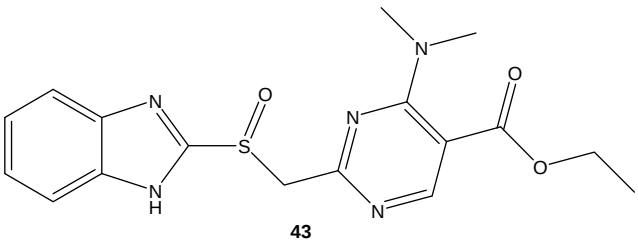
41

Annulations of pyridine ring to an alicycle has also been tried. Yamada *et al.,*[80] have synthesized a series of 2-[(cycloalka[*b*]pyridinyl)sulfinyl]-1*H*-benzimidazoles and tested for the inhibition of pentagastrin induced gastric acid secretion. A novel benzimidazole derivative containing a cyclohepta[*b*]pyridine moiety was found to be the most potent among the congeners, which included five- to eight- membered cycloalka[*b*]pyridine ring system. Of them 2-[(6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridin-9-yl)-sulfinyl]-1*H*-benzimidazole analogs having various substituents on aromatic rings were found to be superior than omeprazole. TY-11345 **42** was selected for further evaluation. Notably, the methysulfinyl linkage has also been modified.



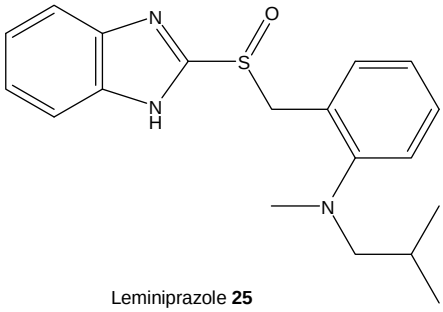
TY 11345 **42**

Replacement of the pyridine ring with less basic isosteric pyrimidine ring has also been reported by Japanese workers[81]. They have evaluated 2-(1*H*-benzoimidazole-2-sulfinylmethyl)-4-dimethylamino-pyrimidine-5-carboxylic acid ethyl ester **43** for its proton pump inhibition. It was found to have marked proton pump inhibitory activity with IC_{50} of 7.5 μm as compared to omeprazole IC_{50} of 5.8 μm.



43

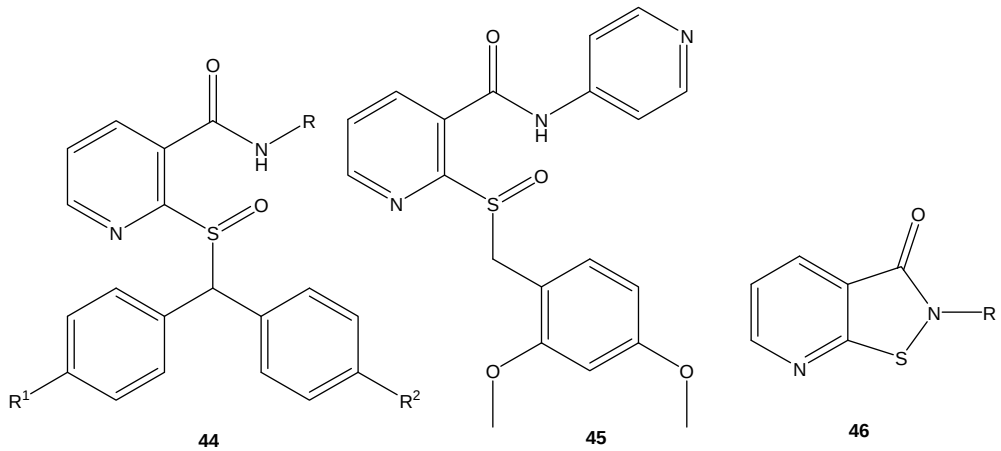
Replacement of the heterocyclic pyridine ring with aromatic carbocycles has also been attempted. Tsukahara *et al*[82]., synthesized [2-(1*H*-benzoimidazole-2-sulfinylmethyl)-phenyl]-isobutyl-methyl-amine (Leminoprazole) **25** which was found to be a potent PPI.



Leminiprazole **25**

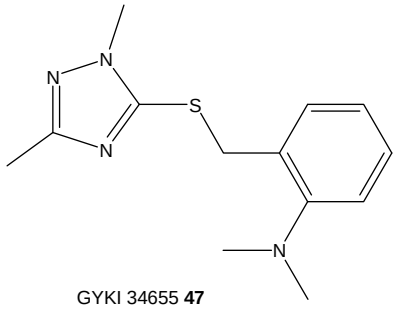
1.7.2. Irreversible Inhibitors - Not Related Structurally to Omeprazole

Terauchi *et al*[83]., have reported the synthesis and evaluation of *N*-substituted 2-(benzhydryl)nicotinamides **44** and *N*-substituted 2-(benzylsulfinyl)nicotinamides **45**, which upon acid activation were converted to their active forms, 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridines **46** responsible for gastric H^+/K^+-ATPase inhibition.[55] Of these, **45** showed *in vivo* and *in vitro* inhibitory activities equivalent to omeprazole and was more stable than omeprazole, lansoprazole and pantoprazole at neutral and weakly acidic pH. Further, these parent nicotinamides, were devoid of any *in vitro* H^+/K^+-ATPase inhibitory activity of themselves.



44 **45** **46**

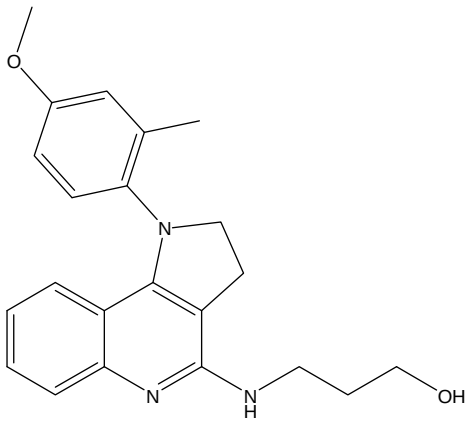
Berzsenyi *et al.*,[84] have synthesized and tested [2-(2,5-dimethyl-2*H*-[1,2,4]triazol-3-ylsulfanylmethyl)phenyl]dimethylamine, (GYKI-34655) **47** as irreversible inhibitor, which was found to be a potent gastric anti-secretory, anti-ulcer and cytoprotective agent.



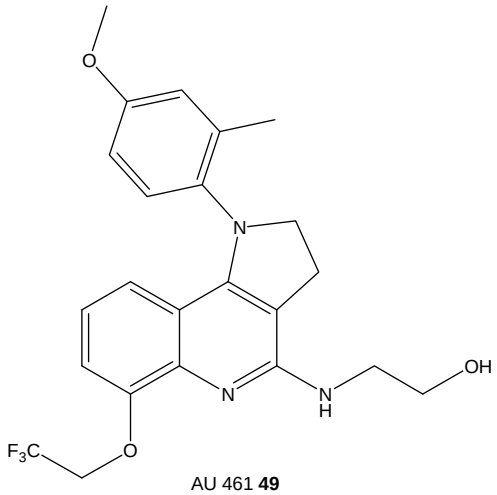
GYKI 34655 **47**

1.7.3. Reversible Inhibitors (Acid Pump Antagonists)

Cheon, *et al.*,[85] have reported the activity of 1-(2-methyl-4-methoxyphenyl)-4-[(3-hydroxypropyl)amino]-6-methyl-2,3-dihydropyrrolo[3,2-*c*]quinoline (DBM-819) **48** as potential reversible inhibitor. DBM-819 successfully reduced histamine and pentagastrin stimulated gastric acid secretion and protected against gastric lesions induced by ethanol, NaOH, indomethacin and aspirin, suggesting that DBM-819 acts as an effective anti-ulcer agent *in vivo*. The same workers have also evaluated 1-(2-methyl-4-methoxyphenyl)-4-[(2-hydroxyethyl)amino]-6-trifluoroethoxy-2,3-dihydropyrrolo[3,2-*c*] quinoline (AU-461) **49**, which was found to be reversible and competitive inhibitor with respect to the activating K^+ cation.[86]

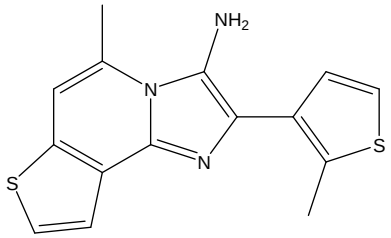


DBM 819 **48**



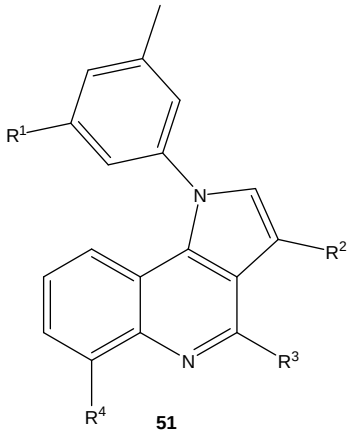
AU 461 **49**

3-Amino-5-methyl-2(2-methyl-3-theinyl)-imidazo[1,2-*a*]thieno[3,2-*c*]pyridine (SPI-447) **50** have also been studied as a reversible inhibitor of proton pump. SPI-447 had no effect on Na^+/K^+-ATPase activity and was K^+ competitive inhibitor of H^+/K^+-ATPase similar to SCH28080 **23**.[69]



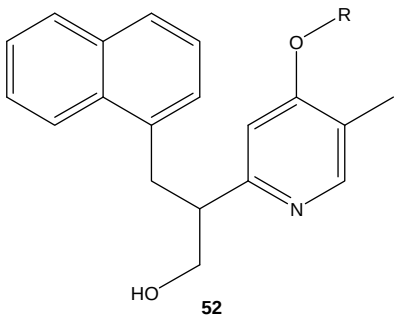
SPI 447 **50**

A series of 1-aryl-3-substitued pyrrolo[3,2-*c*]quinolines **51**, have been found to be inhibitor of H^+/K^+-ATPase. *In vitro* H^+/K^+-ATPase inhibitory activity was dependent on the substituents at the 3-posotion of the pyrrolo[3,2-*c*]quinolines, whereas 1-aryl substituents affected the *in vivo* gastric acid secretion.[87]



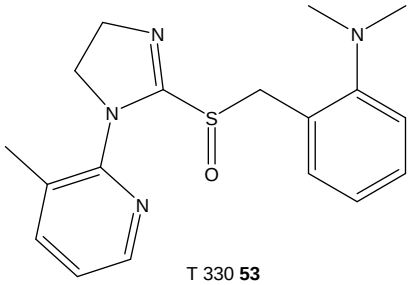
51

Niiyama *et al.*,[88] have synthesized novel 4-substituted pyridine derivatives like 4-alkoxy-, 4-alkylthio and 4-aryloxy-5-methyl-2-[1-(hydroxymethyl)-2-(1-napthyl)-ethyl (ethenyl)] pyridine **52** which were found to have reversible inhibitory activity against H^+/K^+-ATPase.



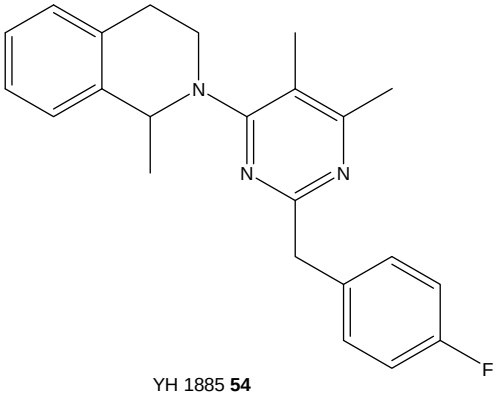
52

Kinoshita, *et al.*,[89] have reported a be novel reversible PPI, 2-[(2-dimethyl-aminobenzyl)sulfinyl]-1-(3-methylpyridine-2-yl-)imidazole **53** (T-330), which was found to possess, anti-secretory activity more potent than omeprazole and ranitidine.



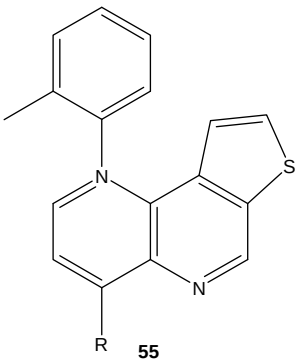
T 330 **53**

Kim, *et al.,*[90] have reported the synthesis and proton pump inhibitory activity of YH-1885 **54** which is now one of the most clinically advanced APA's.



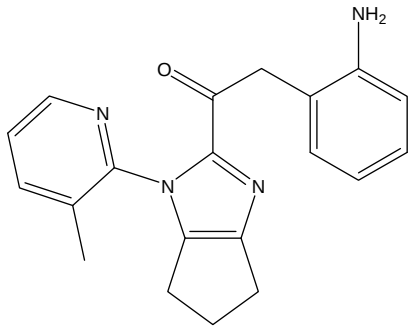
YH 1885 **54**

Condensed napthyridines have also been reported as possible reversible proton pump inhibitors, *e.g.* 4-substituted-1-(2-methylphenyl) thieno [2,3-*c*]-1,5-napthyridines **55.** These compounds were evaluated for their H^+/K^+-ATPase and anti-secretory activity. However, *in vitro* activity of these substituted napthyridines was not high enough to be of further interest.[91]



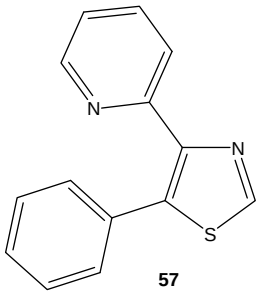
55

Yamada *et al.,*[92] reported the reversible H^+/K^+-ATPase inhibitory activity of 2-[(2-aminobenzyl)sulfinyl]-1-(2-pyridyl)-1,4,5,6-tetrahydrocyclopenta[*d*]imidazoles. Acid degradation study of **56** indicates mechanism of action different from omeprazole.



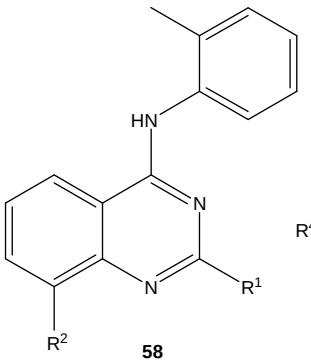
56

Ife *et al.*,[93] have reported 4-(2-pyridyl)-5-phenylthiazoles **57** as reversible, K^+-competitive gastric H^+/K^+-ATPase inhibitors.

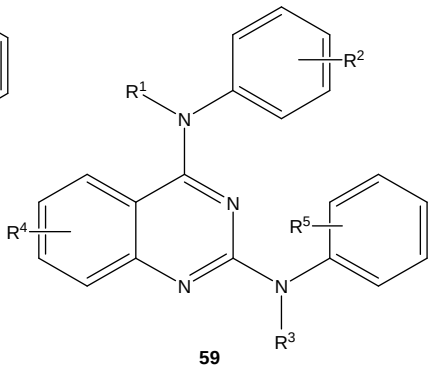


57

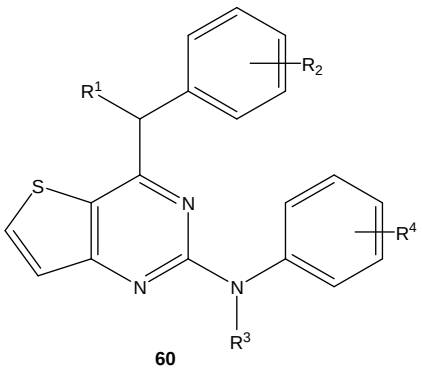
They have further reported reversible proton pump inhibitory activity of 4-(arylamino) quinazolines **58**, 2,4-bis(arylamino)quinazolines **59** and 2,4-bis(arylamino)thieno-pyrimidines **60**. In case of the theinopyrimidines, the [3,2-*d*] isomers proved to be more effective than [2,3-*d*].[94]



58

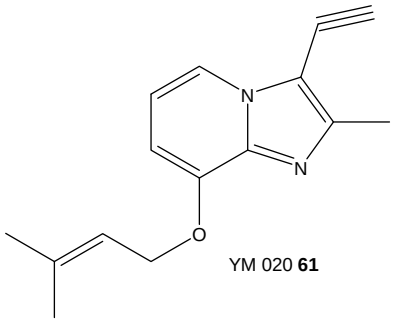


59



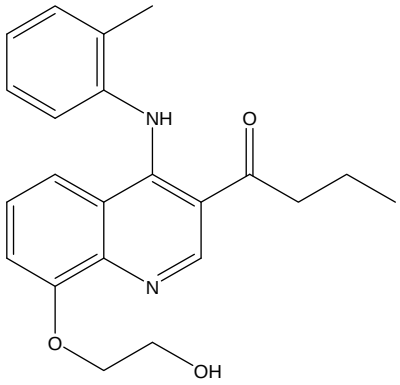
60

Yuki, *et al.*,[95] have reported proton pump inhibitory activity of 2-methyl-8-(3-methyl-but-2-enyloxy)imidazo[1,2-*a*]pyridine-3-carbonitrile (YM-020) **61**.



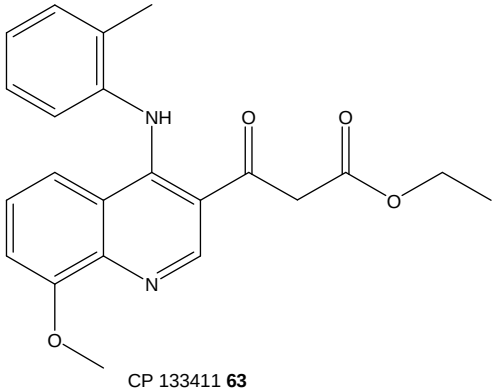
YM 020 **61**

Leach *et al.*,[96] have reported H^+/K^+-ATPase inhibitory activity of 3-butyryl-4-[(2-methylphenyl)amino]-8-(2-hydroxyethoxy)quinoline, SK&F 97574 **62**.[68] It is found to be well tolerated and efficacious in Phase-I studies.



SK & F 97574 **62**

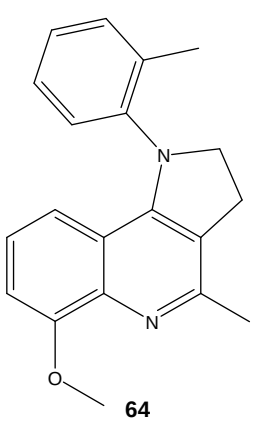
Similar derivatives, 3-[3-(ethoxycarbonyl)propionyl]-8-methoxy-4-[(2-methylphenyl) amino]quinolines, (CP-113411) **63** have also been reported. Besides being reversible inhibitors of gastric proton pump, they also inhibited bone absorption by osteoclasts.[97]



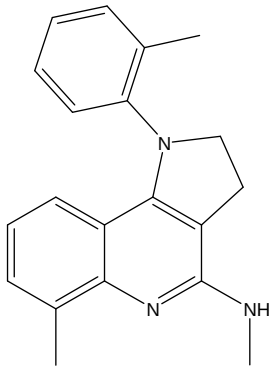
CP 133411 **63**

Ife *et al.*,[98] have reported the synthesis and evaluation of a series of 1-arylpyrrolo[3,2-*c*]-quinolines as inhibitors of H^+/K^+-ATPase. Unsaturation in the five membered ring of this nucleus made little difference, but introduction of heteroatom in the same ring reduced the activity drastically. Of the series, compound **64** showed reversible K^+ competitive

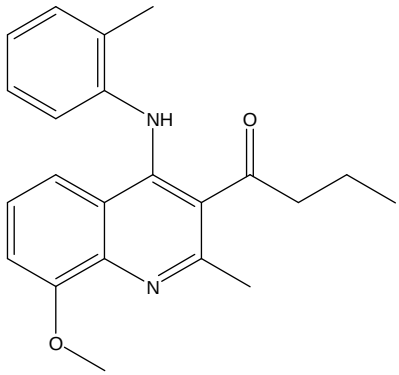
binding to the enzyme.[99] Further, modification of same nucleus by Leach *et al.*,[100] led to discovery of SK & F 96356 **65**, a potent inhibitor of gastric acid secretion. Ife *et al*[98]., studied 3-substituted–4-(phenylamino)quinolines as reversible inhibitors of H^+/K^+-ATPase. From this series, SK & F 96067 **28** was found to be potent inhibitor of histamine stimulated gastric acid secretion.



64

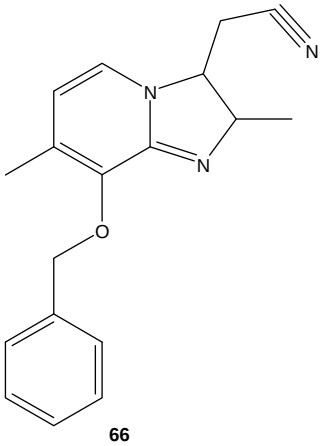


SKF & F 96356 **65**

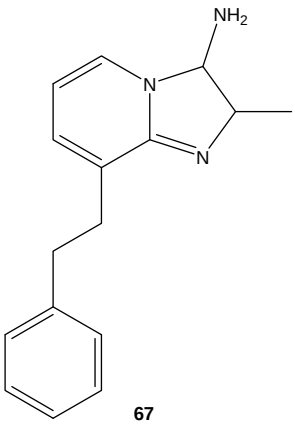


SKF & F 96067 **28**

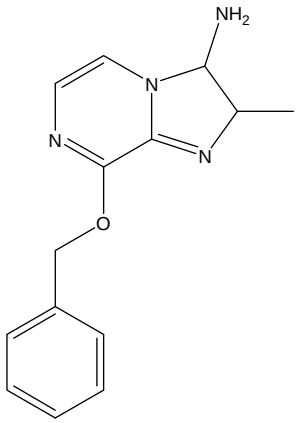
Kaminski *et al.*,[101] identified 3-(cyanomethyl)-2,7-dimethyl-8-(phenylmethoxy)imidazo [1,2-*a*]pyridine **66**, 3-amino-2-methyl-8-(2-phenylethyl)imidazo[1,2-*a*]pyridine **67**, and 3-amino-2-methyl-8-(phenylmethoxy)imidazo[1,2-*a*]pyrazine, SCH-32651 **68**. These analogues exhibit anti-secretory and cytoprotective activity, particularly, SCH 32651 was mentioned as a promising candidate.



66



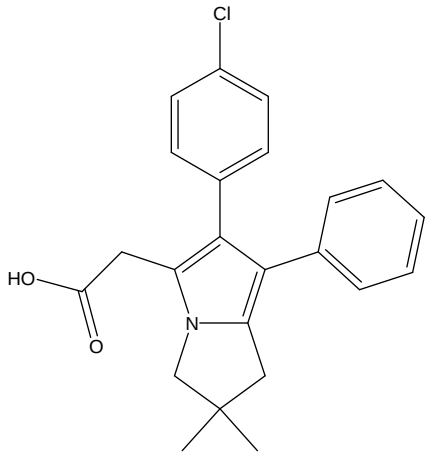
67



SCH 32651 **68**

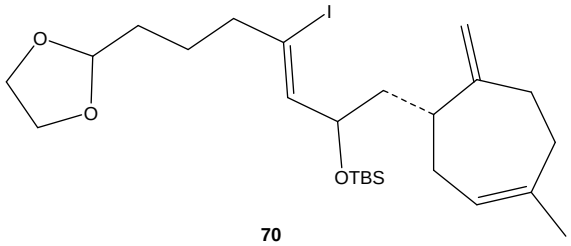
1.7.4. Other Proton Pump Inhibitors under Investigation

Smolka, *et al.*,[102] have reported the synthesis and evaluation of the pyrrolizine derivatives of the type, ML 3000, **69,** which along with the inhibition of H^+/K^+-ATPase also inhibited 5-lipoxygenase.

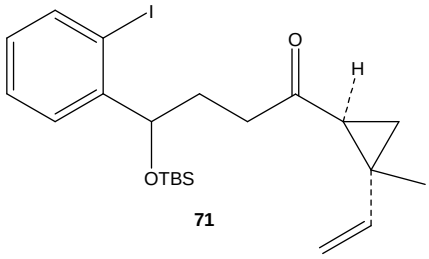


ML 3000 **69**

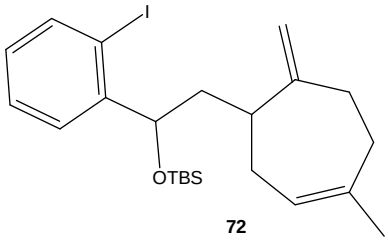
Hayashi *et al.,*[103] have reported the proton pump inhibitory effects of synthetic compounds with the scopadulan ring system, which have ether linkages at C-6, C-13 and/or C-18 positions. *Tert*-butyldimethylsilyl ethers of 5-methylenecycloheptene and related compounds **70-72** were shown to be novel proton pump inhibitors.



70



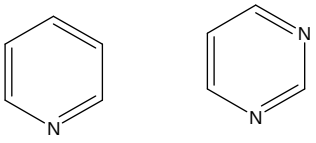
71



72

Jain *et al.,*[104] have designed a variety of novel mononuclear and condensed pyrimidine analogs of omeprazole replacing the pyridine heterocycle of conventional PSMBs with its 3-aza isoster, pyrimidine. The rationale behind their work is that the weakly basic nature of pyrimidine (*pKa*-1.31) as compared to pyridine (*pKa*-5.2), has the N_1 and N_3 of pyrimidine less electron donating than the pyridine nitrogen.[105] This makes the formation of sulfenamide intermediate difficult. (Figure 20)

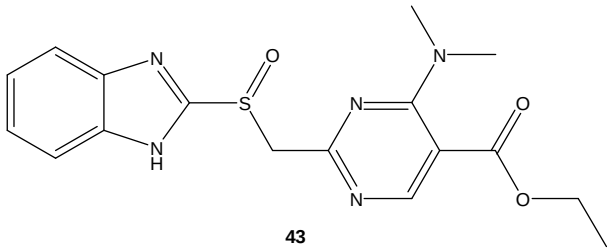
A review of a basic literature on organic & the heterocyclic chemistry reveals that indeed pyrimidine ring is the ring of choice.



Pyridine
pKa : 5.2

Pyrimidine
pKa : 1.31

The weekly basic nature of pyrimidine (pKa 1.31) is striking in relation to pyridine (pKa 5.2). It is understandable as in inductive effect (depletion of π-electrons), caused by insertion of the avidly electron-attracting second nuclear nitrogen atom. Pyrimidine may therefore be likened more to beta-nitropyridine (pKa 0.8), which contains the equally strongly electron-attracting nitro group, than to the parent pyridine. A cursory review of the literature does reveal successful use of this logic**.** Replacement of the pyridine ring with less basic isosteric pyrimidine ring has also been reported by Japanese workers[81]. They have evaluated2-(1*H*-benzoimidazole-2-sulfinylmethyl)-4-dimethylamino-pyrimidine-5-carboxy-lic acid ethyl ester **43** for its proton pump inhibition. It was found to have marked proton pump inhibitory activity with IC_{50} of 7.5 μm as compared to omeprazole IC_{50} of 5.8 μm.



43

It really appears difficult that in this system the formation of the disulfide intermediate is likely. This is owing to the poor availability of electrons on pyrimidine and its nitrogens.

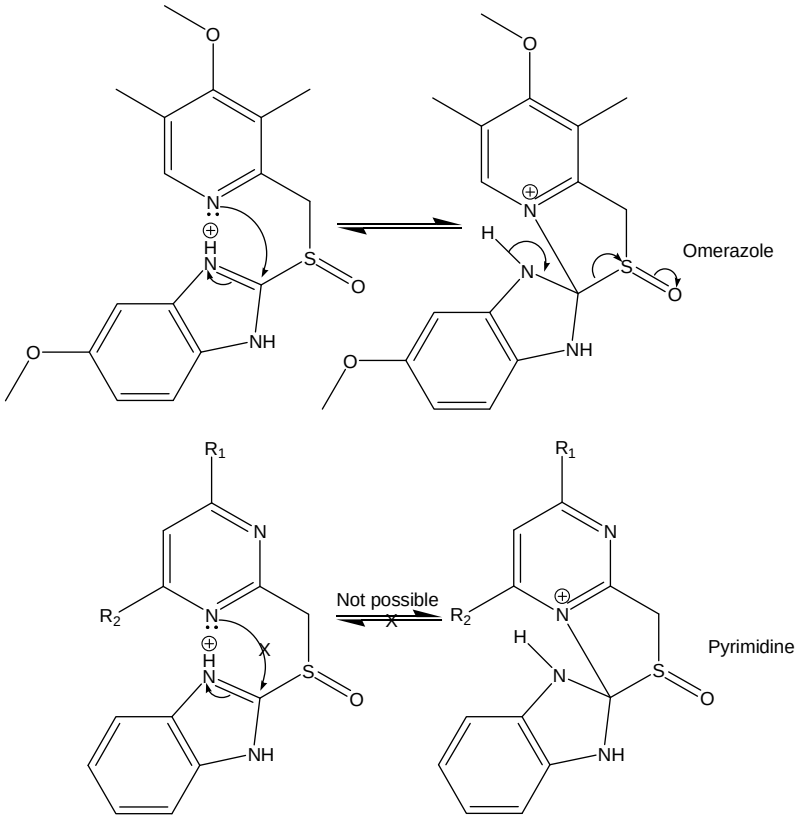
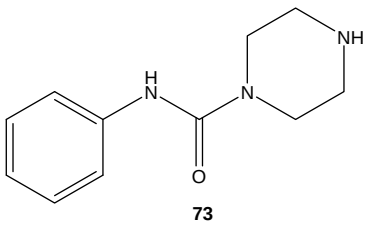


Figure 20: Inability of pyrimidine analog of omeprazole to form sulfenamide intermediate.

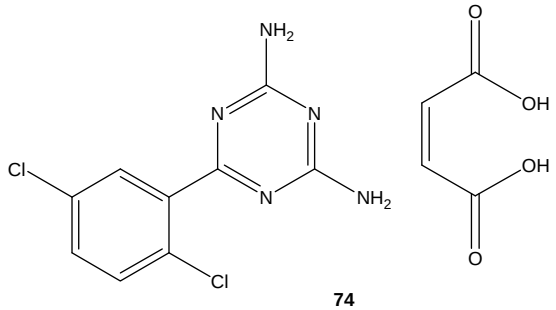
1.7.5 Some More Literature Reports:

Corvi-Mora[106] synthesized derivatives of piperazinyl acetamides **73** possessing anti-ulcer and anti-secretion properties. These compounds were entirely free from anticholinergic activity. Though mechanism of action is not clear, anti-ulcer activity was evaluated successfully in different models like reserpine ulcer with rats and phenylbutazone-histamine ulcer.



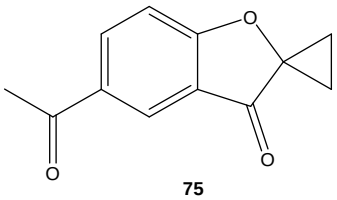
73

Murai *et.al.*,[107] reported the anti-ulcerative activity of benzoguanamine derivatives **74**.



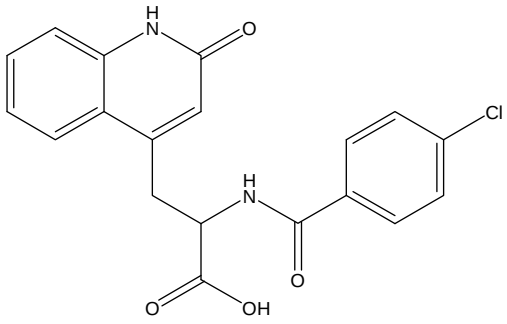
74

Hirosada *et.al.*,[108] reported gastric secretion inhibiting activity of spiro compounds with novel skeleton **75**. These compounds were found to be of value as anti-ulcer, anti-inflammatory and as analgesic.



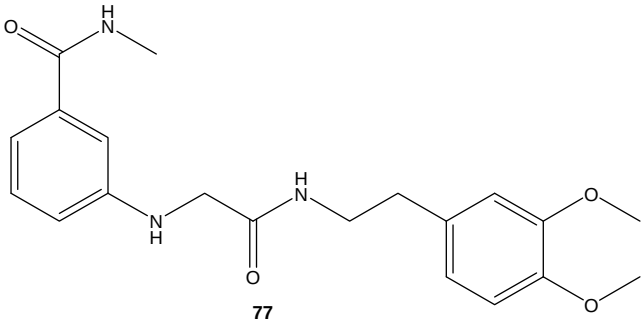
75

Otsubo *et al.*,[109] synthesized the enantiomers of 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propionic acid **76**, new antiulcer agent that enhances mucosal resistance The (+) isomer, rebamipide, was about 1.7 times as potent as the (-)-isomer in antiulcer activity against ethanol-induced gastric ulcers.



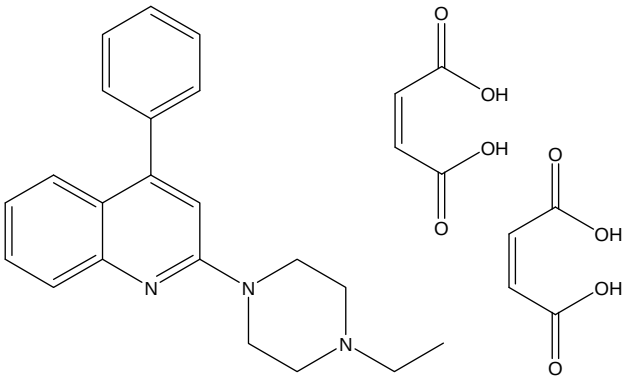
76

Miki, *et al.*,[110] have synthesized derivatives of benzamide, **77** which have exhibited excellent inhibitory effects on several gastric models such as alcohol ulcer, indomethacin ulcer, aspirin ulcer and stress ulcer. Also, these compounds exhibited an inhibitory effect on duodenal ulcer models such as cysteamine ulcer and dulcerozine ulcer.



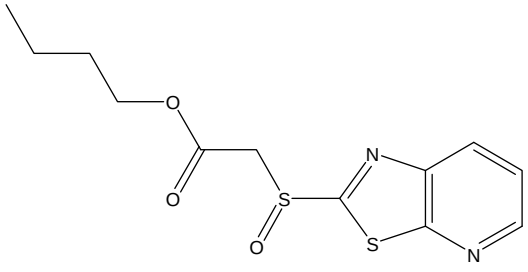
77

Hino, *et al.*,[111] have synthesized a novel class of anti-ulcer agents, substituted 4-phenyl-2-(1-piperazinyl)quinolines. These compounds can be classified into three groups; that is effective on stress-induced ulcers, that is effective on both stress-induced and ethanol-induced ulcer and that is selectively effective on the ethanol-induced ulcer. Among the compounds AS-2646 **78** (fumarate salt), showed potent inhibition of stress induced ulcer and gastric acid secretion.



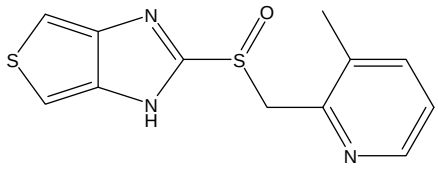
AS 2646 **78**

Katano, *et al.*,[112] have reported the anti-ulcer activity of some pyridothiazole derivatives **79** which exhibited both strong effect of inhibiting the secretion of gastric acid and an enhanced effect on protecting the gastrointestinal mucosa.

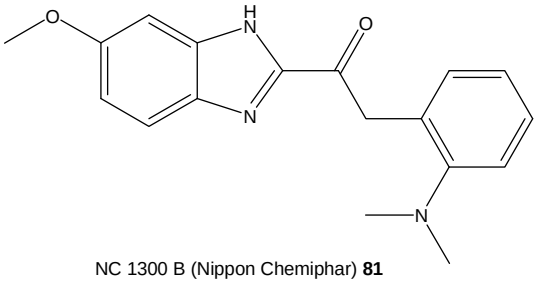


79

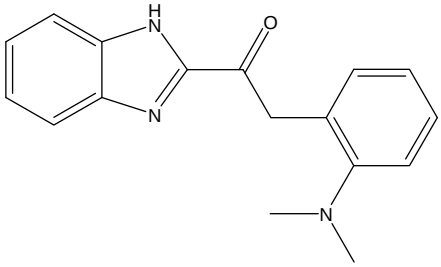
Herling *et al.*,[113] had synthesized structural analogs of PSMBs by replacing benzimidazoles heterocycle by theinoimidazole to get S 1924 **80**. Similarly, aminobenzyl ring has also been tried as a replacement to pyridine ring of PSMBs as in **81** and **82**.



S 1924 (Hoechst) **80**

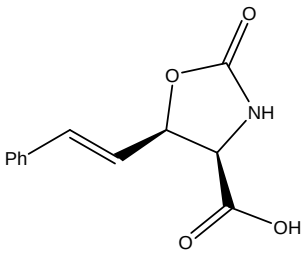


NC 1300 B (Nippon Chemiphar) **81**



NC 1300 (Nippon Chemiphar) **82**

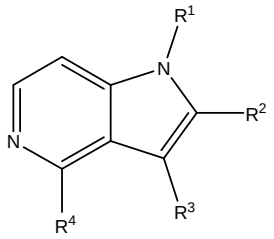
SAR and QSAR of *N*-acyl derivatives of amino acids for inhibition of gastric proton pump using gastric microsomal vesicles, and their effect on pylorus ligation-induced ulcers in rats has been studied. *N*-acylated amino acid derivatives, mostly analogs of proglumide, benzotript and rebamipide have shown potent anti-ulcer properties. It has been found that a significant number of *N*-acylated amino acids showed good degree of inhibition of gastric proton pump. From all of these compounds for their ability to control acid secretions in pylorus-ligated rats, *cis*-5-(2-phenylethenyl)-2-oxo-oxazolidine-4-carboxylic acid **83** was found to be the most potent compound.[114]



83

A novel series of pyrrolo[3,2-*c*]pyridine derivatives **84**, has been synthesized and evaluated for their reversible proton pump inhibitory effects. From this series, compound 3-benzyl-2-methyl-4(1,2,3,4-tetrahydroisoquinolin-2-yl)-1*H*-pyrrolo[3,2-*c*]pyridine hydro chloride was found most potent reversible PPI, which inhibits H^+/K^+ATPase activity by 50% before washout and did not inhibits H^+/K^+ATPase activity after washout. The

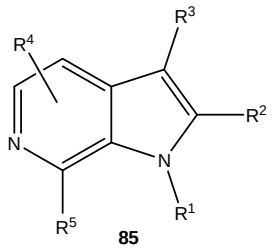
gastric H^+/K^+ATPase inhibitory activity of these compounds completely recovered to non-treated group levels after washout, confirming the reversible inhibition of gastric H^+/K^+ATPase.[115] Another compound from the same series, 2-(2,3-dimethyl-1-propyl-1*H*-pyrrolo[3,2-*c*]pyridine-7-yl)-1,2,3,4-tetrahydroisoquinoline hydrochloride was also found very potent inhibitor of H^+/K^+ATPase with same mechanism of action.[116]



84

R^1= H, alkyl, alkoxy, hydroxy, cycloalkyl, thiazolyl, alkenyl, benzyl *etc.*
R^2 = H, alkyl
R^3 = H, alkyl, alkenyl, benzyl, *etc.*
R^4 = 1,2,3,4-tetrahydroisozuinolinyl, benzyloxy, amino, alkylcarbonyl, phenoxycarbonyl, benzyl *etc.*

Another novel series of pyrrolo[2,3-*c*]pyridine derivatives[117] **85**, analogues to the above series in structure was found to be very potent as reversible inhibitors of H^+/K^+ATPase, especially the compound, 7-(4-fluorobenzyloxy)-2,3-dimethyl-1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*c*]-pyridine hydrochloride which was very potent with ED_{50} of 14.0 mg/kg. Another analogues series[118], pyrrolo[3,2-*b*]pyridine has also been equally found very potent. The compound, 7-(4-fluorobenzyloxy)-1-isobutyl-2,3-dimethyl-1*H*-pyrrolo[3,2-*b*]pyridine hydrochloride was very effective with ED_{50} of 2.4 mg/kg.

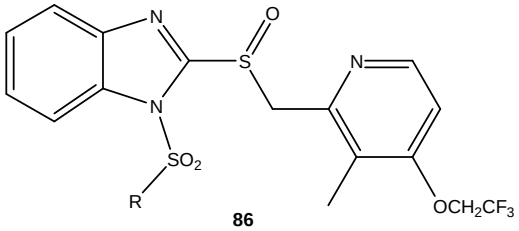


85

R^1 = H, alkyl, alkoxy, alkoxycarbonyl, alkylcarbonyl, alkylamine, naphthyl, quinolinyl, thiazolyl, thiophenyl, isoxazolyl, alkenyl, acetyl, phenyl *etc.*
R^2 = alkylmstraight chain or branched
R^3 = H, alkyl, alkylsulfanyl, *etc.*
R^4 = H, halogen, cyno, hydroxycarbonyl, alkoxycarbonylamino, morpholinocarbonyl *etc.*

Prodrugs of benzimidazole-type proton pump inhibitors of general structure **86** have been studied, to develop agents that slowly hydrolyze to provide benzimidazole type proton pump inhibitors which inhibit exogenously or endogenously gastric acid secretion along

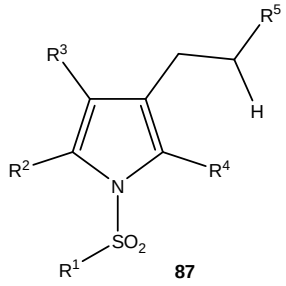
with improved solubility in physiological fluids and improvement in cell penetration. The R substitution on imidazole is expected to undergo cleavage under physiological conditions or under influence of an enzyme to provide the corresponding compound with a free NH group.[119]



86

R = Sub. aryl, sub. pyridine, sub. naphthalene, sub. quinoline, sub. thiazole, sub. benzothiazole or sub. amine *etc.*

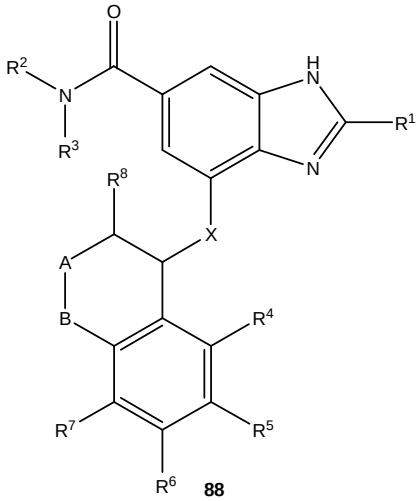
A novel series of pyrrole containing derivatives of general structure **87** has been synthesized and evaluated for their anti-secretary effects. Compound, *tert*-butyl{[5-bromo-1-(pyridine-3-ylsulfonyl)-1*H*-pyrrol-3-yl]methyl}methylcarbamate was a potent compound, from this series with IC_{50} of 210 nM.[120]



87

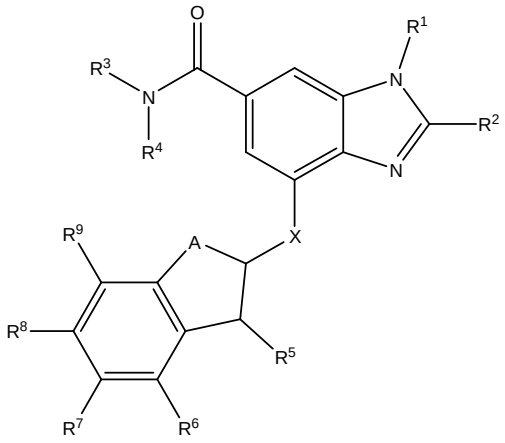
R^1 = nitrogen cantaining heterocyclic group, gernally condensed with benzene ring or a heterocycle
R^2 = C_6-C_{14} aryl groups
R^3 = H
R^4 = alkyl, acyl, helogen, CN, NO_2
R^5 = alkyl group

A series of chromane substituted benzimidazole derivatives of general structure **88** and **89** has been synthesized and evaluated for their acid pump inhibitory activity. Some of the compounds from this series showed high potency with IC_{50} for the inhibition of H^+/K^+ATPase.[121,122]



88

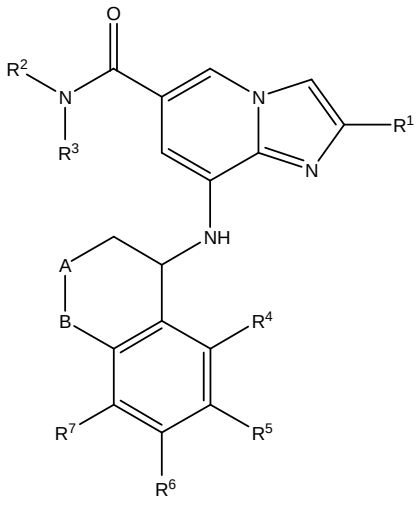
A-B = -O-CH_2-, -S-CH_2-, -CH_2-O- or -CH_2-S-
X = O or NH
R^1 = C_1-C_7 alkyl
R^2, R^3 = H, C_3-C_7 alkyl
R^4, R^5, R^6, R^7 = H, Halogen, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy
R^8 = H, OH, C_1-C_6 alkoxy



89

A = CH_2 or CH_2-CH_2
X = O, NH
R^1 = H, C_1-C_6 alkyl
R^2 = C_1-C_6 alkyl group, C_3-C_7 cycloalkyl
R^5 = H, OH, C_1-C_6 alkyl, C_1-C_6 alkoxy
R^6, R^7, R^6, R^9 = H or halogen

Furthermore, chromane substituted 2-alkyl imidazopyridine derivatives of structure **90** have been evaluated. These compounds showed less toxicity, better property of phototoxicity, good absorption, distribution, good solubility, less protein binding other then acid pump with good metabolic stability.[123]



90

A-B = -O-CH_2-, -S-CH_2-, -CH_2-O- or -CH_2-S-
X = O or NH
R^1 = C_1-C_6 alkyl
R^2 = C_1-C_6 alkyl, C_3-C_7 cycloalkyl group
R^3 = C_1-C_7 alkyl
R^4, R^5, R^6, R^7 = H, Halogen, C_1-C_6 alkyl

1.7.5.1. CCK2/Gastrin-Receptor Antagonists

Gastrin is the only peptide hormone released from the stomach. It mediates the gastric acid secretion. Gastrin stimulated secretion of gastric acid is produced directly by stimulation of Cholecystokinin-2 (CCK2)/gastrin receptors on parietal cells or indirectly after CCK2/gastrin receptors-mediated HA releases from ECL cells. The regulation of gastrin and HA-stimulated gastric acid secretion are key therapeutic targets in controlling acid-peptic disorders. Inhibition of acid secretion through H2-receptor antagonists and PPI's has positive feedback effect on the release of gastrin.[124,125] Numbers of chemically diverse CCK2/gastrin receptor antagonist have been studied for their anti-secretory effects or as inhibitors of panic attacks including L-365260 (Merck) **91**, CR2194 (Rotta) **92** and JB95008 (James Black Foundation) **93**.[126] However, till date, none has been marketed.

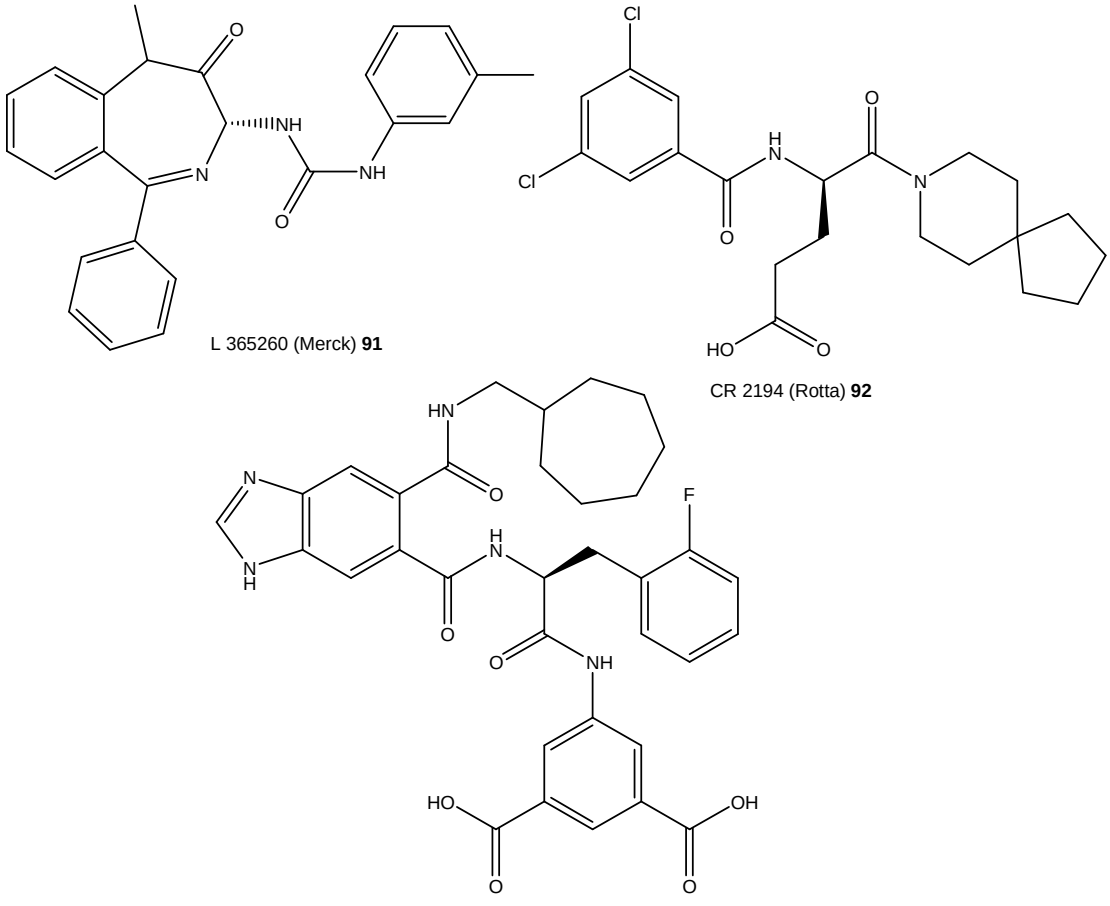


Figure 21. Structures of CCK2/gastrin receptor antagonists.

1.8. Biological Evaluation of PPI's

1.8.1. Studies on Isolated Guinea Pig Mucosa[127]:-

Preparation of tissue and solution:- Isolated guinea pig mucosa is mounted on a plastic funnel with the mucosal surface facing the tube lumen. Each preparation is immersed in an organ bath containing 40 ml of serosal solution having the different compositions.

Measurement of H^+ secretion: This is performed by continuous titration using a radiometer (Copenhagen, Denmark) pH-stat (pHM 82, TTT 80) and Autoburette (ABU 80).

Measurement of K^+ secretion: K^+ content of mucosal solution is determined on a flame-emission photometer.

Experiments with simultaneous measurements of K^+ and H^+ secretion: Histamine is added to serosal solutions followed by sample solutions and secretion rates are calculated.

1.8.2. Effect of H^+/K^+ ATPase Inhibitors on Serum Gastrin Levels[128]: -

Female wistar rats are treated with the H^+/K^+-ATPase inhibitors to cause gastric inhibition. Blood samples are collected and gastrin is determined by radio-immunoassay using a commercially available kit. At the end of the study of 10 weeks, the animals are studied for their gastric acid output using pylorus ligation (Shay technique)

1.8.3. Pylorus Ligation in Rats (Shay rats)[128]:-

A simple and reliable method for production of gastric ulceration in the rat based on the ligature of the pylorus has been published by Shay *et al*. (1945). The ulceration is caused by accumulation of acidic gastric juice in the stomach.

1.9. Conclusion:

In 19th century, light diet consisting of food not stimulating gastric acid secretion was recommended for treating peptic ulcer-related disorders. From then a number of strategies have been designed to control these disorders related to the hypersecretion of acid. These therapeutic strategies extend from simple conventional antacids to the use of more complex and effective proton pump inhibitors (PPI's). Associated effects of antacids like constipation or diarrhea limit their patient compliance and are today mainly used for fast symptomatic relief. Muscarinic antagonists like pirenzepine inhibit gastric acid secretion as well as decrease gastric motility, but clinical use of these drugs is now limited because

of availability of more effective anti-secretory medications. A new era in the treatment of acid-peptic disorders dawned with the launch of H2-receptor antagonist, cimetidine, in 1976. This class of drugs, however, has a short duration of action. Peptic ulcers caused by *H. pylori* can be treated by combination of antibiotics and anti-secretory medications. However, complex drug regimen and associated side effects may limit usefulness. Launch of omeprazole in 1988 introduced a conceptually new approach of inhibition of proton pump in the management of acid-related disorders. PPI's proved to be superior to any of the previously used drugs including H_2-antagonists. Today, almost two decades after introduction of the first PPI, the apparent drawbacks of irreversible proton pump inhibitors, mainly because of their prolonged acid suppression, are becoming a cause of concern. Hence, the researchers worldwide have been attracted toward designing reversible, shorter, and rapid acting acid pump antagonists (APAs). Thus, APAs are the conceivable future drugs for the treatment of acid-peptic disorders.

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2. Aim of the Present Work

2. Aim of the Present Work

A careful study and scrutiny of the review of literature on the currently used Proton Pump Inhibitors (PPI's) especially of the pyridylmethylsulfinyl benzimidazoles (PMSB) types indicate some important drawbacks[1,2] associated with their usage, such as;

1. They have irreversible inhibitory effects on gastric acid secretion and can cause extreme irreversible gastric acid suppression
2. They cause achlorhydria at recommended doses & may lead to enteric infections like typhoid, cholera & dysentery *etc.*
3. They may affect digestion & nutrition
4. They have significant drug interactions
5. They are not recommended for maintenance
6. They have un-predictable action and variation in individual responsiveness of duodenal ulcer patients
7. Hypergastrinemia causes rebound phenomena
8. They can cause cause gastric polyps and carcinoma
9. Their affinity for various cytochrome P-450's can lead to their self inhibition.

Therefore, there is need for the discovery and development of milder, reversible PPI's. This fact has been realized by the medicinal chemists' worldover.

An investigation into the mechanism of action of these PPI's can throw some light on the probable reasons for these drawbacks. These molecules rearrange in the strongly acidic environment of the parietal cells.[3] Covalent binding of the rearranged inhibitor to the H^+/K^+-ATPase results in inactivation of proton pump.[4] In the covalent binding, a disulfide linkage of the drug is formed with the active site of the cystine-rich H^+/K^+-ATPase (Proton Pump). One of these sites has been identified as cystine-813 (and probably cystine-822) of H^+/K^+-ATPase as shown in Fig. 1.

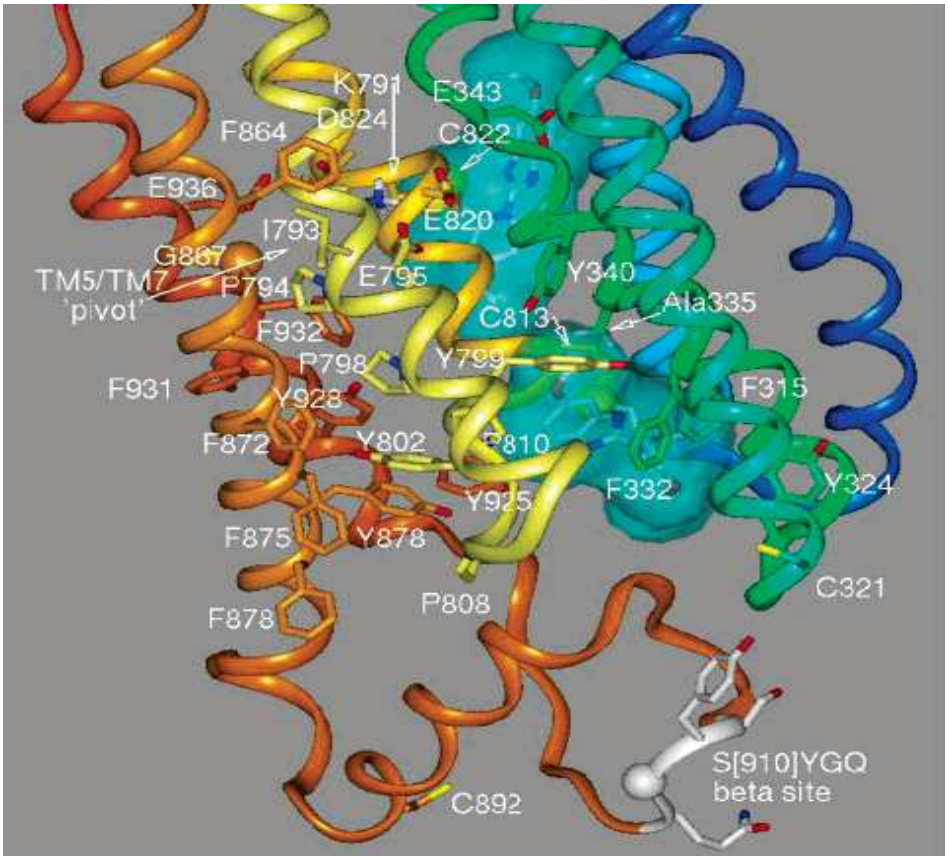


Figure 1. Membrane domain of the H^+/K^+-ATPase E2-P model with pantoprazole, bound at Cys813 and Cys822 (stick with Connolly surfaces in cloud). A known site of *a* subunit interaction (*36*, *37*), S [910] YGQ, is highlighted (white ribbon) in the TM7/TM8 loop. Cys813, Cys892, and Cys321 are labeled (*38*) by various proton pump inhibitors (all at Cys813, Omeprazole at Cys892, and lansoprazole at Cys321) and are solvent-accessible in the model. Labeling at the latter two sites is not correlated with inhibition (*3*). The crossover point ("pivot") between TM5 near Ile793 and TM7 near Gly867 (gold sphere) is apparently conserved in the P2-type ATPases. An extensive array of aromatic side chains (in stick form) replaces non-aromatic Ca^{2+}-ATPase residues and affects the spacing between helices. TM9 and TM10 are omitted for clarity.[5]

The entire cascade for the formation of disulfide is initiated by donation of an electron pair from the basic pyridinyl 'N' atom to the electron deficient 'C' of benzimidazole (Figure 2).

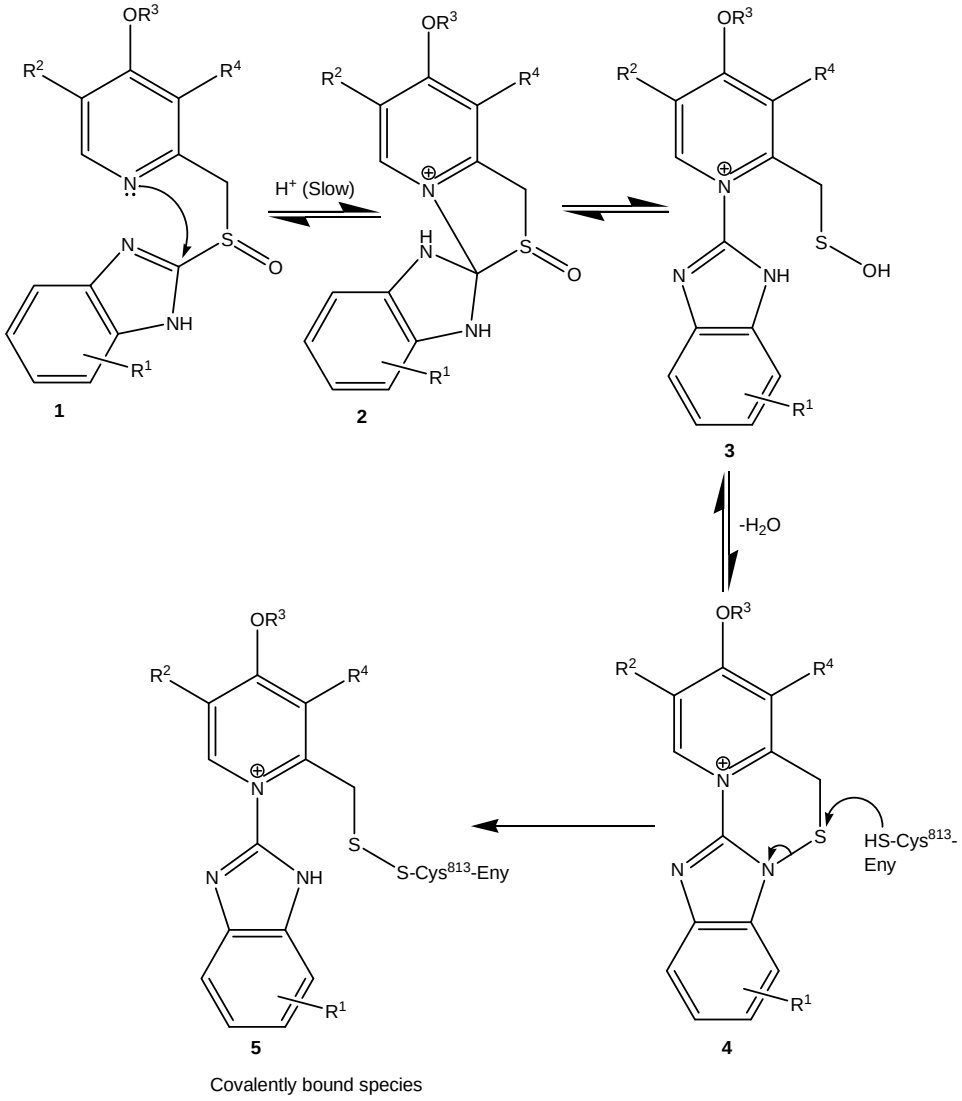


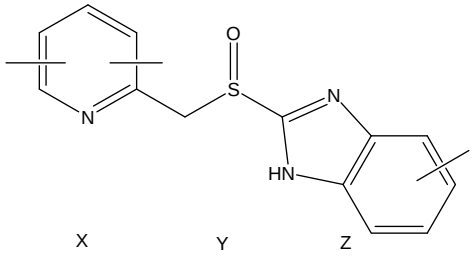
Figure 2. Reaction mechanism proposed for the acid transformation of pyridinylmethyl-sulfinyl benzimidazoles (PMSB's) to sulfenamide.

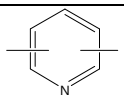
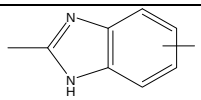
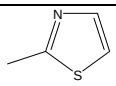
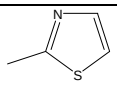
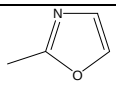
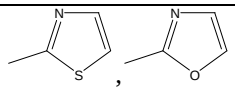
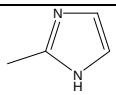
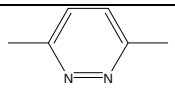
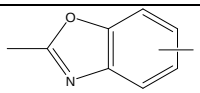
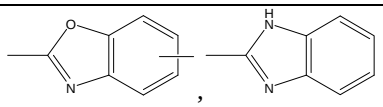
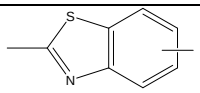
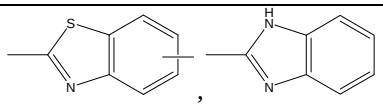
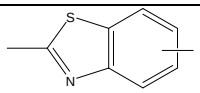
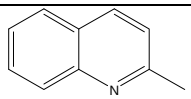
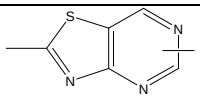
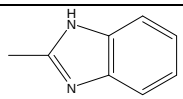
Therefore, there is a need to develop better analogs of the existing PPI's in which the formation of this disulfide intermediate can be avoided, so as to obtain reversible Proton pump inhibition & thus overcome the drawbacks of the currently available PPI's.

2.1 Structural changes done so far

Earlier, medicinal chemists worldwide have tried a variety of changes in the skeleton of PMSB moiety based on the principles of the isosteric/bioisosteric replacement of the group as well as the heterocyclic ring system to achieve subtle changes in the nature of this PMSB nucleus, which may alter its irreversible binding (inhibition) to the proton pump to a reversible one.

Table 4. Modifications reported in the PMSB nucleus

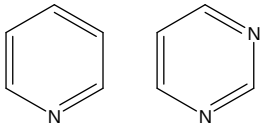


 **X** **(pyridine nucleus)[6]**	 **Y** **(methylsulfinyl linkage)[6]**	 **Z** **(benzimidazole nucleus)[7]**
	-CH_2S-	
	-CH_2CH_2-	 ,
	-CH_2-	
	-CH=CH-	 ,
	-CH_2S-	 ,
	-CH_2SCH_2-	
	-NHCO-	

One of the changes worth trying is the replacement of the pyridine ring of the PSMB skeleton with the less basic pyrimidine ring.

2.2. Bacisity of Pyridine *vs* Pyrimidine

As suggested above, one of the options that has not been tried is the 3-aza analog of pyridine *i.e.* pyrimidine, which is it's logical bioisoster.



Pyridine
pKa : 5.2

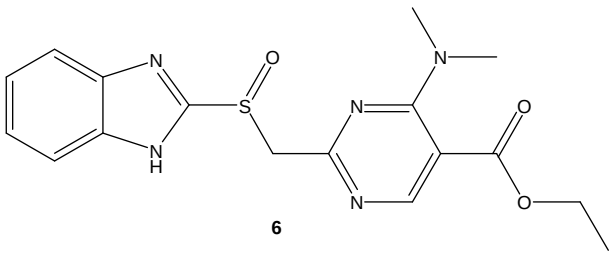
Pyrimidine
pKa : 1.31

A review of a basic literature on organic & the heterocyclic chemistry reveals that indeed pyrimidine ring is the ring of choice. This is because

1. Pyrimidine is weakly basic or rather acidic than pyridine (lower pKa)
2. Electronegativity of the additional N_3 Nitrogen depletes electrons on N_1.
3. Depletion of the π-electrons is caused by an insertion of the second electron attracting nitrogen.

The weakly basic nature of pyrimidine (pKa-1.31) is striking in relation to pyridine (pKa-5.2).[8] It is understandable as an inductive effect (depletion of π-electrons), caused by insertion of the avidity electron-attracting second nitrogen atom at N_3. Pyrimidine may therefore be likened more to β-nitropyridine (pKa-0.8), which contains the equally strong electron-attracting nitro group, than to the parent pyridine.

A cursory review of the literature does reveal only one successful use of this logic[9] (structure-6).



6

(Roussel Morishita Co. Ltd., under clinical trials)

2.3. Proposed Series of Compounds

Theoretically, it really appears that in this pyrimidine system the formation of the disulfide intermediate is difficult. This is owing to the poor availability of electrons on pyrimidine 'N_1' & 'N_3' nitrogens (Fig. 3).

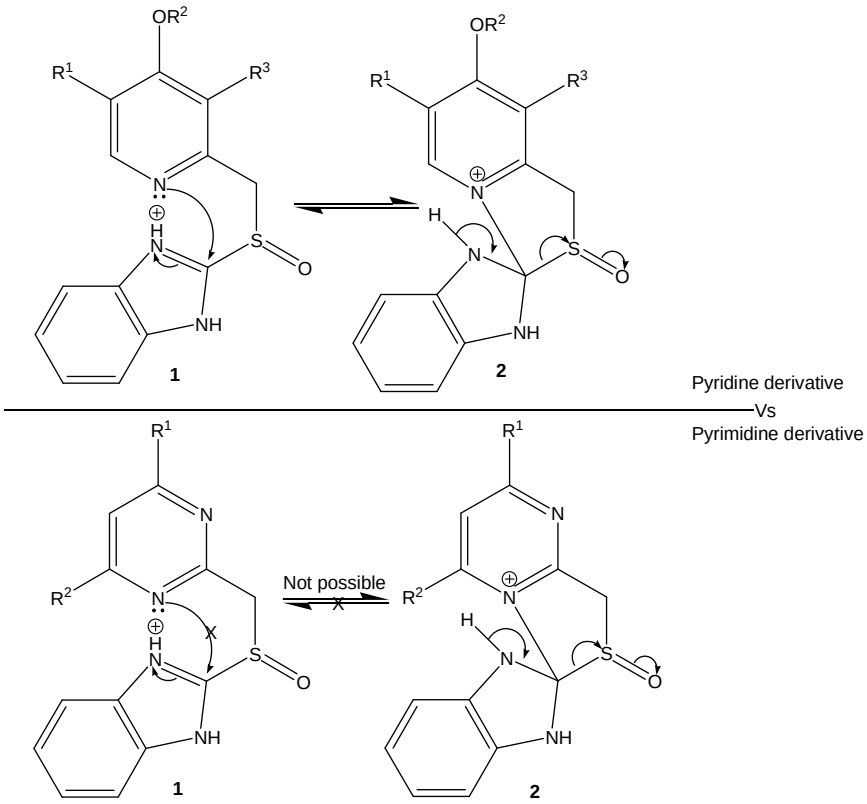


Figure 3. Pyridine derivative *vs* Pyrimidines derivative in acidic medium

Thus, one can envisage that this system though can bind the proton pump; the binding may not as strong as the PMSB pyridine system and may be even loose and reversible. Therefore, series of pyrimidine analogues of the existing drug PMSB skeleton was planned for the proposed work.

It was decided to utilize the availability of 2-chloromethylpyrimidines, especially the condensed 2-chloromethylpyrimidin-4(3*H*)-ones to condense them with 2-mercapto-benzimidazole to get the corresponding pyrimidinylmethylsulfinyl benzimidazoles of the following general type **7** (Figure 4).

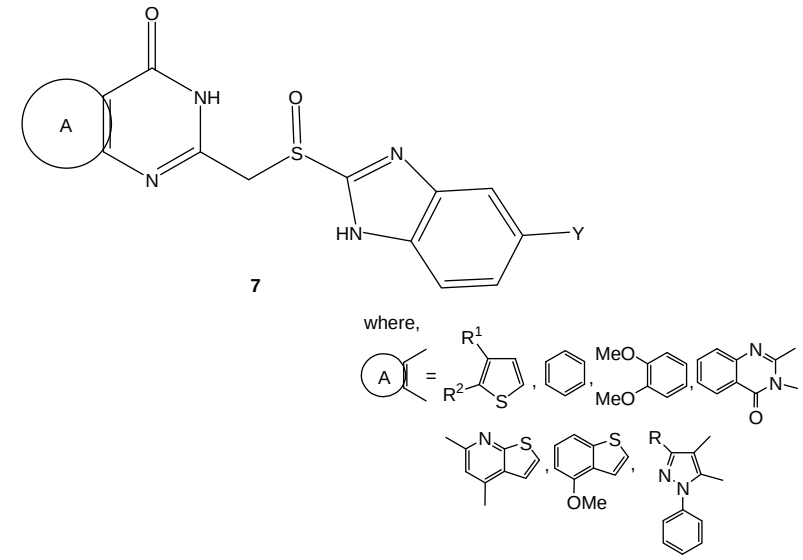


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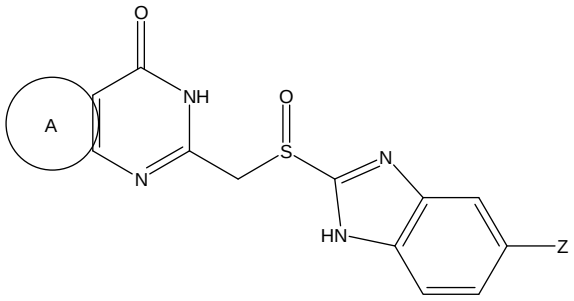
Figure 4. Proposed series of compounds

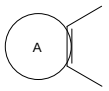
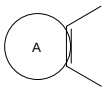
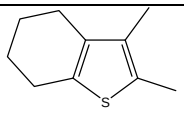
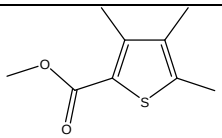
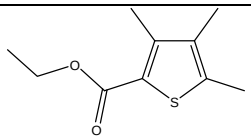
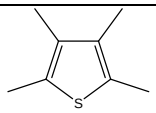
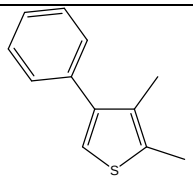
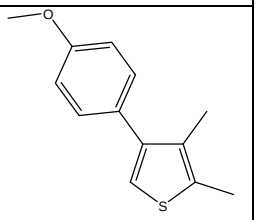
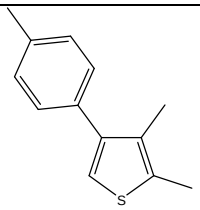
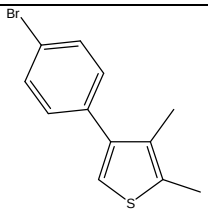
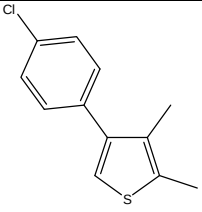
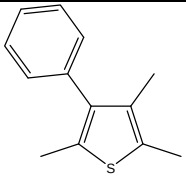
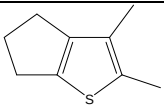
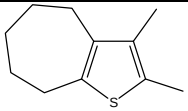
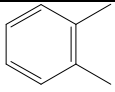
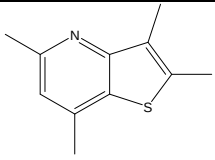
The choice of this particular system is due to the reason that main building block of this system namely, condensed 2-chloromethylpyrimidin-4-(3*H*)-ones are easy to prepare. The other precursor, 2-mercaptobenzimidazole is either preparable or easily accessible, commercially.

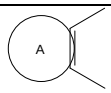
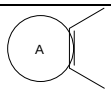
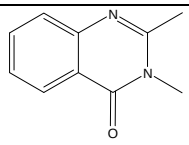
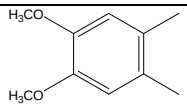
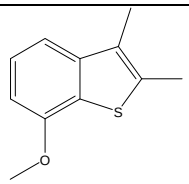
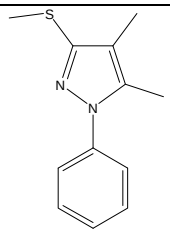
Thus, a series of 2-(1*H*-benzimidazol-2-ylsulfinyl)-3*H*-pyrimidin-4-ones and 2-(5-methoxy-1*H*-benzimidazol-2-ylsulfinyl)-3*H*-pyrimidin-4-ones was planned to be synthesized, characterized and evaluated for antiulcer activity, using a suitable animal model. The following series was envisaged (Table 5).

Table 5. Proposed series of compounds synthesized for biological evaluation



Z = H, OCH_3

S. No.		S. No.	
1.		2.	
3.		4.	
5.		6.	
7.		8.	
9.		10.	
11.		12.	
13.		14.	

S. No.		S. No.	
15.		16.	
17.		18.	

2.4 Pharmacological Activity

Antiulcer and anti acid secretary activity of the newly synthesized compounds on rats was planned using a simple and reliable method for production of gastric ulceration based on the ligature of the pylorus as per method published by Shay *et al*[10]. The ulceration is caused by accumulation of acidic gastric juice in the stomach. The intensity of ulceration is expressed in terms of ulcer index.

2.5 Establishing Quantitative Structure Activity Relationships (QSAR)

QSAR is one of the most effective lead optimization techniques of rational drug design since last three decades. It quantitatively correlates the effects of structural changes all around the molecule on its exhibited biological activity. In simple words it provides a mathematical near to accurate picture on the optimal desirable structural features of a lead molecule with best biological activity.

Thus, structures of all the synthesized and biologically evaluated molecules shall be built in 3D using standard drug design softwares, shall be minimized and refined and various physicochemical 2D and 3D properties of these molecules shall be computed and correlated with the observed biological activity data through systematic multiparameter statistical regression analysis, to evolve out meaningful mathematical equations of Qunatitative Structure Activity Relationships (QSAR). Systematic interpretation of the QSAR data should help in probing into the optimal physico-chemical/structural requirements for the highest antiulcer activity in this series of compounds. This is one of the logical and rational approaches to the drug design leading to lead optimization.

2.6 References

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3. Results and Discussion

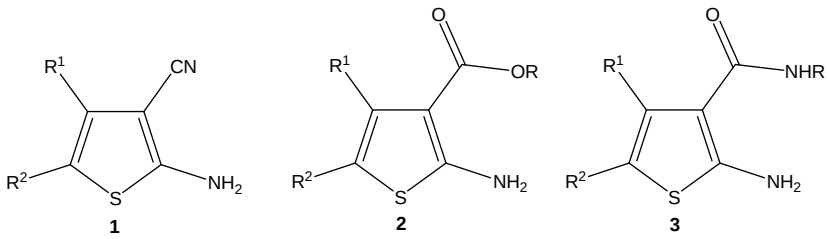
3.1 Synthesis of Starting Materials

3.1.1 Synthesis of thiophene *o*-aminoesters (The Gewald reaction)

3.1.2 Synthesis of other *o*-aminoesters substrates

3.1.1 Synthesis of thiophene *o*-aminoesters (The Gewald reaction)

Excellent synthetic methodologies for a variety of substituted 2-aminothiophenes, have been developed by Gewald and coworkers.[1] The 2-amino-3-cyano **1**, 2-amino-3-carbethoxy **2** and 2-amino-3-carbamoyl **3** thiophenes obtained by the Gewald reaction are of considerable importance for the generation of thienopyridines, thienopyrimidines and thienodiazepines. These molecules especially thieno[2,3-*d*]pyrimidines have exhibited antimalarial[2], anti-bacterial[3], anti-inflammatory[4], anticonvulsant properties[5], CNS depressant[6], hypnotic[7] and anti-platelet aggregating[8] activity. Besides this the thienodiazepines have exhibited good antidepressant activities[9], antianxiety[10], antipsychotic[11] and anticonvulsant activities.[12]



These molecules show great promise in biomedicine[13], because of their application in pharmaceuticals, agriculture, pesticides and dyes. The Gewald methods offer considerable improvements over all the other existing methods for 2-aminothiophenes.

Notably, Gewald has described four synthetic methods for 2-amino-3-carbalkoxythiophenes. These methods involve:

3.1.1a Condensation of α-mercaptoketones or α-mercaptoaldehydes with alkyl cyanoacetates.[14]

3.1.1b Treatment of aldehydes or ketones with alkylcyanoacetates and elemental sulfur.[15]

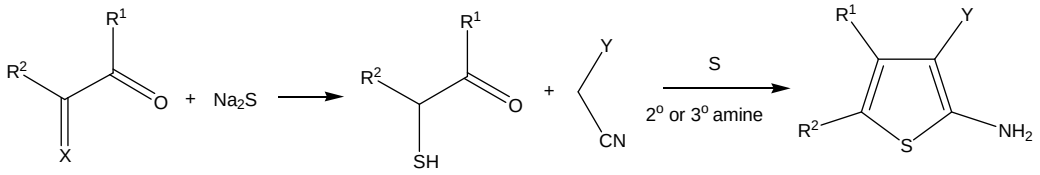
3.1.1c Cyclization of acryonitriles (obtained from condensing aldehydes or ketones with alkylcyanoacetates) with elemental sulfur.[15]

3.1.1d The reaction of enamines (derived from ketones and morpholine or piperidine with alkyl cyanoacetate) and elemental sulfur.[15]

3.1.1a Condensation of α-Mercaptoketones or Aldehydes with Alkyl Cyanoacetates

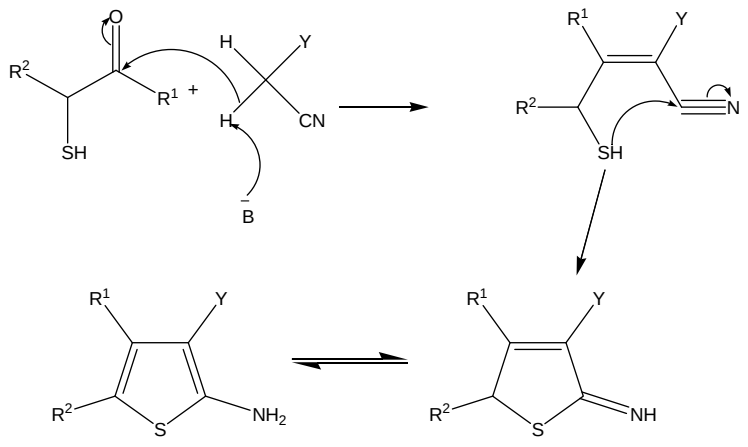
In one of the versions[16-18] of the Gewald reaction, an α-mercaptoketone or aldehyde is treated with an active methylene nitrile bearing an electron withdrawing group, such as; methyl/ethyl cyanoacetates, malonitrile, benzoylacetonitrile or *p*-nitrobenzyl cyanide in solvents such as ethanol, dimethylformamide or dioxane in the presence of a catalyst such as triethylamine, diethylamine or piperidine at around 50°C (Scheme-1). The α-mercaptoketone or aldehyde is often generated *in situ* by the reaction of an alkali sulfide with an appropriate α-halocarbonyl compound. The detailed mechanism of the reaction has not been demonstrated, but it seems likely that the aldol-type condensation occurs first, followed by an attack of the thiolate on the cyano group (Scheme-2). This particular version of Gewald reaction has several drawbacks such as;

i. it utilizes the starting compounds which are unstable and difficult to prepare
ii. this methodology is limited to aliphatic α-mercapto derivatives
iii. non-activated nitriles such as cyanoacetic acid and benzyl cyanide do not undergo the Gewald reaction.



X = Halo, R^1, R^2 = H, alkyl, aryl, cycloalkyl, heteroaryl
Y = CN, COOEt, COPh, CONHR

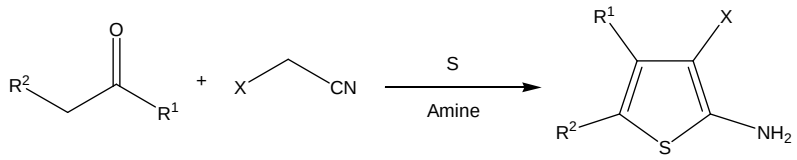
Scheme 1



Scheme-2. Mechanism for Scheme-1

3.1.1b Treatment of Aldehydes or Ketones with Alkyl Cyanoacetates and Elemental Sulfur

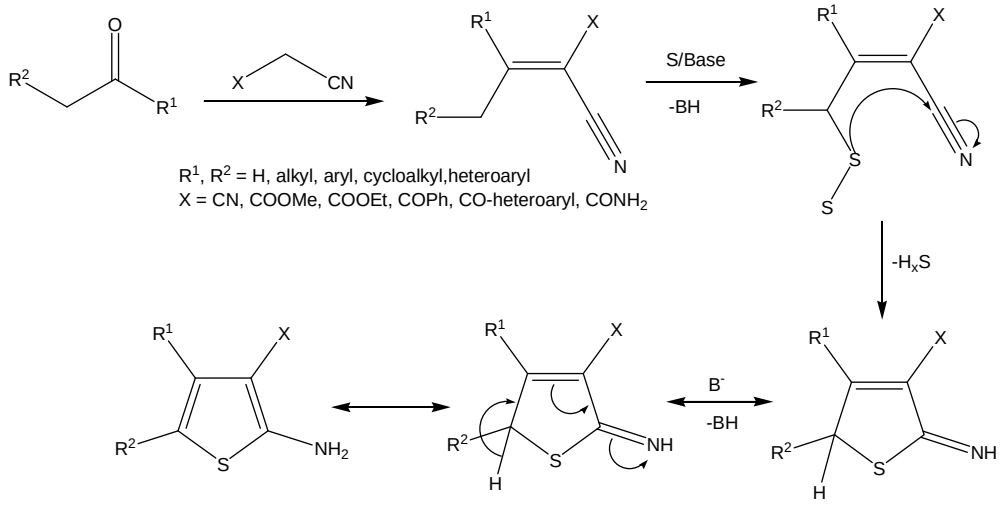
This method involves a one pot procedure which is extensively used for the synthesis of numerous 2-amino-3-carbonylthiophenes.[19-21] Here, the methodology involves condensation of methyl aldehydes, methyl ketones, or 1,3 dicarbonyl compounds with activated acetonitriles such as malonitrile, cyanoacetic ester, cyanoacetamide and its *N*-substituted derivatives, hetroarylacetonitriles, α-cyanoketones & sulfur in presence of a secondary or tertiary aliphatic amine at room temperature. Ethanol, dimethylformamide, dioxane, excess ketone such as methyl ethyl ketone or cyclohexanone are preferred solvents, while the 2^o or 3^o amines employed may be diethylamine, morpholine or triethylamine. Only 0.5-1.0 molar equivalents of the amine, based on the amount of nitrile is used. The yields of the product are much higher by this method (Scheme-3).



R^1, R^2 = H, alkyl, aryl, cycloalkyl,heteroaryl
X = CN, COOMe, COOEt, COPh, CO-heteroaryl, $CONH_2$

Scheme 3

Alternatively, a two step procedure is preferable. An, α, β-unsaturated nitrile is first prepared through Knovenagel condensation of the carbonyl compound and an active methylene nitrile and then treated with sulfur and amine (Scheme-4). This two step version of the Gewald reaction gives higher yields. Even more important is that certain ketones such as alkyl aryl ketones, do not give thiophene in the one pot-modification,[22,23] but give acceptable yields by this two-step technique (Scheme-4).

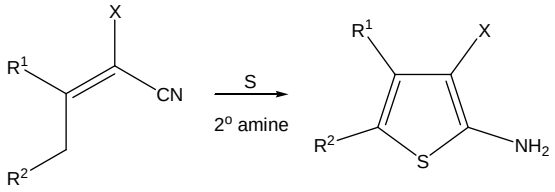


Scheme-4. Mechanism for Scheme-3

Both of these one and two steps variants have been employed out with numerous ketones and aldehydes and active methylene nitriles. Cyclic and heterocyclic ketones have been used extensively. It was however, observed that significantly lower yields (40-65%) were obtained with cyclic ketones having rings larger than six-membered. This trend is suggestive of increasing non-bounded repulsive interaction between methylene protons in middle and large sized rings fused to a planar five membered rings.[24]

3.1.1c Cyclization of Acryonitriles with Elemental Sulfur

Acryonitriles, obtained through the condensation of aldehydes or ketones with alkyl cyanoacetates, using either diethylamine or morpholine, get cyclized into substituted 2-aminothiophenes through the action of 'S' in presence of a 2^o amine. (Scheme-5)

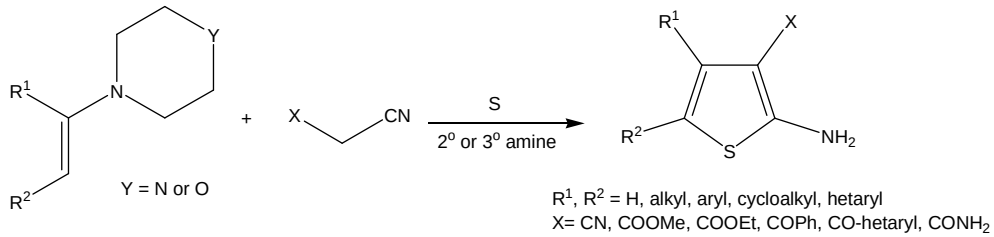


$R^{1,}$ R^2 = H, alkyl, aryl, cycloalkyl, heteroaryl
X = CN, COOMe, COOEt, COPh, CO-heteroaryl, $CONH_2$,

Scheme-5

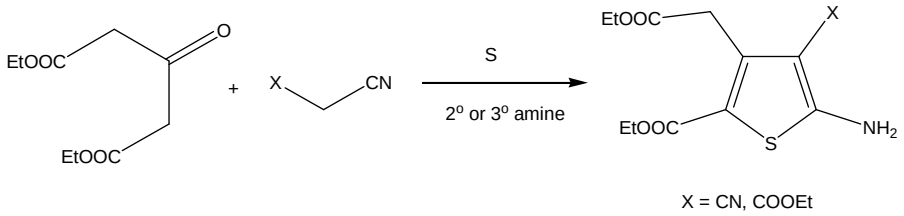
3.1.1d Treatment of Enamines with Alkyl Cyanoacetates and Elemental Sulfur

Enamines derived from ketones and 2^o aliphatic amines like morpholine or piperidine undergo the Gewald reaction with activated acetonitriles and elemental sulfur to give 2-aminothiophenes (Scheme-6).



Scheme-6

Sabnis and Rangekar[25,26] have developed the synthesis of versatile synthons in more than 90% yield by condensing diethyl acetonedicarboxylate with sulphur and an activated acetonitrile (Scheme-7). These compounds have demonstrated tremendous application in synthesizing novel dyes and many biologically active compounds.

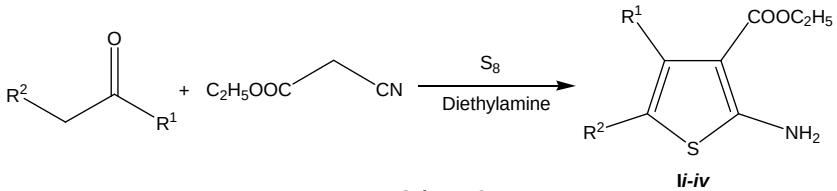


Scheme-7

In the present work, two different variants have been used to prepare thiophene-*o*-amino esters **(I*i-xii*)**

Method A

It is one pot condensation reaction involving reaction of ketone, ethylcyanoacetate and sulphur in the presence of diethylamine as catalyst at ambient temperature (method 4.1.1b). Secondary amine used here is 0.5-1.0 mole equivalent of the amount of nitrile used (Scheme-8).

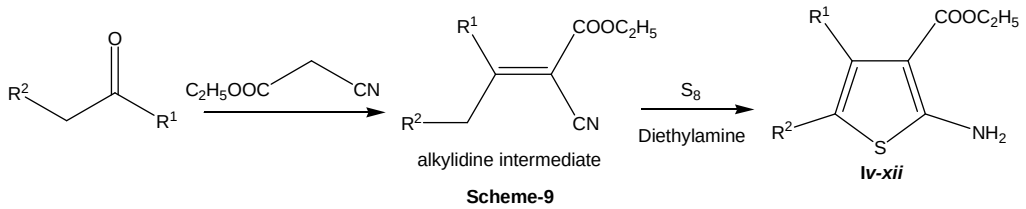


Scheme 8

Method B

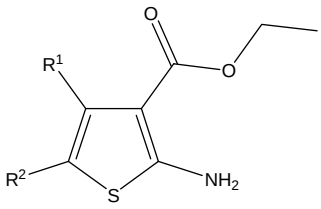
This method is two-step process (method 4.1.1b). First step is the prior condensation of ketone with an ethylcynoacetate, under the catalysis of sodium acetate to obtain an α,β-unsaturated nitrile (Knoevenagel condensation product which is otherwise known as alkylidine intermediate) in a suitable solvent like benzene. In this step, water molecules

formed during the reactions were removed using Dean-Stark condenser. In the second step, the alkylidine intermediate is reacted with sulphur in ethanol containing a secondary amine base such as diethylamine at around 50°C to afford the corresponding *o*-aminothiophene (Scheme-9).



Scheme-9

Table-6: Physical data of 2-amino-3-carbethoxythiophenes (I*i-xii*) synthesized.



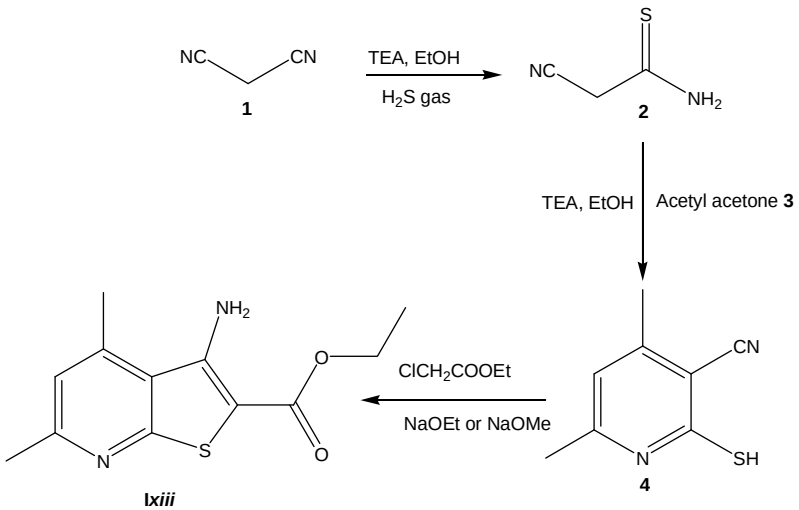
Sr. No	R^1	R^2	Mol.For. Sol. of recryst	Yield (%)	M.P. (°C)	Time (hrs)	Route
I*i*	$-(CH_2)_4-$		$C_{11}H_{15}NO_2S$ (E)	80	110-112	3	A
I*ii*	$-CH_3$	$-COOCH_3$	$C_{10}H_{13}NO_4S$ (E)	70	80-82	2-3	A
I*iii*	$-CH_3$	$-COOC_2H_5$	$C_{11}H_{15}NO_4S$ (T)	50	103-105	2	A
I*iv*	$-CH_3$	$-CH_3$	$C_9H_{13}NO_2S$ (E)	50	92-93	3	A
I*v*	$-C_6H_5$	H	$C_{13}H_{13}NO_2S$ (E)	75	95-97	15-18	B
I*vi*	$4-CH_3OC_6H_4$	H	$C_{14}H_{15}NO_3S$ (E)	73	96-99	15-18	B
I*vii*	$4-CH_3C_6H_4$	H	$C_{14}H_{15}NO_2S$ (E)	89	102-104	15-18	B
I*viii*	$4-BrC_6H_4$	H	$C_{13}H_{12}BrNO_2S$ (E)	76	78-80	15-18	B
I*ix*	$4-ClC_6H_4$	H	$C_{13}H_{12}ClNO_2S$ (E)	80	102-104	15-18	B
I*x*	$-C_6H_5$	$-CH_3$	$C_{14}H_{15}NO_2S$ (E)	76	91-93	15-18	B
I*xi*	$-(CH_2)_3-$		$C_{10}H_{13}NO_2S$ (E)	59	82-84	15-18	B
I*xii*	$-(CH_2)_5-$		$C_{12}H_{17}NO_2S$ (E)	71	75-77	15-18	B

E =Ethanol, T = Toluene

3.1.2 Synthesis of other *o*-aminoester substrates:

3.1.2a. Synthesis of 3-amino-2-carbethoxy-4, 6-dimethylthieno[2,3-*b*]pyridine[28] (I*xiii*)

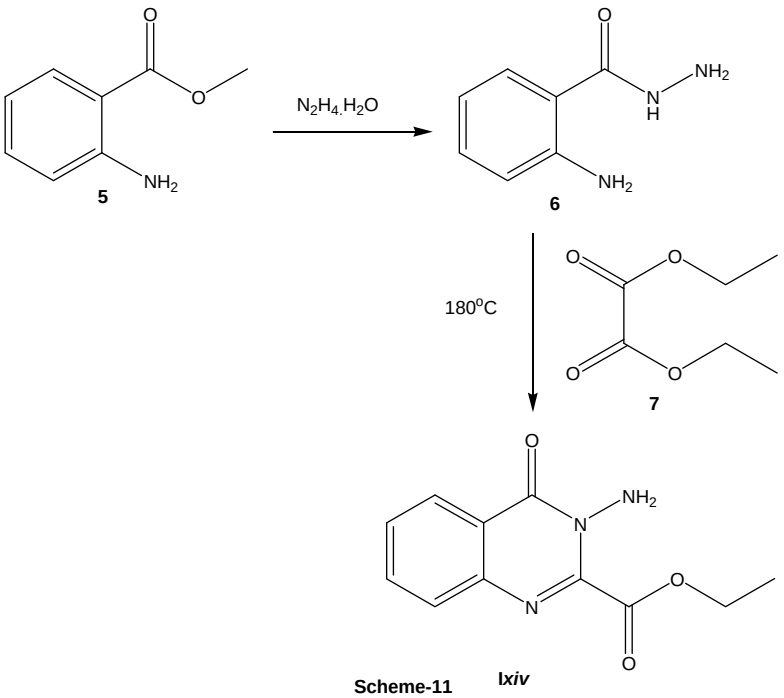
The synthesis of thiocyanoacetamide **2** was carried out through the reaction of malononitrile **1** and H_2S gas using triethylamine as a base. The 4,6-dimethyl-3-cyano-2-mercaptopyridine **4** was synthesized by suspending thiocyanoacetamide and acetyl-acetone **3** in absolute ethanol under basic condition.[27] Synthesis of 3-amino-2-carbethoxy-4,6-dimethylthieno[2,3-*b*]pyridine **I*xiii*** was carried out by reacting 4,6-dimethyl-3-cyano-2-mercaptopyridine **4** and ethyl chloroacetate under strong basic conditions like using sodium ethoxide (Scheme-10).



Scheme-10

3.1.2b Synthesis of 3-amino-2-carbethoxyquinazolin-4-one[29] (I*xiv*)

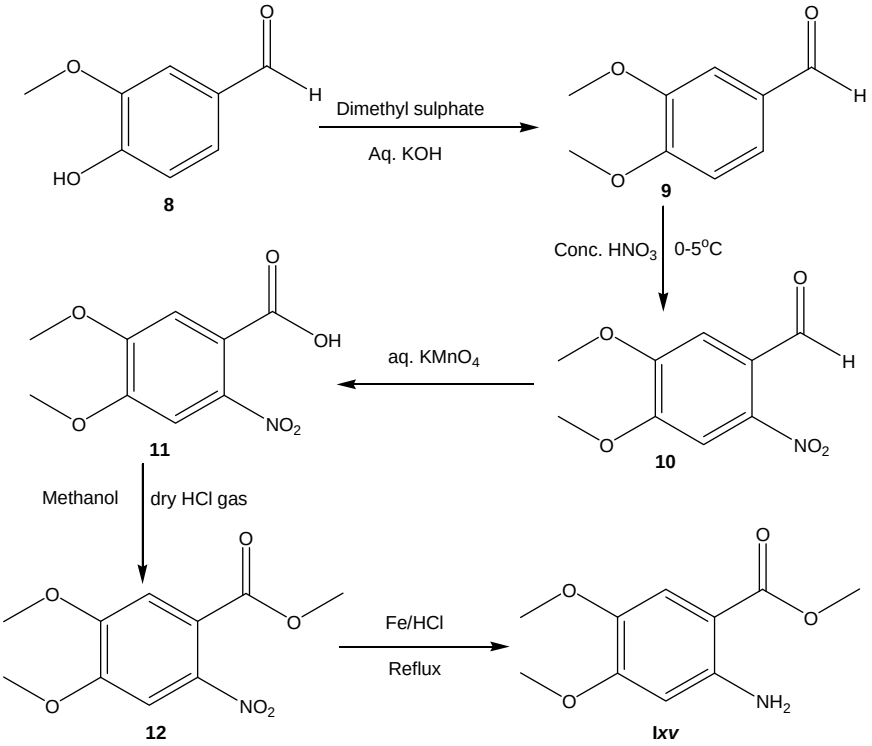
First step to synthesize 3-amino-2-carbethoxyquinazolin-4-one **I*xiv*** was to reflux methyl anthranilate **5** and hydrazine hydrate for 2 hrs. On cooling solid crystals of anthranilic acid hydrazide **6** were obtained. The mixture of anthranilic acid hydrazide and diethyl oxalate **7** were heated under reflux with stirring in an oil bath at 180^{o}C. After completion of the reaction, excess of diethyl oxalate was removed in *vacuo* to give a semi-solid product which became crystalline on treatment with ethanol, characterized as 3-amino-2-carbethoxyquinazolin-4-one **I*xiv*** (Scheme-11).



Scheme-11 **I*xiv***

3.1.2.c Synthesis of methyl-2-amino-4,5-dimethoxybenzoate[30] (I*xv*)

Synthesis of methyl-2-amino-4,5-dimethoxybenzoate was carried out through a series of following steps. The synthesis starts with *o*-methylation of vanillin **8**, with dimethyl sulphate in presence of aq. KOH. In the next step, selective nitration of veratraldehyde **9** under controlled conditions was carried out using fuming nitric acid at 0°C to get 3,4-dimethoxy-6-nitrobenzaldehyde/6-nitroveratraldehyde **10**. The 3,4-dimethoxy-6-nitro-bezoic acid **11** was prepared by the oxidation of the aldehyde group of 6-nitroveratraldehyde using potassium permanganate as a oxidizing agent. The next step involves the esterification of the benzoic acid by passing dry HCl gas in methanol to get methyl 4,5-dimethoxy-2-nitrobenzoate **12**. The next step involved the reduction of the nitro group with the use of iron powder (activated 80#mesh) and catalytic amount of conc. HCl in ethanol at 80°C to get target compound, 2-animo-4,5-dimethoxy methylbenzoate **I*xv*** (Scheme-12).



Scheme-12

3.1.2.d. Synthesis of 2-carbethoxy-3-amino-4-methoxybenzo(*b*)thiophene (I*xvi*)

a. Synthesis of 2-nitro-6-methoxybenzonitrile[31] 14

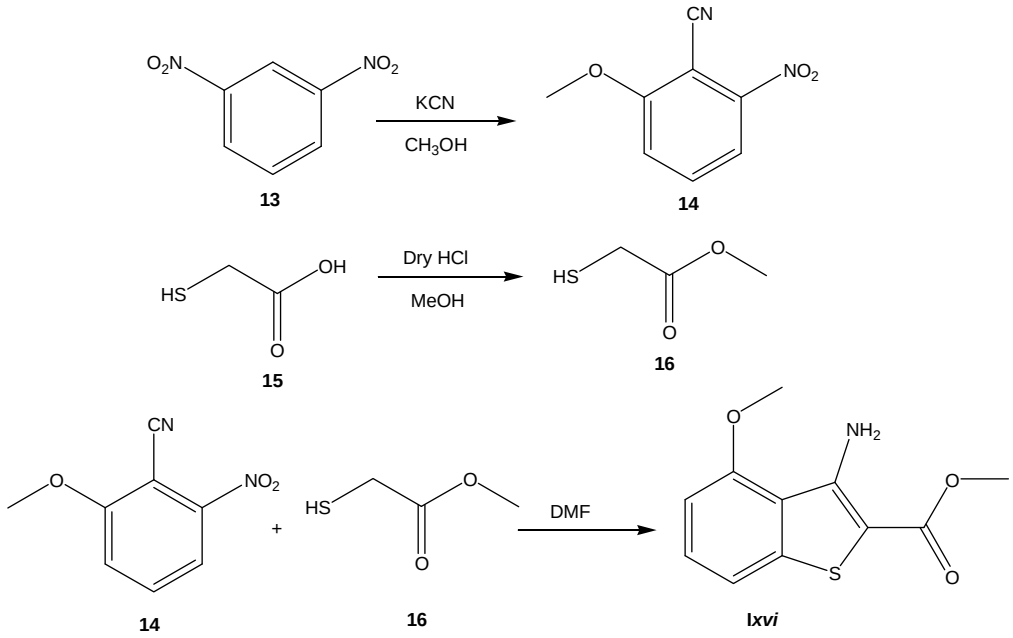
m-Dinitrobenzene **13** was reacted with potassium cyanide in water and the purple mixture was allowed to stand at RT for 2-3 days. The black precipitate separated out was collected. The filtrate was diluted with cold water & allowed to stand overnight to obtain the second crop of the product. The combined precipitates were extracted with chloroform, which on evaporation gave the 2-nitro-6-methoxy benzonitrile **14** as a red powder.

b. Synthesis of methyl thioglycolate[32] 16

Dry HCl gas was bubbled in methanol containing thioglycolic acid **15** under ice-cold conditions for 5-6 hrs. Next day the reaction mixture was boiled on water bath for 2 hrs, cooled to RT and reaction mixture was quenched with ice water & extracted with chloroform. Chloroform extracts on evaporation gave yellow colored methyl thioglycolate **16**.

c. Synthesis of 2-carbethoxy-3-amino-4-methoxybenzo(*b*)thiophene[33] (I*xvii*)

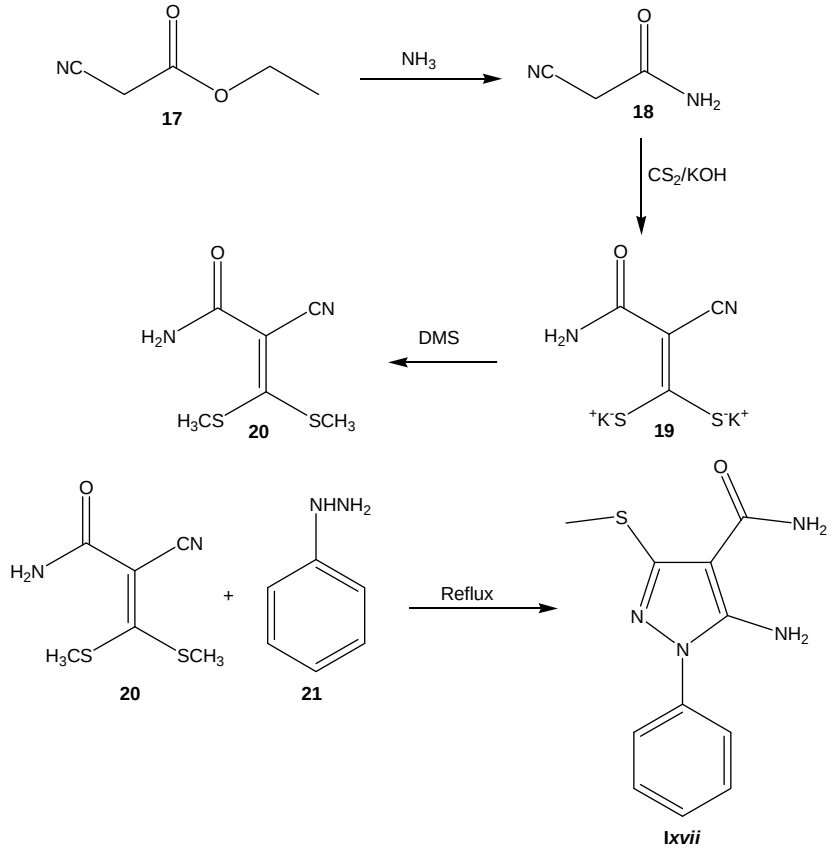
Reaction of 2-nitro-6-methoxy benzonitrile **14** and methyl thioglycolate **16** under basic condition and with continuous stirring at 0^{o}C gave the title compound in high yield (Scheme-13).



Scheme-13

3.1.2e. Synthesis of 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole[34] (I*xv*)

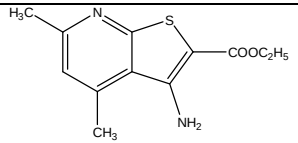
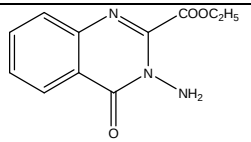
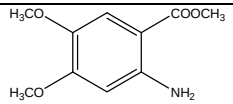
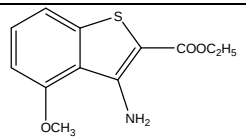
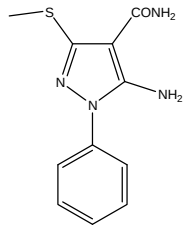
The 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole was synthesized by series of steps as under. The ethyl cyanoacetate **17** and ammonia were reacted to get cyanoacetamide **18**. Then cyanoacetamide and carbon disulfide were reacted in presence of aq. KOH to form the potassium dithiolate salt **19**, which was then *S*-methylated with dimethyl sulphate to yield ethyl-2,2-di-(methylthio)methylene cyanoacatamide **20**. The ethyl-2,2-di-(methylthio)methylene cyanoacetamide was then refluxed with phenyl hydrazine **21** in ethanol to form 5-amino-4-carboxamide-3-(methylthio)pyrazole **I*xvii*** as a sole product (Scheme-14).



lxvii

Scheme-14

Table 7: Physical data of other *o*-aminoesters (I*xiii-xvii*) synthesized

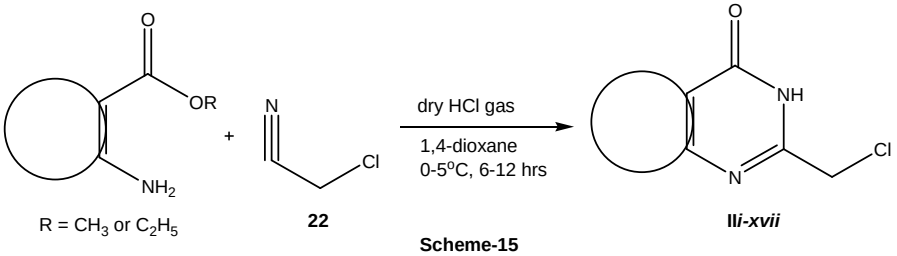
Compd No.	Compound	M.P (^oC)	Yield (%)	Mol. Formula (Solv. of Crystn.)	IR (cm^{-1})	Mass (m/e)	NMR (δppm)
I*xiii*		152-156	90	$C_{12}H_{14}N_2O_2S$ (E)	3435, 3332(γ_{NH}), 2979(γ_{C-H}), 1668($\gamma_{C=O}$)	250(M^+), 222, 204, 176, 149, 132	1.38 (3H, t, $COOCH_2CH_3$, *J* = 5.1 & 6.9), 2.57 (3H, s, CH_3), 2.71 (3H, s, CH_3), 4.32 (2H, q, $COOCH_2CH_3$, *J* = 6.9 & 7.2), 6.14 (2H, s, NH_2), 6.82 (1H, s, Ar-*H*)
I*xiv*		137-138	44	$C_{11}H_{11}N_3O_3$ (E)	3476, 3334(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$), 1687(γ_{CONH})	218(M^+), 204, 161, 144, 218, 204, 161, 144	1.45 (3H, t, $COOCH_2CH_3$, *J* = 7.2), 4.50 (2H, q, $COOCH_2CH_3$, *J* = 6.9 & 7.2), 5.15 (2H, s, br, NH_2), 7.48-8.29 (4H, m, Ar-*H*)
I*xv*		120-122	47	$C_{11}H_{15}NO_4$ (E)	3476, 3373(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$).	--	--
I*xvi*		140-143	80	$C_{11}H_{11}NO_3S$ (E)	3484, 3376(γ_{NH}), 2947(γ_{C-H}), 1670($\gamma_{C=O}$)	--	--
I*xvii*		146-150	56	$C_{11}H_{12}N_4OS$ (E)	3449, 3394(γ_{NH}), 3138(γ_{C-H}), 1662(γ_{CONH})	248(M^+), 231, 216, 198, 186, 157	--

E= Ethanol

3.2 Synthesis of condensed pyrimidine intermediates.

3.2.1 Synthesis of condensed 2-chloromethylpyrimidin-4(3*H*)-ones (II*i-xvii*)

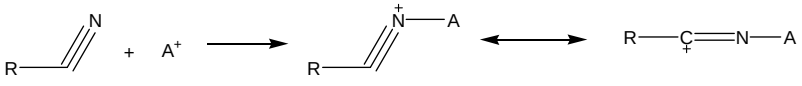
The condensed 2-chloromethylpyrimidine-4(3*H*)-ones **II*i-xvii*** were planned to be synthesized through the dry HCl gas catalyzed one pot condensation of the appropriate 2-amino-3-carbethoxy substrates **I*i-xvii*** and chloroacetonitrile **22** as described in earlier reports on HCl gas catalyzed one pot condensation by Shishoo *et al*.[35-38] (Scheme-15)



Scheme-15

The proposed mechanism is as follows (Scheme-16);

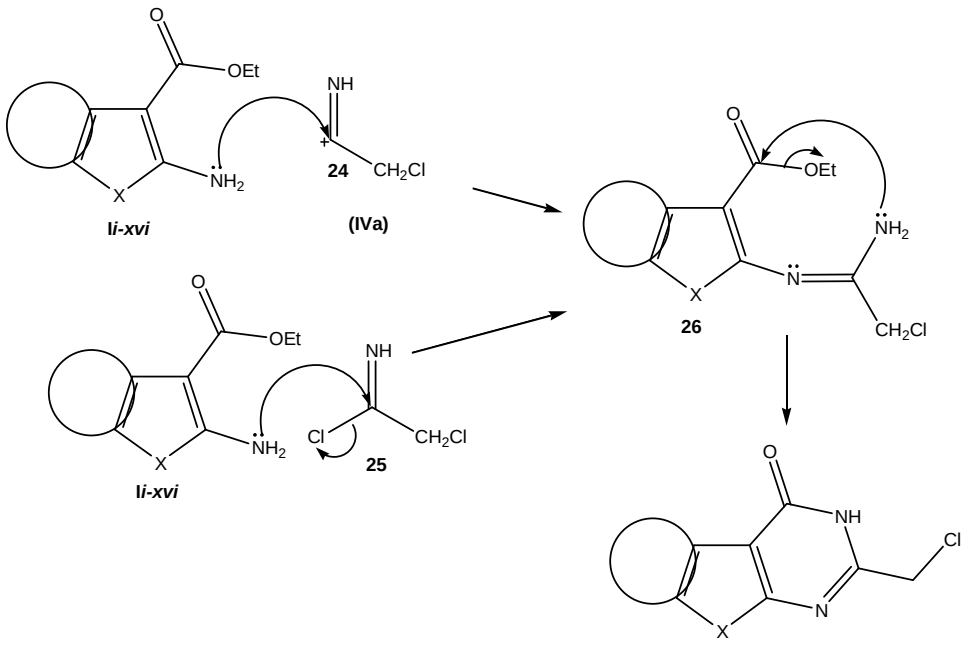
The interaction of the nitrile **22a** with a lewis A^+ under anhydrous conditions leads to the formation of species **23**, with enhanced electrophilicity. This enhanced reactivity of nitriles towards nucleophiles in the presence of acids, particularly halogen acids is well known. This enhanced reactivity has been appropriately exploited for the synthesis of condensed pyrimidines through their reaction with appropriate *o*-aminocarbonyl compounds **(I*i-xvii*).**



22a **23**

Scheme-16

Cyclization reactions with nitriles under acidic conditions presumably proceed *via* the formation of transient amidine intermediate **26** resulting from the reaction of *o*-aminocarbonyl compounds with the protonated nitrile **24** or imidoyl halide intermediate **25** (Scheme-17).



Ii-xvi **Ii-xvi** **IIi-xvii**

X = S or -CH=CH-

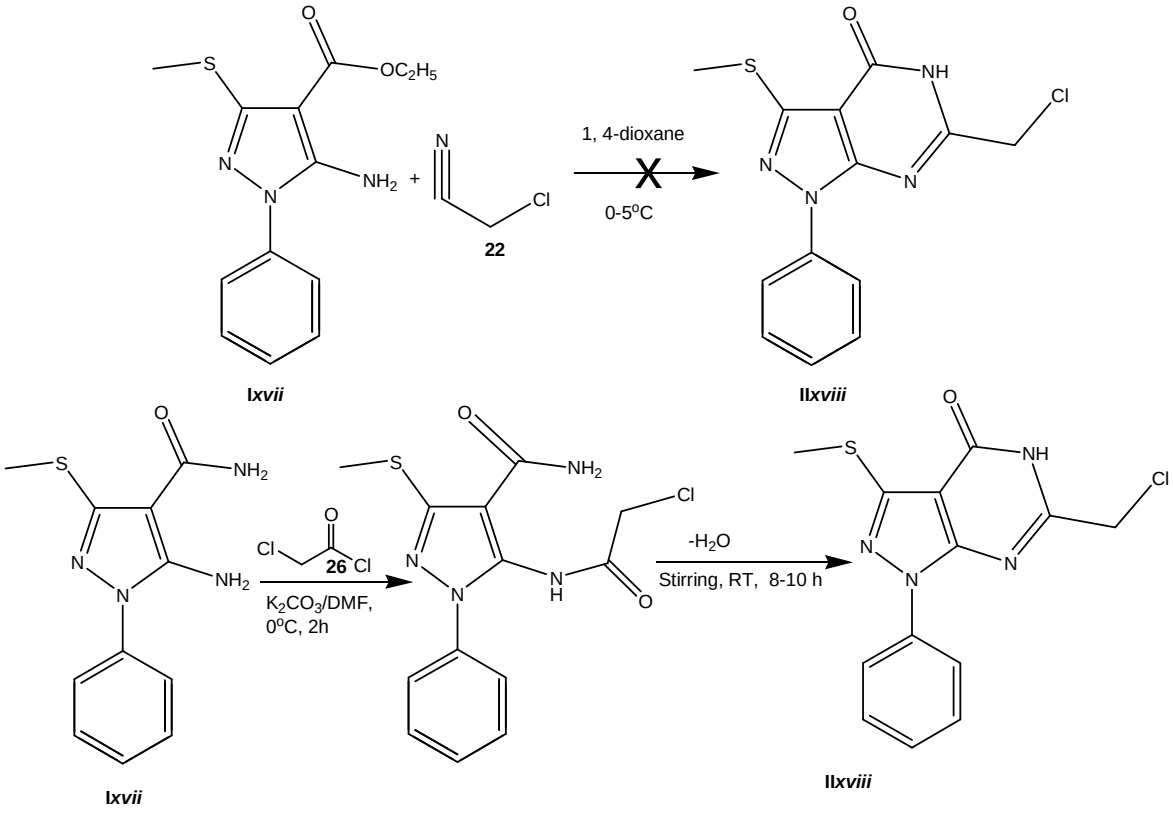
Scheme-17

The imidoyl halide intermediate enhances the electrophilicity of the nitrile carbon tremendously and thus, helps in their facile condensation with the aminocarbonyl compounds. This is then followed by intramolecular cyclization.[36] This cyclization is also probably facilitated by the protonation of the carbonyl group.

For the condensation of 2-amino-3-carbethoxy substrate with nitriles under the influence of dry HCl gas, some productive modification in experimental procedure has been tried, that involves the mixing the two reactants in saturated solution of HCl in dioxane (7M) and stirring the mixture for few hrs at 0-5^{o}C, followed by further stirring for 2 hrs at RT and thereafter heating on a boiling water-bath for 2 hrs. The reaction mixture was then worked up as usual, involving pouring on ice-water mixture and basification with conc. ammonium hydroxide. This modification not only reduced the reaction time and labour but also gave excellent yields of the desired products. In fact only once a stock solution of dioxane saturated with HCl was to be prepared and then it could be used simultaneously for 4-5 different condensations at a time. This has also curtailed the use of multiple HCl generation assemblies.

3.2.2 Synthesis of 2-chloromethyl-5-substituted-7-phenylpyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (II*xviii*)

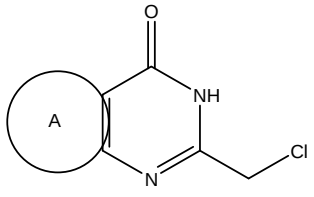
However, when *o*-aminoester of the nitrogen containing heterocycle pyrazole was tried to cyclize with nitriles under acidic conditions, the reaction failed to proceed. So modification was done by preparing pyrazole *o*-aminoamides as the starting material and reacted with chloroacetylchloride **26** in presence of potassium carbonate and cyclized *in situ* in presence of water to get the corresponding 2-chlormethyl derivative (Scheme-16).



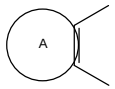
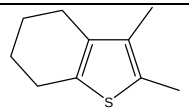
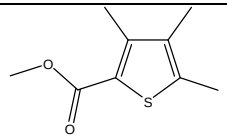
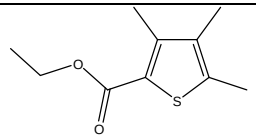
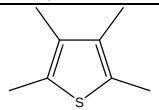
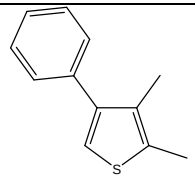
I*xvii* II*xviii* I*xvii* II*xviii*

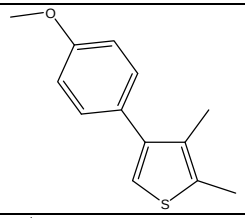
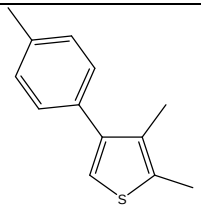
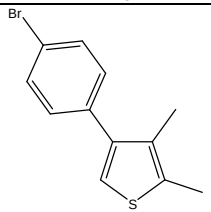
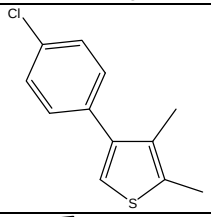
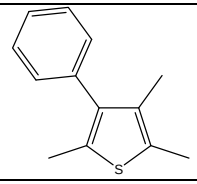
Scheme-16

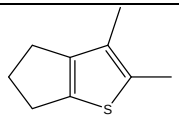
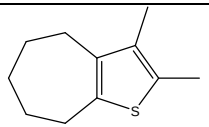
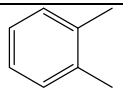
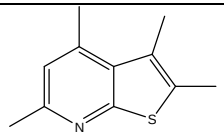
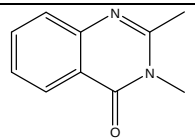
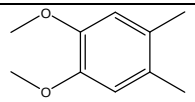
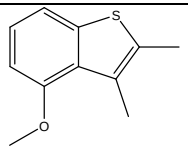
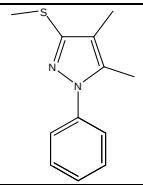
Table-8: Physical data of condensed 2-chloromethylpyrimidin-4(3*H*)-ones (II*i-xviii*) synthesized



II*i-xvii*

Compd. No.		Yield (%)	M. P (^{o}C)	Mol. formula (Solv. of crystn.)	IR (cm^{-1})	Mass (m/e)	NMR (δppm)
II*i*		90	273-276	$C_{11}H_{11}ClN_2OS$ (D)	3014(γ_{Ar-H}), 1662(γ_{CONH}), 754(γ_{C-Cl}).	255(M^+), 221, 149	1.62 (4H, s, C*H*$_2$ at 6 & 7), 2.77 (2H, s, C*H*$_2$ at 4), 3.02 (2H, s, C*H*$_2$ at 8), 4.55 (2H, s, C*H*$_2$ at 2), 10.60 (1H, s, N*H* at 3)
II*ii*		90	250-252	$C_{10}H_9ClN_2O_3S$ (M-C)	2863(γ_{ArH}), 1724(γ_{COO}), 1664(γ_{CONH}), 915, 763, 686(γ_{C-Cl})	--	--
II*iii*		86	243-245	$C_{11}H_{11}ClN_2O_3S$ (T-M)	2864(γ_{CH-}), 1725(γ_{COO-}), 1670(γ_{CONH}), 249(γ_{CH2}), 763 (γ_{C-Cl})	286(M^+)	1.41 (3H, t, OCH_2CH_3, *J* = 7), 2.95 (3H, s, C*H*$_3$ at 5). 4.38 (2H, quartlet, *J* = 7, OCH_2CH_3), 4.57 (2H, s, C*H*$_2$), 10.62 (1H, s, N*H*)
II*iv*		97	253-255	$C_9H_9ClN_2OS$ (D)	2917(γ_{C-H}), 662(γ_{CONH}), 1211(γ_{CH2}) and 769(γ_{C-Cl})	229(M^+)	2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 4.51 (2H, s, C*H*$_2$), 10.03 (1H, s, br, N*H*)
II*v*		87	221-223	$C_{13}H_9ClN_2OS$ (D)	2855(γ_{C-H}), 663(γ_{CONH}), 1294(γ_{CH2}), 1046 and 748(γ_{C-Cl})	276(M^+)	4.58 (2H, s, C*H*$_2$), 7.31-7.52 (5H, m, Ar-*H* and 1*H* at 6 position), 12.69 (1H, s, br, N*H*)

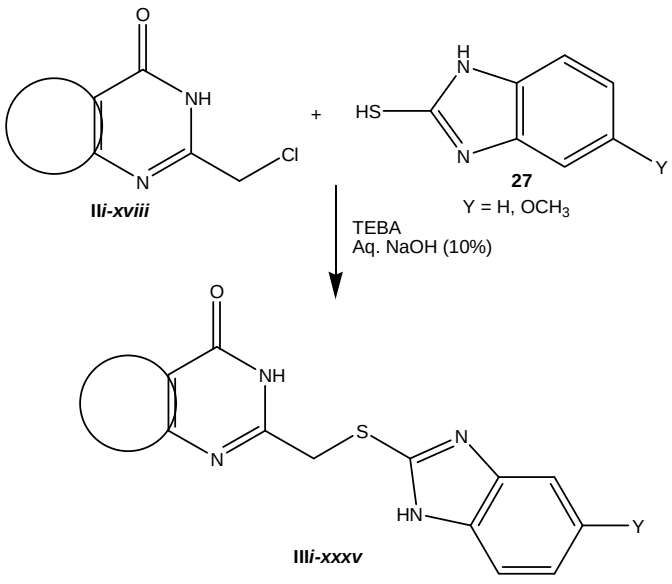
Compd. No.	A	Yield (%)	M. P (^oC)	Mol. formula (Solv. of crystn.)	IR (cm^{-1})	Mass (m/e)	NMR (δppm)
II*vi*		87	208-210	$C_{14}H_{11}ClN_2O_2S$ (T-M)	3094(γ_{ArH}), 2945($_{C-H}$), 1672(γ_{CONH}), 715(γ_{C-Cl})	--	3.84 (3H, s, Ar-OC*H*$_3$), 4.49 (2H, s, C*H*$_2$ at 2), 7.14-7.54 (5H, m, Ar-*H* at 6)
II*vii*		86	258-260	$C_{14}H_{11}ClN_2OS$ (E-C)	3028(γ_{ArH}), 1651(γ_{CONH}), 762 (γ_{C-Cl})	290(M^+)	2.39 (3H, s, C*H*$_3$), 4.53 (2H, s, C*H*$_2$), 7.13 (1H, s, C*H*), 7.19-7.46 (4H, m, Ar-*H*), 10.43 (1H,s, N*H*)
II*viii*		76	247-249	$C_{13}H_8BrClN_2OS$ (E-C)	3120(γ_{Ar-H}), 1651(γ_{CONH}), 756(γ_{C-Cl})	--	--
II*ix*		78	229-231	$C_{13}H_8Cl_2N_2OS$ (T-M)	3107(γ_{Ar-H}), 1649(γ_{CONH}), 756(γ_{C-Cl})	--	4.55 (2H, s, C*H*$_2$ at 2), 7.40-7.55 (5H, m, Ar-*H* & *H* at 6)
II*x*		94	261-264	$C_{14}H_{11}ClN_2OS$ (E-C)	3035(γ_{CH2}),1658(γ_{CONH}), 728(γ_{C-Cl})	--	2.40 (3H, s, C*H*$_3$ at 6), 4.42 (2H, s, *CH*$_2$ at 2), 7.38-7.44 (5H, m, Ar-*H*)

Compd. No.	A	Yield (%)	M. P (°C)	Mol. formula (Solv. of crystn.)	IR (cm^{-1})	Mass (m/e)	NMR (δppm)
II*xi*		73	278-280	$C_{10}H_9ClN_2OS$ (T-M)	3015(γ_{CH2}), 1678(γ_{CONH}), 754(γ_{C-Cl})	--	2.46 (2H, m, C*H*$_2$ at 6, *J* = 7.0), 2.94 (4H, m, CH_2 at 5 & 7), 4.50 (2H, s, C*H*$_2$, at 2), 12.56 (1H, s, br, N*H*)
II*xii*		84	188-190	$C_{12}H_{13}ClN_2OS$ (M-C)	2924(γ_{C-H}), 1670(γ_{CONH}), 1471(γ_{C-H}), 736, 752 (γ_{C-Cl})	--	--
II*xiii*		70	257-259	$C_9H_7ClN_2O$ (E-C)	1699(γ_{CONH})	--	--
II*xiv*		58	273-275	$C_{12}H_{10}Cl\,N_3OS$ (E-C)	3443, 3338 (γ_{NH}), 2946(γ_{C-H}), 1672(γ_{CONH}), 760(γ_{C-Cl})	281(M+1), 279(M^+), 244, 216	--
II*xv*		50	240-243	$C_{11}H_7Cl\,N_4O_2$ (E-C)	2896(γ_{C-H}), 1686(γ_{CONH}), 778(γ_{C-Cl})	--	4.62 (2H, s, C*H*$_2$), 7.22-8.35 (4H, m, Ar-*H*), 9.62 (1H, s, N*H*)
II*xvi*		87	240-242	$C_{11}H_{11}ClN_2O_3$ (E-C)	3012 (γ_{C-H}), 1666 (γ_{CONH}), 780 (γ_{C-Cl})	254(M^+), 239, 219	--
II*xvii*		58	265-267	$C_{12}H_9Cl\,N_2O_2S$ (E-C)	2782(γ_{C-H}), 1670(γ_{CONH}), 735 (γ_{C-Cl})	--	--
II*xviii*		74	275-277	$C_{13}H_{11}Cl\,N_4OS$ (E-C)	2849(γ_{C-H}), 1676(γ_{CONH}), 765(γ_{C-Cl})	--	2.50 (3H, s, C*H*$_3$), 4.35 (2H, s, *CH*$_2$Cl), 7.12-7.91 (5H, m, Ar-*H*)

E = Ethanol, C = Chloroform, D = Dimethylformamide, T = Toluene, M = Methanol,

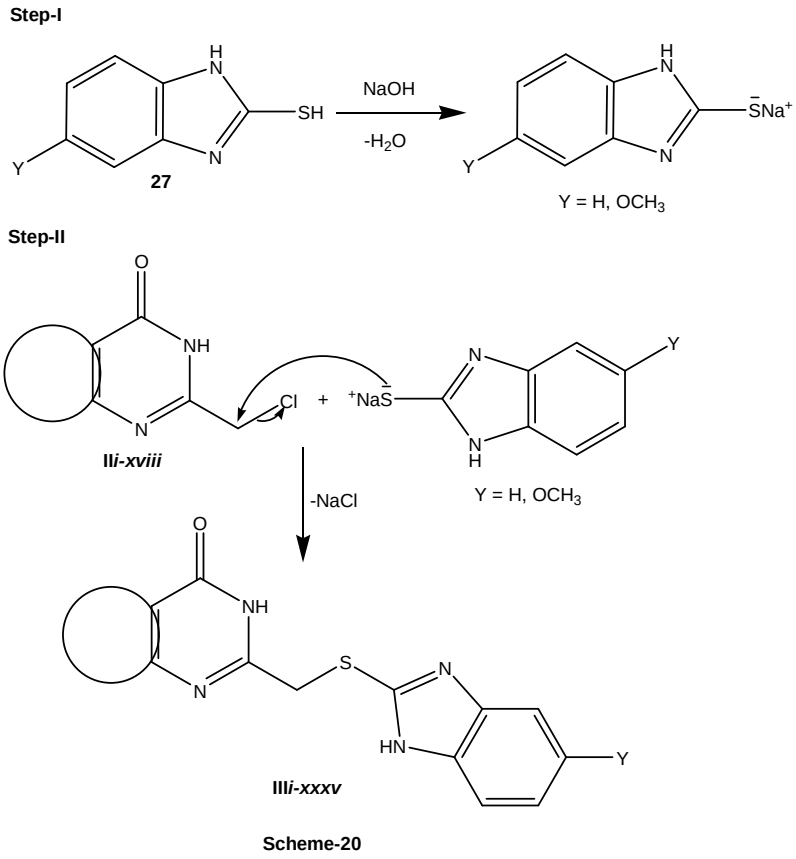
3.3 Condensation of 2-chloromethylpyrimidin-4(3*H*)-ones and 2-mercaptobenzimidazoles to get corresponding condensed pyrimidinylmethylthiobenzimidazoles (III*i-xxxv*)

The appropriate condensed 2-chloromethylpyrimidines-4(3*H*)-one **II*i-xviii*** were condensed with the 2-mercaptobenzimidazole and 2-mercapto-5-methoxy **27** benzimidazole under basic conditions. Literature reports indicate use of NaOMe/MeOH as one of the preferred reaction condition for this condensation. However, it was decided to utilize the green chemical, eco-friendly technique of Phase Transfer Catalysis (PTC), which involved aq. NaOH (10%) and methylene dichloride as two phases. Triethyl Benzylammonium Chloride (TEBA.Cl) was the PTC employed. The reaction was facile at stirring condition at RT within an hour; the product was formed completely and in excellent yield and purity (Scheme-19).



Scheme-19

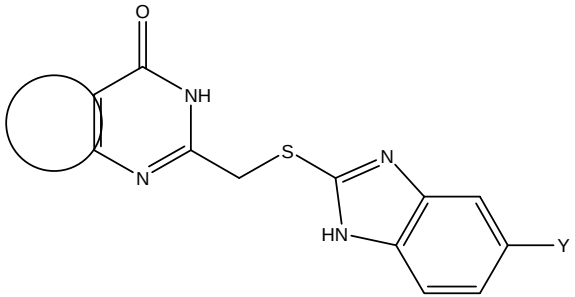
The proposed mechanism is as follows (Scheme-20):

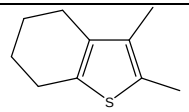
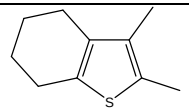
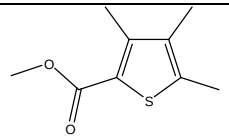
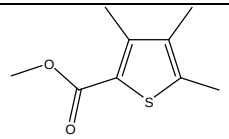
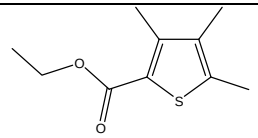


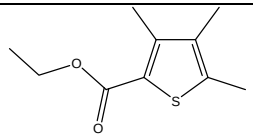
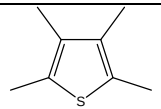
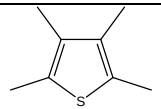
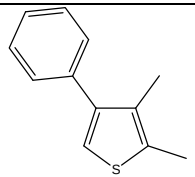
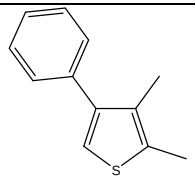
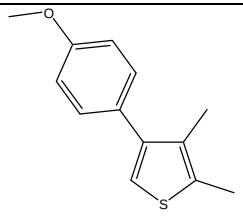
Scheme-20

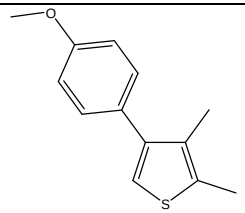
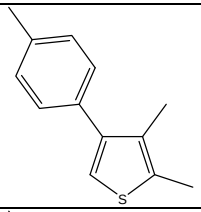
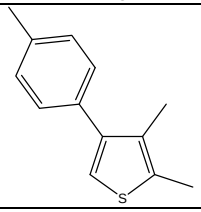
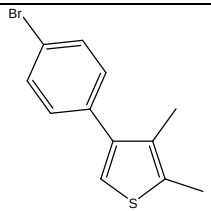
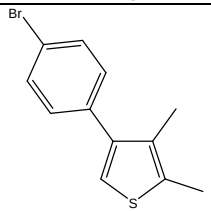
In a typical procedure, the appropriate 2-mercaptobenzimidazole was taken in 20 ml of 10% aq. NaOH and stirred at RT. This was followed by addition of a pinch of TEBA. chloride. Thereafter, added the solution of dropwise condensed 2-chloromethylpyrimidin-4(3*H*)-ones (**II*i-xviii***) dissolved in methylenedichloride (MDC). When the addition of 2-chloromethylpyrimidin-4(3*H*)-one was complete, the reaction mixture was further stirred at RT for 6-8 hrs. The two phases were separated and the organic layer was washed with water and dried over anhydrous sodium sulphate. The dichlomethane was removed under reduced pressure to give the crude product. The dry crude solid was recrystallized from mixture of solvents such as methanol-chloroform or methanol-MDC.

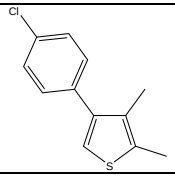
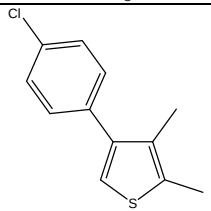
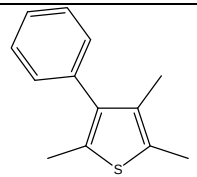
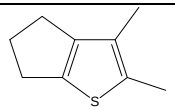
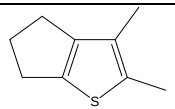
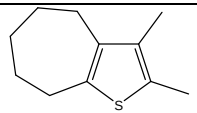
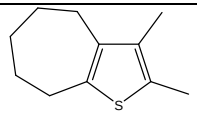
Table-9. Physical data of condensed pyrimidinylmethylthiobenzimidazoles (III*i-xxxv*) synthesized

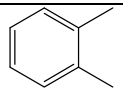
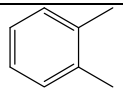
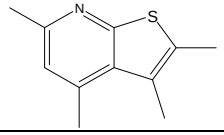
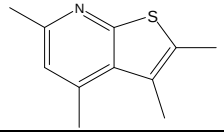
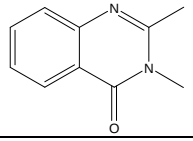
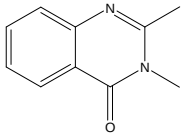
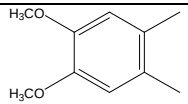
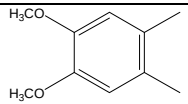


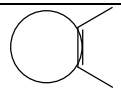
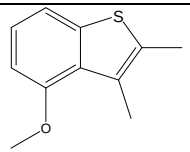
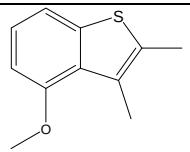
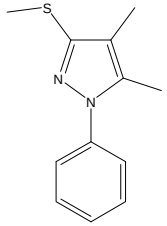
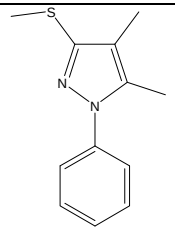
S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*i*		H	$C_{18}H_{16}N_4OS_2$ (C-M)	264-267	3247(γ_{NH}), 2939(γ_{C-H}), 1680(γ_{CONH}), 743(γ_{C-S})	368(M^+), 335, 307, 150	1.83-1.88 (4H, m, C*H*$_2$ at 6 & 7), 2.76 (2H, t, C*H*$_2$ at 5, *J* = 5.64), 2.95 (2H, t, C*H*$_2$ at 8, *J* = 5.80), 4.39 (2H, s, CH_2 at SC*H*$_2$), 7.39-7.16 (4H, m, Ar-*H*), 12.50 (1H, s, N*H*), 13.17 (1H, s, N*H*)
III*ii*		OCH_3	$C_{19}H_{18}N_4O_2S_2$ (C-M)	210-215	3266(γ_{NH}), 2940(γ_{C-H}), 1670(γ_{CONH}), 643(γ_{C-S}).	398(M^+), 365, 219, 180	1.83-1.88 (4H, m, C*H*$_2$ at 6 & 7), 2.75 (2H, t, C*H*$_2$ at 5, *J* = 5), 2.97 (2H, t, C*H*$_2$ at 8, *J* = 5), 3.87 (3H, s, OC*H*$_3$), 4.32 (2H, s, CH_2 at SC*H*$_2$), 6.81-7.25 (3H, m, Ar-*H*), 7.57 (1H, s, N*H*), 12.21 (1H, s, N*H*)
III*iii*		H	$C_{17}H_{14}N_4O_3S_2$ (C-M)	258-260	3282(γ_{NH}), 2956(γ_{C-H}), 1667(γ_{CONH}), 741(γ_{C-S})	386(M^+), 353, 150	2.90 (3H, s, C*H*$_3$ at 5), 3.87 (3H, s, C*H*$_3$ of C*H*$_3$OOC), 4.36 (2H, s, C*H*$_2$ at SCH_2), 7.17 (2H, q, *H* at imidazole, *J* = 3.2), 7.53 (2H, q, imidazole, *J* = 3.16)
III*iv*		OCH_3	$C_{18}H_{16}N_4O_4S_2$ (C-M)	230-235	3339(γ_{NH}), 2943(γ_{C-H}), 1690(γ_{CONH}), 613(γ_{C-S})	416(M^+), 383, 210, 180	2.68 (3H, s, C*H*$_3$ at 5), 3.86 (3H, s, OC*H*$_3$), 3.88 (3H, s, C*H*$_3$-O-CO-), 4.65 (2H, s, CH_2 at SC*H*$_2$), 7.05 (1H, dd, C*H* at imidazole, *J* = 2.30 & 6.64), 7.15 (1H, d, C*H* at imidazole, *J* = 2.2), 7.54 (1H, d, C*H* at imidazole, *J* = 8.92), 12.47 (1H, br s, N*H*), 13.45 (1H, s, N*H*).
III*v*		H	$C_{18}H_{16}N_4O_3S_2$ (C-M)	270-272	3237(γ_{NH}), 2945(γ_{C-H}), 1716(γ_{COOEt}), 1673(γ_{CONH}), 740(γ_{C-S})	400(M^+), 365, 215, 150	--

S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*vi*		OCH_3	$C_{19}H_{18}N_4O_4S_2$ (C-M)	257-260	3247(γ_{NH}), 2985(γ_{C-H}), 1690(γ_{CONH}), 650(γ_{C-S})	430(M^+), 397, 369, 180	1.38 (3H, t, CH_3 of C*H*$_3$-CH_2-O-, *J* = 6), 2.67 (3H, s, C*H*$_3$ at 5), 3.84 (3H, s, OC*H*$_3$), 4.20-4.38 (4H, m, CH_3-C*H*$_2$-O and SC*H*$_2$), 6.82 (1H, dd, C*H* at Imidazole *J* = 6.8 & 2.04); 7.03 (1H, s, CH at imidazole), 7.42 (1H, d, CH at imidazole), 10.84 (1H, br s, N*H*), 12.94 (1H, br s, N*H*)
III*vii*		H	$C_{16}H_{14}N_4OS_2$ (C-M)	272-275	3263(γ_{NH}), 2917(γ_{C-H}), 1669(γ_{CONH}), 605(γ_{C-S})	--	--
III*viii*		OCH_3	$C_{17}H_{16}N_4O_2S_2$ (C-M)	130-134	3306(γ_{NH}), 2966(γ_{C-H}), 1683(γ_{CONH}), 601(γ_{C-S})	368(M^+), 339, 180	2.38 (3H, s, C*H*$_3$ at 5), 2.47 (3H, s, C*H*$_3$ at 6), 3.80 (3H, s, OC*H*$_3$), 4.27 (2H, s, CH_2 at SC*H*$_2$), 6.77-6.80 (3H, m, Ar-*H*), 10.19 (1H, s, N*H*), 13.25 (1H, s, N*H*)
III*ix*		H	$C_{20}H_{14}N_4OS_2$ (C-M)	224-227	3044(γ_{C-H}), 1680(γ_{CONH}), 741(γ_{C-S})	390(M^+), 357, 272, 150	4.39 (2H, s, CH_2 at SC*H*$_2$), 7.10 (1H, s, *H* at 6), 7.16-7.54 (9H, m, Ar-*H*), 12.60 (1H, s, N*H*), 13.78 (1H, s, N*H*)
III*x*		OCH_3	$C_{21}H_{16}N_4O_2S_2$ (C-M)	165-170	3091(γ_{NH}), 2988(γ_{C-H}), 1685(γ_{CONH}), 620(γ_{C-S})	420(M^+), 387, 256, 180	3.82 (3H, s, OC*H*$_3$), 4.36 (2H, s, CH_2 at SC*H*$_2$), 6.81 (1H, dd, C*H* at Imidazole, *J* = 6.44 & 2.36), 7.11 (1H, s, CH at 6), 7.01-7.56 (7H, m, Ar-*H*), 11.90 (1H, s, N*H*), 13.30 (1H, s, N*H*)
III*xi*		H	$C_{21}H_{16}N_4O_2S_2$ (C-M)	244-248	3256(γ_{NH}), 2839(γ_{C-H}), 1663(γ_{CONH}), 746(γ_{C-S})	421(M^+), 387, 359, 159	3.82 (3H, s, OC*H*$_3$), 4.43 (2H, s, C*H*$_2$ at SCH$_2$), 6.84 (2H, d, *H* at imidazole, *J* = 8.6), 7.08 (1H, s, H at 7), 7.17 (2H, q, H at imidazole, *J* = 3.16), 7.47-7.52 (4H, m, Ar-*H*), 12.40 (1H, s, N*H*), 13.25 (1H, s, N*H*)

S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*xii*		OCH_3	$C_{22}H_{18}N_4O_3S_2$ (C-M)	140-142	3242(γ_{NH}), 2941(γ_{C-H}), 1690(γ_{CONH}), 603(γ_{C-S}).	450(M^+), 272, 180.	3.34 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 4.34 (2H, s, CH_2 at SC*H*$_2$), 7.06 (H, s, *H* at thiophene), 6.89-7.50 (7H, m, Ar-*H*), 12.35 (1H, br s, N*H*), 13.20 (1H, s, N*H*)
III*xiii*		H	$C_{21}H_{16}N_4OS_2$ (C-M)	260-262	3229(γ_{NH}), 3032(γ_{C-H}), 1685(γ_{CONH}), 743(γ_{C-S})	404(M^+), 371, 343, 150	2.36 (3H, s, CH_3), 4.42 (2H, s, C*H*$_2$ at SCH_2), 7.10 (1H, s, H at 6), 7.15-7.43 (8H, m, Ar-*H*), 12.54 (1H, s, N*H*), 13.37 (1H, s, N*H*)
III*xiv*		OCH_3	$C_{22}H_{18}N_4O_2S_2$ (C-M)	245-247	3246(γ_{NH}), 2878(γ_{C-H}), 1659(γ_{CONH}), 627(γ_{C-S})	401(M^+), 270, 180	--
III*xv*		H	$C_{20}H_{13}BrN_4OS_2$ (C-M)	247-249	3235(γ_{NH}), 2940(γ_{C-H}), 1647(γ_{CONH}), 756(γ_{C-S})	470(M^+), 437, 150	4.42 (2H, s, C*H*$_2$ at SCH_2), 7.16-7.20 (3H, m, *H* at imidazole), 7.43-7.56 (6H, m, Ar-*H*), 12.45 (1H, s, N*H*), 13.55 (1H, s, N*H*)
III*xvi*		OCH_3	$C_{21}H_{15}BrN_4O_2S_2$ (C-M)	135-141	3262(γ_{NH}), 2945(γ_{C-H}), 1690(γ_{CONH}), 666(γ_{C-S})	500(M^+), 322, 180	3.82 (3H, s, OC*H*$_3$), 4.37 (2H, s, CH_2 at SC*H*$_2$), 6.80 (1H, dd, C*H* at imidazole, *J* = 2.4 & 6.5), 6.99 (1H, s, C*H* at imidazole), 7.14 (1H, s, C*H* at imodazole), 7.38-7.51 (5H, m, 4Ar-*H* and 1*H* at 6 of thiophene), 12.50 (1H, br s, N*H*)

S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*xvii*		H	$C_{20}H_{13}ClN_4OS_2$ (C-M)	227-230	3279(γ_{NH}), 1662(γ_{CONH}), 743(γ_{C-S})	424(M^+), 391, 363, 150	--
III*xviii*		OCH_3	$C_{21}H_{15}ClN_4O_2S_2$ (C-M)	128-130	3194γ_{NH}), 3090(γ_{C-H}), 1680(γ_{CONH}), 622(γ_{C-S})	454(M^+), 421, 276, 180	3.82 (3H, s, OC*H*$_3$), 4.38 (2H, s, CH_2 at SC*H*$_2$), 7.15 (1H, s, C*H* at 6), 6.80 (1H, dd, C*H* at imidazole, *J* = 2.4 & 6.36), 7.00 (1H, d, C*H* at imidazole, *J* = 2.16), 7.40 (1H, d, C*H* at imidazole, *J* = 8.76), 7.32-7.52 (4H, m, Ar-*H*)
III*xix*		H	$C_{21}H_{16}N_4OS_2$ (C-M)	263-265	3243(γ_{NH}), 2937(γ_{C-H}), 1656(γ_{CONH}), 740(γ_{C-S})	404(M^+), 371, 343, 150	2.20 (3H, s, C*H*$_3$ at 6), 4.52 (2H, s, C*H*$_2$ at C*H*$_2$S), 7.10-7.60 (9H, m, Ar-*H*), 12.25 (1H, s, N*H*), 13.00 (1H, s, N*H*)
III*xx*		H	$C_{17}H_{14}N_4OS_2$ (C-M)	258-260	3247(γ_{NH}), 2943(γ_{C-H}), 1685(γ_{CONH}), 741(γ_{C-S})	354(M^+), 321, 293, 205, 150	2.45 (2H, m, CH_2 at 6), 2.94 (4H, m, C*H*$_2$ at 5 & 7), 4.40 (2H, s, C*H*$_2$ at SCH_2), 7.19 (2H, m, *H* at imidazole), 7.51 (2H, m, *H* at imidazole), 12.90 (1H, s, N*H*), 13.45 (1H, s, N*H*)
III*xxi*		OCH_3	$C_{18}H_{16}N_4O_2S_2$ (C-M)	162-165	3235(γ_{NH}), 2992(γ_{C-H}), 1668(γ_{CONH}), 665(γ_{C-S})	384(M^+), 351, 205, 180.	--
III*xxii*		H	$C_{19}H_{18}N_4OS_2$ (C-M)	264-266	3212(γ_{NH}), 2916(γ_{C-H}), 1685(γ_{CONH}), 737(γ_{C-S})	382(M^+), 349, 232, 150	1.68 (6H, m, C*H*$_2$ at 6, 7, & 8), 1.88 (2H, t, C*H*$_2$ at 5, *J* = 3.36), 3.30 (2H, t, C*H*$_2$ at 9, *J* = 5.36), 4.36 (2H, s, C*H*$_2$ at SCH_2), 7.20 (2H, m, *H* at imidazole), 7.56 (2H, m, *H* at imidazole, *J* = 3.16), 12.50 (1H, s, N*H*), 13.40 (1H, s, N*H*)
III*xxiii*		OCH_3	$C_{20}H_{20}N_4O_2S_2$ (C-M)	226-230	3190(γ_{NH}), 2918(γ_{C-H}), 1685(γ_{CONH}), 650(γ_{C-S})	412(M^+), 379, 232, 180	1.66 (4H, m, C*H*$_2$ at 6 & 7), 1.87 (2H, m, C*H*$_2$ at 5), 2.85 (2H, m, C*H*$_2$ at 8), 3.29 (2H, m, C*H*$_2$ at 9), 3.84 (3H, s, C*H*$_3$ at OCH_3), 4.34 (2H, s, S*CH*$_2$), 6.79-7.43 (3H, m, Ar-*H*), 12.30 (1H, s, N*H*), 13.21 (1H, s, N*H*)

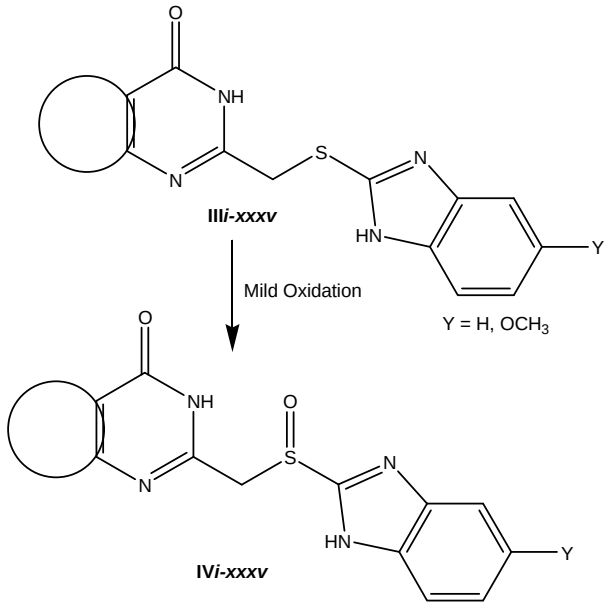
S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*xxiv*		H	$C_{16}H_{12}N_4OS$ (C-M)	196-198	3145(γ_{NH}), 1670(γ_{CONH}), 742(γ_{C-S})	308(M^+), 275, 247, 163.	4.53 (2H, s, C*H*$_2$ at C*H*$_2$S), 7.11-8.09 (8H, m, Ar-*H*).
III*xxv*		OCH_3	$C_{17}H_{14}N_4O_2S$ (C-M)	177-179	3061(γ_{NH}), 1675(γ_{CONH}), 772(γ_{C-S}).	--	--
III*xxvi*		H	$C_{19}H_{15}N_5OS_2$ (C-M)	120-122	2922(γ_{C-H}), 1685(γ_{CONH}), 1570(γ_{C-C}), 738(γ_{C-S})	--	--
III*xxvii*		OCH_3	$C_{20}H_{17}N_5O_2S_2$ (C-M)	178-180	2922(γ_{C-H}), 1654(γ_{CONH}), 1570(γ_{C-C}), 785(γ_{C-S})	423(M^+), 390, 362, 180.	--
III*xxviii*		H	$C_{18}H_{12}N_6O_2S$ (C-M)	180-182	2923(γ_{C-H}), 1670(γ_{CONH}), 1606(γ_{C-C}), 742(γ_{C-S})	--	--
III*xxix*		OCH_3	$C_{19}H_{14}N_6O_3S$ (C-M)	154-156	2923(γ_{C-H}), 1672(γ_{CONH}), 1607(γ_{C-C}), 774(γ_{C-S})	--	--
III*xxx*		H	$C_{18}H_{16}N_4O_3S$ (C-M)	200-202	3201(γ_{NH}), 1662(γ_{CONH}), 1608(γ_{C-C}), 742(γ_{C-S})	--	--
III*xxxi*		OCH_3	$C_{19}H_{18}N_4O_4S$ (C-M)	115-118	2926(γ_{C-H}), 1647(γ_{CONH}), 785(γ_{C-S})	398(M^+), 365, 220, 180	--

S. No.		Y	Mol. Formula (Sol. of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
III*xxxii*		H	$C_{19}H_{14}N_4O_2S_2$ (C-M)	155-158	2916(γ_{C-H}), 1663(γ_{CONH}), 736(γ_{C-S})	--	--
III*xxxiii*		OCH_3	$C_{20}H_{16}N_4O_3S_2$ (C-M)	115-120	2923(γ_{C-H}), 1669(γ_{CONH}), 738(γ_{C-S})	--	--
III*xxxiv*		H	$C_{20}H_{16}N_6OS_2$ (C-M)	240-242	2922(γ_{C-H}), 1691(γ_{CONH}), 739(γ_{C-S})	--	--
III*xxxv*		OCH_3	$C_{21}H_{18}N_6O_2S_2$ (C-M)	82-85	3123(γ_{C-H}), 1685(γ_{CONH}), 1589(γ_{C-C}), 758(γ_{C-S})	450(M^+), 417, 272, 180	--

3.4 Mild Oxidation of Thio Derivatives (III*i-xxxv*) to Sulfinyl Derivatives Using *m*-Chloroper-Benzoic Acid (*m*-CPBA) (IV*i-xxxv*)

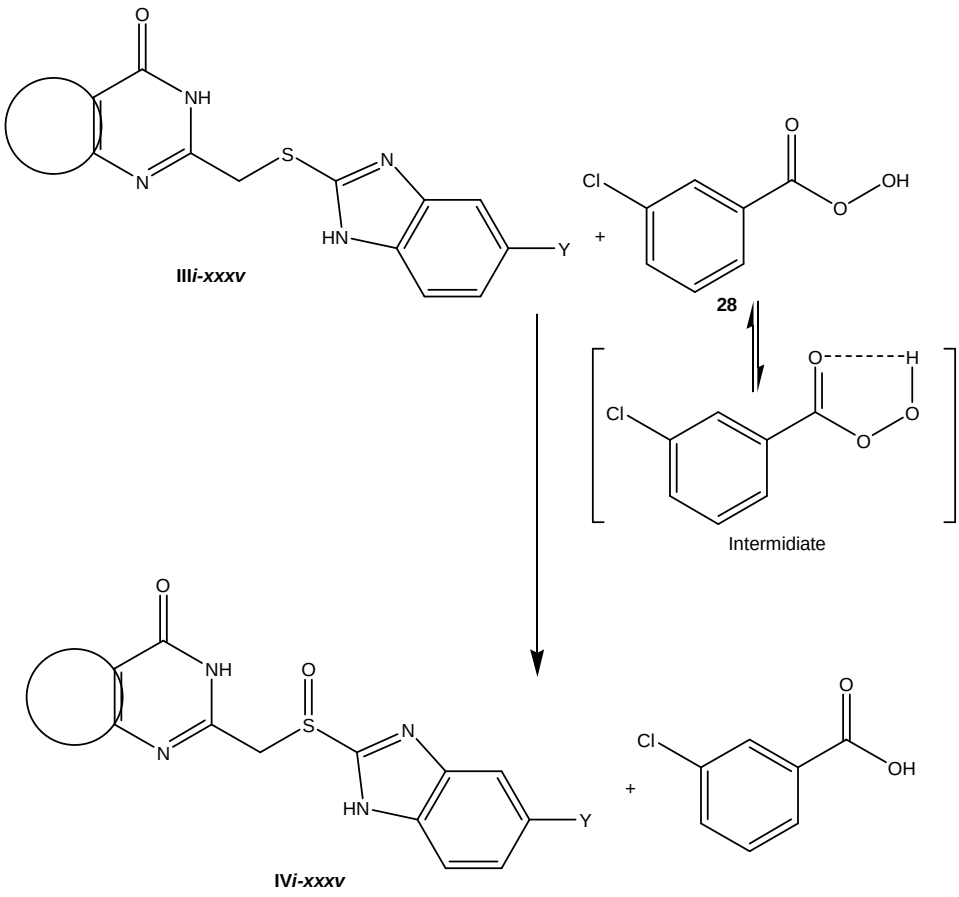
The organic sulfides may be selectively oxidized to sulfinyl derivatives by using *m*-CPBA **28**. Since oxidation using per acids occurs under very mild conditions, it can be successfully applied to the preparation of base sensitive sulfoxides.[39-41] Oxidation of sulfides with 1.2 moles of *m*-CPBA in methanol & dichloromethane at 0^{o}C gave the corresponding sulfinyl derivative in quantitative yields. Further, the reaction doesn't proceed further to the sulfonyls under these reactions conditions using this reagent.

Thus, the target 2-(1*H*-benzimidazol-2-yl)methylsulfinylcondensedpyrimidin-4(3*H*)-ones and 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)methylsulfinylcondensedpyrimidin-4(3*H*)-ones (**IV*i-xxxv***) were synthesized by mild oxidation of 2-(1*H*-benzimidazol-2-yl) and 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)methylthiocondensedpyrimidin-4(3*H*)-ones (**III*i-xxxv***) using *m*-chloroperbenzoic acid as oxidizing agent (Scheme-21).



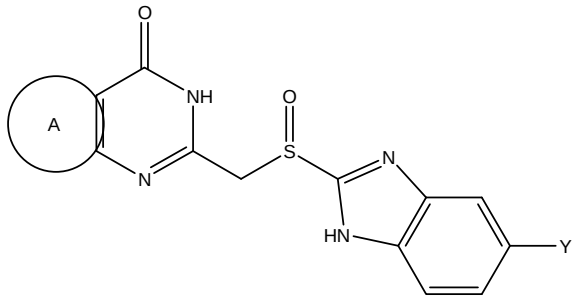
Scheme-21

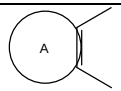
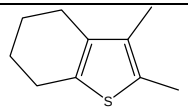
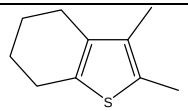
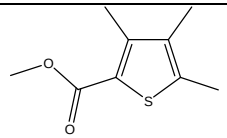
The proposed reaction of oxidation involves a nucleophilic attack by the sulfide on a cyclic hydrogen-bonded form of the peracid[42-43] (Scheme-22).

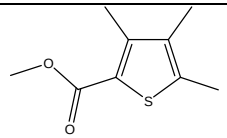
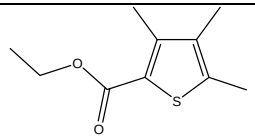
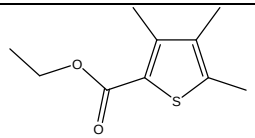
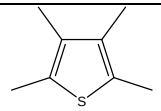
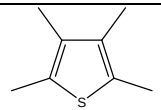
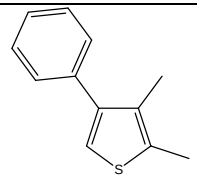

O
NH
N
S
N
HN
Y
III*i-xxxv*
Cl
O
O
OH
+
28
O
H
Cl
O
O
Intermidiate
O
NH
O
S
N
N
HN
Y
IV*i-xxxv*
+
Cl
O
OH

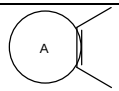
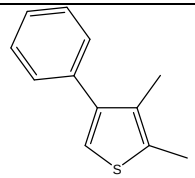
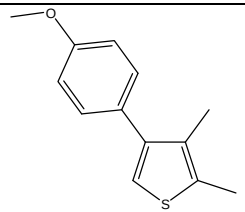
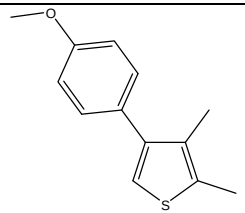
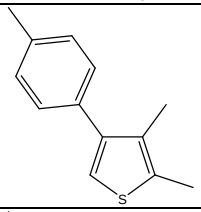
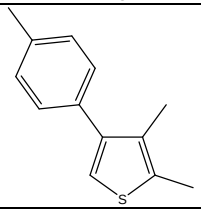
Scheme-22

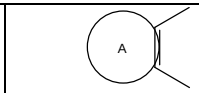
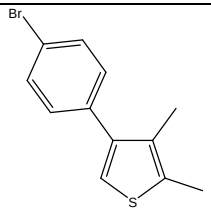
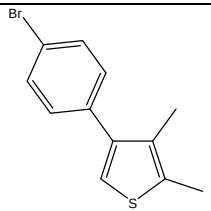
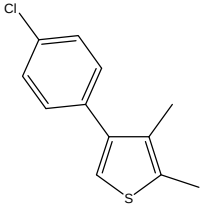
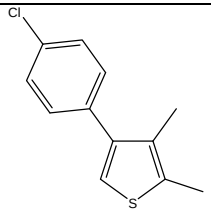
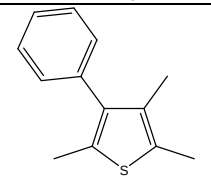
Table-10. Physical data of 2-(1*H*-benzimidazol-2-yl)methylsulfinylcondensedpyrimidin-4(3*H*)-ones and 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)methylsulfinylcondensedpyrimidin-4(3*H*)-one (IV*i-xxxv*)

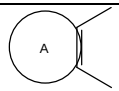
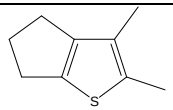
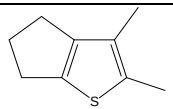
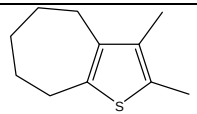
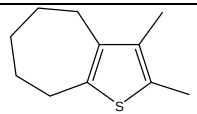
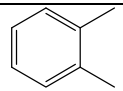
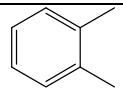
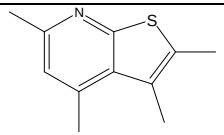


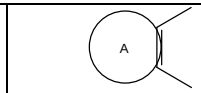
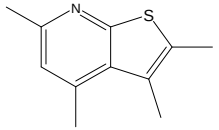
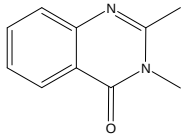
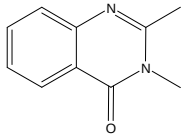
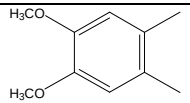
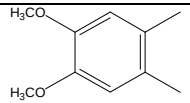
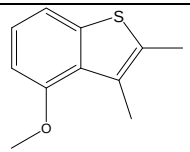
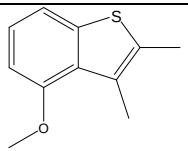
S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*i*		H	$C_{18}H_{16}N_4O_2S_2$ (C-M)	216-218	3235(γ_{NH}), 2946(γ_{C-H}), 1655(γ_{CONH}), 1060(γ_{S-O}), 747(γ_{C-S})	368, 364, 336, 218, 150	1.79-1.83 (4H, m, C*H*$_2$ at 6 & 7), 2.54-2.79 (4H, m, C*H*$_2$ at 5 & 8), 5.58 (2H, s, CH_2 at SC*H*$_2$), 7.39-7.16 (4H, m, Ar-*H*), 12.60 (1H, s, N*H*), 13.80 (1H, s, N*H*)
IV*ii*		OCH_3	$C_{19}H_{18}N_4O_3S_2$ (C-M)	194-196	3054(γ_{NH}), 2934(γ_{C-H}), 1681(γ_{CONH}), 1045(γ_{S-O}), 716(γ_{C-S})	--	--
IV*iii*		H	$C_{17}H_{14}N_4O_4S_2$ (C-M)	210-214	3237(γ_{NH}), 3078(γ_{C-H}), 1726(γ_{COO}), 682(γ_{CONH}), 1097(γ_{S-O}), 743(γ_{C-S})	--	--

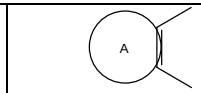
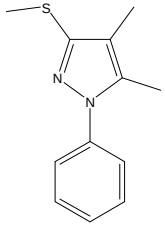
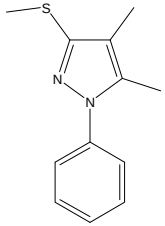
S. No.	A	Y	Mol. Formula (Sol of recryst.)	M.P (°C)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*iv*		OCH_3	$C_{18}H_{16}N_4O_5S_2$ (C-M)	200-205	3008(γ_{C-H}), 1722(γ_{COO}), 1659(γ_{CONH}), 1026(γ_{S-O}), 761(γ_{C-S})	--	2.67 (3H, s, C*H*$_3$ at 5), 3.88 (3H, s, OC*H*$_3$), 3.87 (3H, s, C*H*$_3$-O-CO-), 5.50 (2H, s, CH_2 at SC*H*$_2$), 7.03 (1H, dd, C*H* at imidazole, *J* = 2.30 & 6.62), 7.17 (1H, d, C*H* at imidazole, *J* = 2.2), 7.52 (1H, d, C*H* at imidazole, *J* = 8), 12.40 (1H, br s, N*H*), 13.20 (1H, s, N*H*)
IV*v*		H	$C_{18}H_{16}N_4O_4S_2$ (C-M)	220-222	3239(γ_{NH}), 2946(γ_{C-H}), 1717(γ_{COOEt}), 1670(γ_{CONH}), 1038(γ_{S-O}), 741(γ_{C-S})	--	--
IV*vi*		OCH_3	$C_{19}H_{18}N_4O_5S_2$ (C-M)	200-207	3175(γ_{NH}), 2978(γ_{C-H}), 1715(γ_{COO-}), 1659(γ_{CONH}), 1029(γ_{S-O}), 754(γ_{C-S})	--	--
IV*vii*		H	$C_{16}H_{14}N_4O_2S_2$ (C-M)	160-162	3379(γ_{NH}), 3057(γ_{C-H}), 1681(γ_{CONH}), 1055(γ_{S-O}), 740(γ_{C-S})	--	--
IV*viii*		OCH_3	$C_{17}H_{16}N_4O_3S_2$ (C-M)	200-202	3174(γ_{NH}), 2893(γ_{C-H}), 1651(γ_{CONH}), 1050(γ_{S-O}), 801(γ_{C-S})	--	--
IV*ix*		H	$C_{20}H_{14}N_4O_2S_2$ (C-M)	207-210	3193(γ_{NH}), 2971(γ_{C-H}), 1680(γ_{CONH}), 1046(γ_{S-O}), 740(γ_{C-S})	--	--

S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*x*		OCH_3	$C_{21}H_{16}N_4O_3S_2$ (C-M)	195-198	3120(γ_{NH}), 2883(γ_{C-H}), 1677(γ_{CONH}), 1046(γ_{S-O}), 697(γ_{C-S})	--	--
IV*xi*		H	$C_{21}H_{16}N_4O_3S_2$ (C-M)	214-218	3335(γ_{NH}), 3058(γ_{C-H}), 1677(γ_{CONH}), 1046(γ_{S-O}), 745(γ_{C-S})	--	--
IV*xii*		OCH_3	$C_{22}H_{18}N_4O_4S_2$ (C-M)	220-225	3119(γ_{NH}), 2997(γ_{C-H}), 1675(γ_{CONH}), 1045(γ_{S-O}), 703(γ_{C-S})	--	--
IV*xiii*		H	$C_{21}H_{16}N_4O_2S_2$ (C-M)	215-217	3195(γ_{NH}), 3055(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 739(γ_{C-S})	--	2.43 (3H, s, C*H*$_3$), 5.50 (2H, s, C*H*$_2$ at SCH_2), 7.15-7-75 (9H, m, *H* at 6 and Ar-*H*), 12.56 (1H, s, N*H*), 13.50 (1H, s, N*H*)
IV*xiv*		OCH_3	$C_{22}H_{18}N_4O_3S_2$ (C-M)	203-207	3123(γ_{NH}), 2895(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 768(γ_{C-S})	--	--

S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*xv*		H	$C_{20}H_{13}BrN_4O_2S_2$ (C-M)	214-217	3189(γ_{NH}), 3074(γ_{C-H}), 1678(γ_{CONH}), 1045(γ_{S-O}), 746(γ_{C-S})	--	--
IV*xvi*		OCH_3	$C_{21}H_{15}BrN_4O_3S_2$ (C-M)	214-216	3118(γ_{NH}), 2894(γ_{C-H}), 1677(γ_{CONH}), 1044(γ_{S-O}), 772(γ_{C-S})	496, 481, 466, 218, 180	--
IV*xvii*		H	$C_{20}H_{13}ClN_4O_2S_2$ (C-M)	180-182	3280(γ_{NH}), 1665(γ_{CONH}), 1056(γ_{S-O}), 743(γ_{C-S})	--	--
IV*xviii*		OCH_3	$C_{21}H_{15}ClN_4O_3S_2$ (C-M)	214-219	3123(γ_{NH}), 2895(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 768(γ_{C-S})	--	--
IV*xix*		H	$C_{21}H_{16}N_4O_2S_2$ (C-M)	180-182	3351(γ_{NH}), 3077(γ_{C-H}), 1681(γ_{CONH}), 1054(γ_{S-O}), 742(γ_{C-S})	--	--

S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*xx*		H	$C_{17}H_{14}N_4O_2S_2$ (C-M)	221-225	3218(γ_{NH}), 2960(γ_{C-H}), 1661(γ_{CONH}), 1057(γ_{S-O}), 745(γ_{C-S})	--	--
IV*xxi*		OCH_3	$C_{18}H_{16}N_4O_3S_2$ (C-M)	210-212	3394(γ_{NH}), 2954(γ_{C-H}), 1659(γ_{CONH}), 1029(γ_{S-O}), 808(γ_{C-S})	--	--
IV*xxii*		H	$C_{19}H_{18}N_4O_2S_2$ (C-M)	196-199	3250(γ_{NH}), 2916(γ_{C-H}), 1651(γ_{CONH}), 1057(γ_{S-O}), 744(γ_{C-S})	--	1.68-1.71 (6H, m, C*H*$_2$ at 6, 7, & 8), 1.88 (2H, t, C*H*$_2$ at 5, *J* = 3.30), 3.30 (2H, t, C*H*$_2$ at 9, *J* = 5.32), 5.35 (2H, s, C*H*$_2$ at SCH_2), 7.20-7.69 (4H, m, *H* at imidazole), 12.30 (1H, s, N*H*), 13.20 (1H, s, N*H*)
IV*xxiii*		OCH_3	$C_{20}H_{20}N_4O_3S_2$ (C-M)	155-160	3269(γ_{NH}), 2909(γ_{C-H}), 1672(γ_{CONH}), 1048(γ_{S-O}), 804(γ_{C-S})	408, 392, 380, 363, 245, 180	--
IV*xxiv*		H	$C_{16}H_{12}N_4O_2S$ (C-M)	175-177	3059(γ_{NH}), 1676(γ_{CONH}), 1052(γ_{S-O}), 741(γ_{C-S}).	--	--
IV*xxv*		OCH_3	$C_{17}H_{14}N_4O_3S$ (C-M)	110-112	3351(γ_{NH}), 3076(γ_{C-H}), 1681(γ_{CONH}), 1029(γ_{S-O}), 776(γ_{C-S}).	--	--
IV*xxvi*		H	$C_{19}H_{15}N_5O_2S_2$ (C-M)	140-143	3345(γ_{NH}), 3030(γ_{C-H}), 1685(γ_{CONH}), 1040(γ_{S-O}), 768(γ_{C-S})	--	--

S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*xxvii*		OCH_3	$C_{20}H_{17}N_5O_3S_2$ (C-M)	160-162	3358(γ_{NH}), 2980(γ_{C-H}), 1682(γ_{CONH}), 1055(γ_{S-O}), 760(γ_{C-S})	--	--
IV*xxviii*		H	$C_{18}H_{12}N_6O_3S$ (C-M)	170-172	3059, 2909(γ_{C-H}), 1677(γ_{CONH}), 1053(γ_{S-O}), 741(γ_{C-S})	--	4.70 (2H, s, C*H*$_2$ at SCH_2), 7.02-8.06 (8H, m, Ar-*H*), 12.44 (1H, s, N*H*), 13.55 (1H, s, N*H*)
IV*xxix*		OCH_3	$C_{19}H_{14}N_6O_4S$ (C-M)	157-159	3184, 2922(γ_{C-H}), 1686(γ_{CONH}), 1061(γ_{S-O}), 779(γ_{C-S})	--	3.81 (3H, s, OC*H*$_3$), 4.65 (2H, s, C*H*$_2$ at SCH_2), 7.02-8.06 (8H, m, Ar-*H*), 12.45 (1H, s, N*H*), 13.42 (1H, s, N*H*)
IV*xxx*		H	$C_{18}H_{16}N_4O_4S$ (C-M)	122-124	2916(γ_{C-H}), 1655(γ_{CONH}), 1064(γ_{S-O}), 746(γ_{C-S})	380, 366, 351, 203, 150	--
IV*xxxi*		OCH_3	$C_{19}H_{18}N_4O_5S$ (C-M)	105-107	2916(γ_{C-H}), 1663(γ_{CONH}), 1026(γ_{S-O}),	--	--
IV*xxxii*		H	$C_{19}H_{14}N_4O_3S_2$ (C-M)	195-197	3068(γ_{C-H}), 1683(γ_{CONH}), 1022(γ_{S-O}), 739(γ_{C-S})	392, 363, 245, 180	--
IV*xxxiii*		OCH_3	$C_{20}H_{16}N_4O_4S_2$ (C-M)	125-127	2917(γ_{C-H}), 1675(γ_{CONH}), 1028(γ_{S-O}), 784(γ_{C-S})	--	--

S. No.		Y	Mol. Formula (Sol of recryst.)	M.P (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
IV*xxxiv*		H	$C_{20}H_{16}N_6O_2S_2$ (C-M)	132-134	2980(γ_{C-H}), 1670(γ_{CONH}), 1029(γ_{S-O}), 752(γ_{C-S})	--	--
IV*xxxv*		OCH_3	$C_{21}H_{18}N_6O_3S_2$ (C-M)	172-175	2923(γ_{C-H}), 1678(γ_{CONH}), 1025(γ_{S-O}), 756(γ_{C-S})	404, 270, 180	--

3.5. Spectral Discussion

3.5.1. 2-Chloromethylthieno[2,3-*d*]pyrimidines

The 2-chloromethylthienopyrimidines are colorless to buff white colored solid, with high melting points generally above 200^{o}C. These compounds are soluble in mixture of chloroform and methanol and hot DMF and practically insoluble in methanol, hexane or ethanol.

Infra red (IR) spectra:

IR spectra of 2-chloromethylthienopyrimidines exhibit bands of medium faint intensity around 3200-3100 cm^{-1} due to asymmetric and symmetric N-H stretching vibrations. Intense absorption bands observed in all these spectras around 1680-1650 may be due to N-H deformation vibrations. The IR spectra of some compounds exhibited a strong absorption band around 1730-1720 cm^{-1} due to C=O stretching. Stretching due to C-Cl was observed between 760-730 cm^{-1} (Table-8).

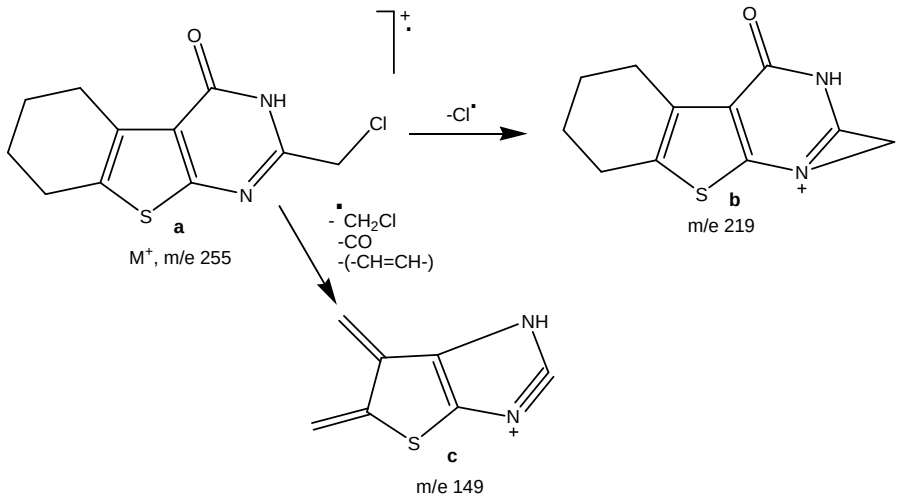
The ^{1}H NMR spectra:

The ^{1}H NMR spectra of 2-chloromethylthienopyrimidines were taken in $CDCl_3$. All the compounds showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons of the chloromethyl linkage appear downfield as a singlet at around 4.4 to 4.6 ppm. Since this methylene group is attached to electronegative atom, the proton signal appear downfield than the normal position. The NH proton present in all the compounds at the 3 position of the pyrimidine ring is observed as a singlet between 10 to 13 ppm. All the aromatic protons present in the molecules were observed as a multiplet at around 7-8 ppm (Table-8).

The Mass Spectra:

The fragmentation pattern of the synthesized compounds 2-chloromethyl-thienopyrimidines, under electron impact ionization has also been studied. It is very interesting and many prominent fragment ion peaks are revealed in the mass spectra of these compounds. The mass spectrum of 2-chloromethyl-5,6,7,8-tetrahydro-benzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **II*i*** was clearly showing the molecular ion peak (**a**) 255 m/e, which is the base peak as well. The major mode of fragmentation is loss of chloride ion to give daughter ion (**b**) m/e 219. The molecular ion (**a**) also loses

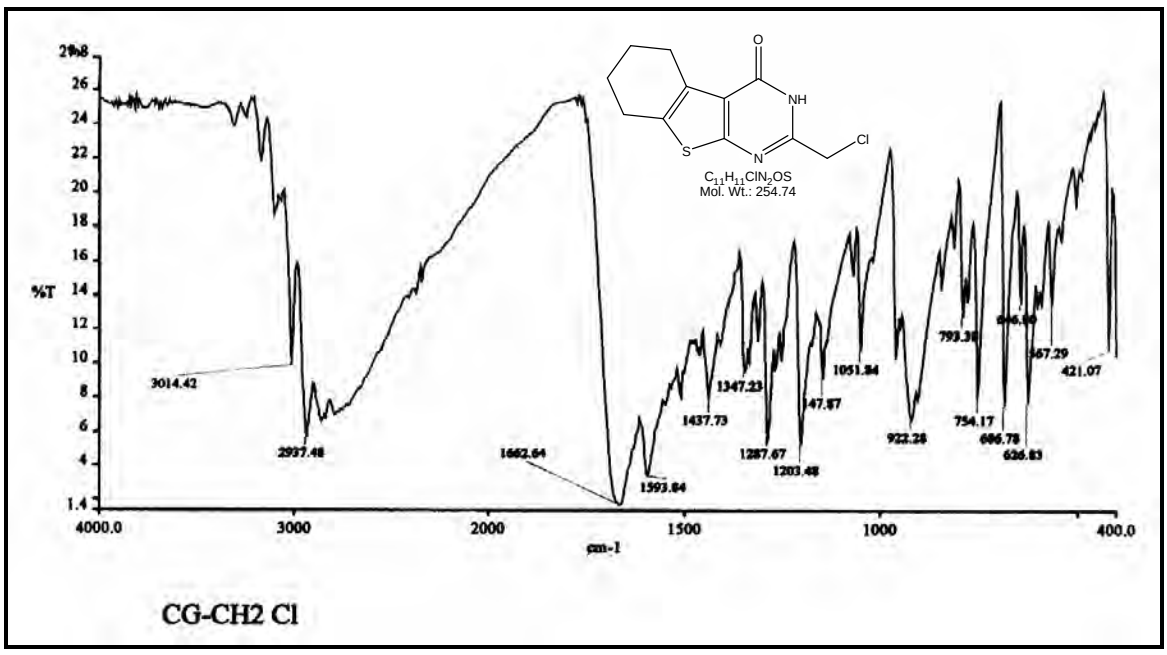
neutral CO and ethyl ion along with chloromethyl ion to give fragment (**c**) m/e 149. The fragmentation patten of compound **II*i*** was given in Scheme-23 (Table-8).



Scheme-23

Specimen IR Spectra of some 2-chloromethylthienopyrimidines:

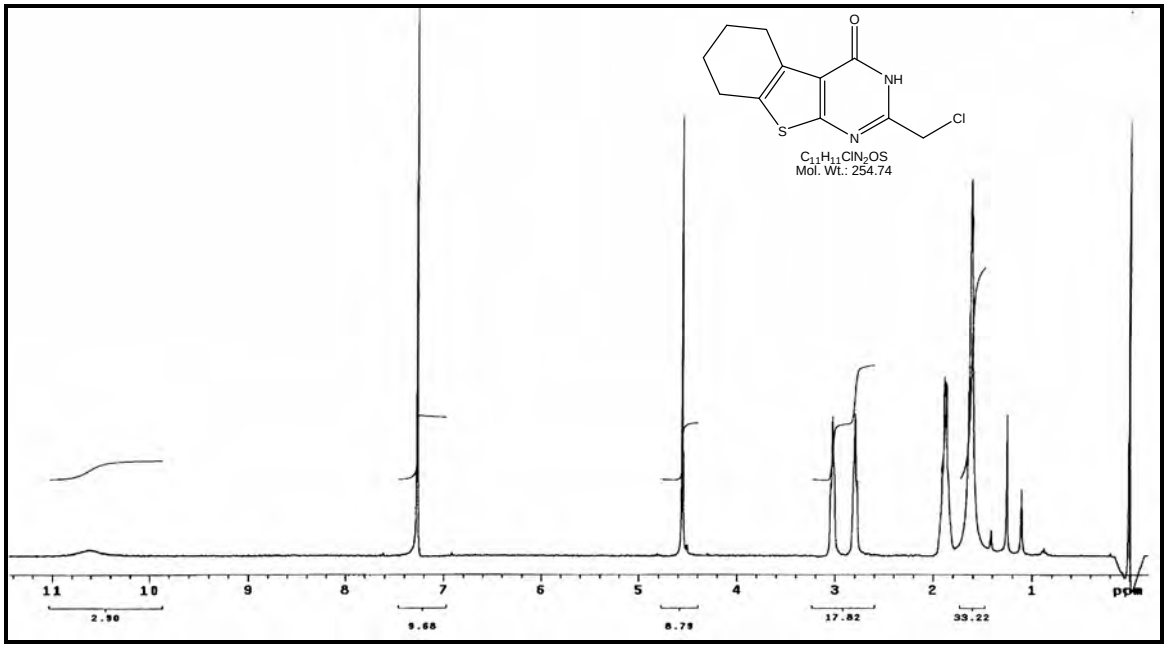
1. IR spectrum of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **II*i***



(KBr)/cm^{-1}: 3014($\gamma_{Ar\text{-}H}$), 1662(γ_{CONH}), 754($\gamma_{C\text{-}Cl}$).

Specimen ^{1}H NMR spectra of 2-chloromethylthienopyrimidines:

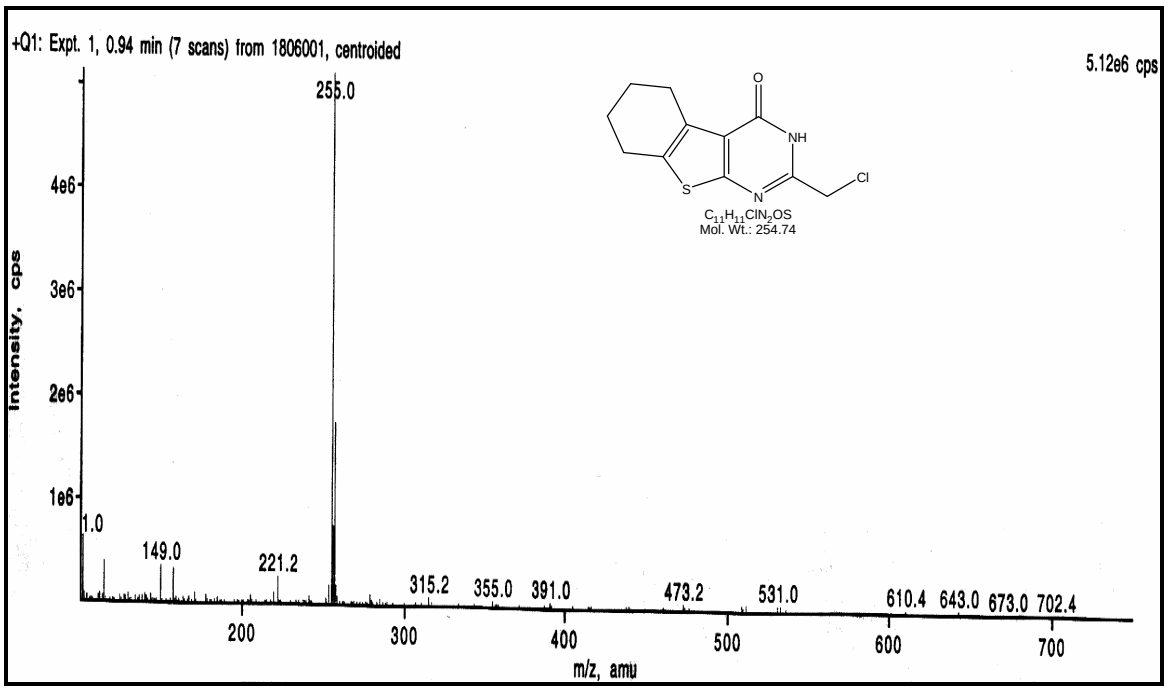
2. ^{1}H NMR spectrum of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **II*i***



^{1}H NMR ($CDCl_3$)δppm: 1.62 (4H, s, CH_2 at 6 & 7), 2.77 (2H, s, CH_2 at 4), 3.02 (2H, s, CH_2 at 8), 4.55 (2H, s, CH_2 at 2), 10.60 (1H, s, N*H* at 3).

Specimen Mass spectra of 2-chloromethylthienopyrimidines:

3. Mass spectra of 2-chloro-methyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one.



MS m/e: 255(M^+), 219, 149.

3.5.2 Condensed products of 2-chloromethylpyrimidin-4(3*H*)-ones with 2-mercapto benzimidazoles (III*i-xxxv*)

The condensed products of 2-chloromethylpyrimidines with 2-mercaptobenzimidazole and 2-mercapto-5-methoxybenzimidazole are colorless to buff white colored solid, with high melting points generally above 250^{o}C but some of the compounds melt in the range of 210-220^{o}C. These compounds are soluble in mixture of MDC and methanol and chloroform and methanol, practically insoluble in methanol, ethanol and hexane (Table-9).

Infra red (IR) spectra:

IR spectra of all the thio compounds reveal characteristic $\gamma_{(C-S)}$ bands due to stretching vibrations in between the regions of 700-600 cm^{-1}. Besides these the usual bands are observed in the IR spectra of all the compounds, characteristic of $\gamma_{(C-H),}$ around 3030 to 2890 cm^{-1}, as well as bands corresponding to $\gamma_{(NH)}$ and $\gamma_{(CONH)}$ between 1680-1575 cm^{-1} (Table-9).

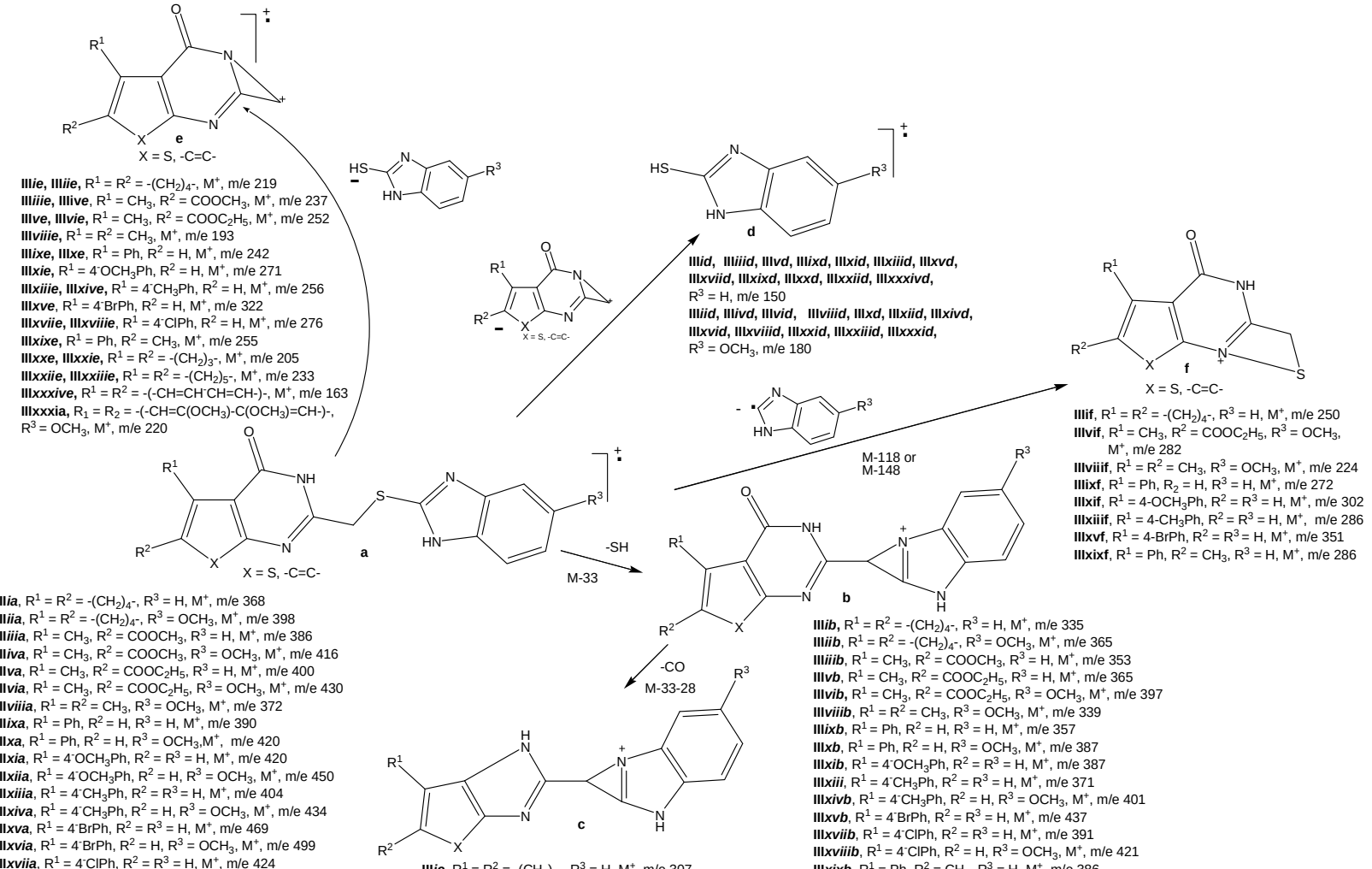
The ^{1}H NMR spectra:

The ^{1}H NMR spectra of condensed thio derivatives were taken in DMSO-d_6. All the compounds showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons of the thiomethyl linkage appear as a singlet at around 4.35 to 4.40 ppm. Since this methylene group is attached to electronegative atom, the proton signal appear downfield than its normal position. The NH protons present in these compounds at the 3 position of the pyrimidine ring and on the benzimidazole ring were observed much downfield, falling in the range of 12-14 ppm. However, in some of the compounds the NH protons are not seen in the spectra this is probably due to very faint or broad peck of these protons. All the aromatic protons present in the molecules were observed as a multiplet at 7-8 ppm (Table-9).

The Mass spectra:

The fragmentation pattern of the condensed thio derivatives under electron impact has also been studied. The condensed product exhibits some common fragmentation pathways. Prominent molecular ion peaks were observed in most of the compounds. In some of the spectra, (M+1) and (M+2) peaks were also observed. The common fragmentation pattern is depicted in Scheme-24. This decomposition of molecular ion (**a**)

involves loss of SH to give the daughter ion (**b**). Subsequent, loss of neutral CO from the daughter ion (**b**) gives the fragment (**c**). Further, breaking of carbon and sulfur bond in the molecular ion (**a**) gives two daughter ions (**d**) and (**e**). In some compounds, molecular ion (**a**) gives daughter ion (**f**) by the loss of benzimidazole ion. The fragmentation pattern of these compounds complies with the assigned structure to the compounds (Table-9).



IIIie, **IIIiie**, $R^1 = R^2 = -(CH_2)_4-$, M^+, m/e 219
IIIiiie, **IIIive**, $R^1 = CH_3$, $R^2 = COOCH_3$, M^+, m/e 237
IIIve, **IIIvie**, $R^1 = CH_3$, $R^2 = COOC_2H_5$, M^+, m/e 252
IIIviiie, $R^1 = R^2 = CH_3$, M^+, m/e 193
IIIixe, **IIIxe**, $R^1 = Ph$, $R^2 = H$, M^+, m/e 242
IIIxie, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = H$, M^+, m/e 271
IIIxiiie, **IIIxive**, $R^1 = 4\text{-}CH_3Ph$, $R^2 = H$, M^+, m/e 256
IIIxve, $R^1 = 4\text{-}BrPh$, $R^2 = H$, M^+, m/e 322
IIIxvie, **IIIxviiie**, $R^1 = 4\text{-}ClPh$, $R^2 = H$, M^+, m/e 276
IIIxixe, $R^1 = Ph$, $R^2 = CH_3$, M^+, m/e 255
IIIxxe, **IIIxxie**, $R^1 = R^2 = -(CH_2)_3-$, M^+, m/e 205
IIIxxiie, **IIIxxiiie**, $R^1 = R^2 = -(CH_2)_5-$, M^+, m/e 233
IIIxxxive, $R^1 = R^2 = -(-CH=CH-CH=CH-)-$, M^+, m/e 163
IIIxxxia, $R_1 = R_2 = -(-CH=C(OCH_3)-C(OCH_3)=CH-)-$, $R^3 = OCH_3$, M^+, m/e 220

IIIid, **IIIiiid**, **IIIvd**, **IIIixd**, **IIIxid**, **IIIxiiid**, **IIIxvd**, **IIIxviid**, **IIIxixd**, **IIIxxd**, **IIIxxiid**, **IIIxxxivd**, $R^3 = H$, m/e 150
IIIiid, **IIIivd**, **IIIvid**, **IIIviiid**, **IIIxd**, **IIIxiid**, **IIIxivd**, **IIIxvid**, **IIIxviiid**, **IIIxxid**, **IIIxxiiid**, **IIIxxxid**, $R^3 = OCH_3$, m/e 180

IIIif, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = H$, M^+, m/e 250
IIIvif, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R^3 = OCH_3$, M^+, m/e 282
IIIviiif, $R^1 = R^2 = CH_3$, $R^3 = OCH_3$, M^+, m/e 224
IIIixf, $R^1 = Ph$, $R_2 = H$, $R^3 = H$, M^+, m/e 272
IIIxif, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 302
IIIxiiif, $R^1 = 4\text{-}CH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 286
IIIxvf, $R^1 = 4\text{-}BrPh$, $R^2 = R^3 = H$, M^+, m/e 351
IIIxixf, $R^1 = Ph$, $R^2 = CH_3$, $R^3 = H$, M^+, m/e 286

IIIia, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = H$, M^+, m/e 368
IIIiia, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = OCH_3$, M^+, m/e 398
IIIiiia, $R^1 = CH_3$, $R^2 = COOCH_3$, $R^3 = H$, M^+, m/e 386
IIIiva, $R^1 = CH_3$, $R^2 = COOCH_3$, $R^3 = OCH_3$, M^+, m/e 416
IIIva, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R^3 = H$, M^+, m/e 400
IIIvia, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R^3 = OCH_3$, M^+, m/e 430
IIIviiia, $R^1 = R^2 = CH_3$, $R^3 = OCH_3$, M^+, m/e 372
IIIixa, $R^1 = Ph$, $R^2 = H$, $R^3 = H$, M^+, m/e 390
IIIxa, $R^1 = Ph$, $R^2 = H$, $R^3 = OCH_3$,M^+, m/e 420
IIIxia, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 420
IIIxiia, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 450
IIIxiiia, $R^1 = 4\text{-}CH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 404
IIIxiva, $R^1 = 4\text{-}CH_3Ph$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 434
IIIxva, $R^1 = 4\text{-}BrPh$, $R^2 = R^3 = H$, M^+, m/e 469
IIIxvia, $R^1 = 4\text{-}BrPh$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 499
IIIxviia, $R^1 = 4\text{-}ClPh$, $R^2 = R^3 = H$, M^+, m/e 424
IIIxviiia, $R^1 = 4\text{-}ClPh$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 454
IIIxixa, $R^1 = Ph$, $R^2 = CH_3$, $R^3 = H$, M^+, m/e 404
IIIxxa, $R^1 = R^2 = -(CH_2)_3-$, $R^3 = H$, M^+, m/e 354
IIIxxia, $R^1 = R^2 = -(CH_2)_3-$, $R^3 = OCH_3$, M^+, m/e 384
IIIxxiia, $R^1 = R^2 = -(CH_2)_5-$, $R^3 = H$, M^+, m/e 382
IIIxxiiia, $R^1 = R^2 = -(CH_2)_5-$, $R^3 = OCH_3$, M^+, m/e 412
IIIxxxiva, $R^1 = R^2 = -(-CH=CH-CH=CH-)-$, $R^3 = H$, M^+, m/e 308
IIIxxxia, $R_1 = R_2 = -(-CH=C(OCH_3)-C(OCH_3)=CH-)-$, $R^3 = OCH_3$, M^+, m/e 398

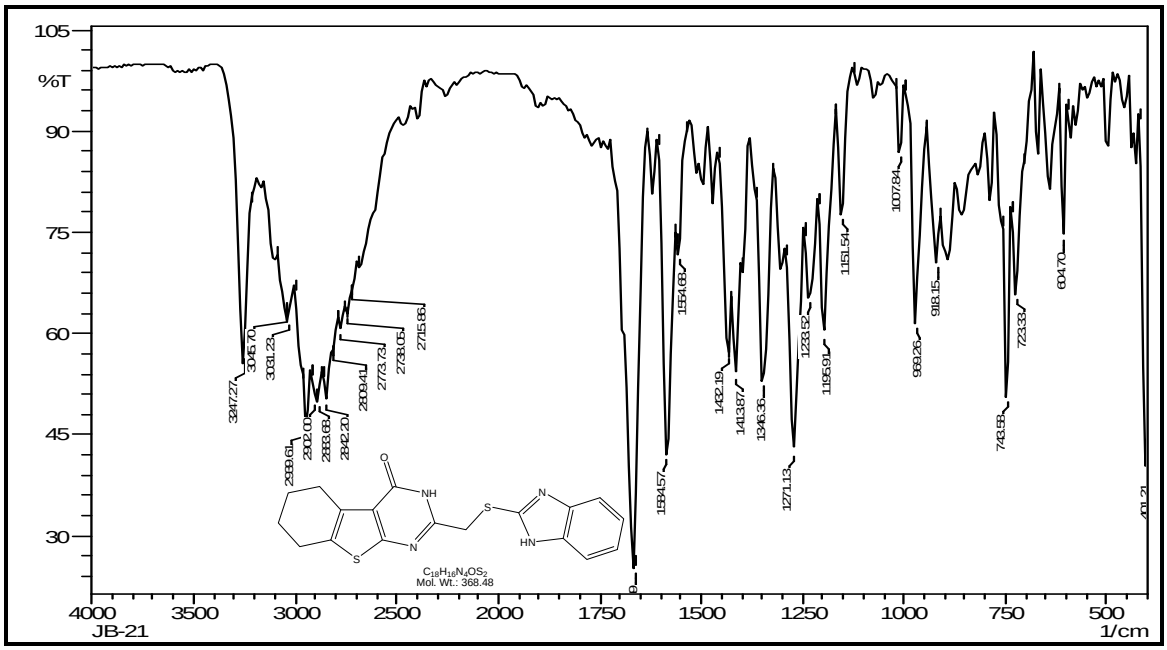
IIIib, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = H$, M^+, m/e 335
IIIiib, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = OCH_3$, M^+, m/e 365
IIIiiib, $R^1 = CH_3$, $R^2 = COOCH_3$, $R^3 = H$, M^+, m/e 353
IIIvb, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R^3 = H$, M^+, m/e 365
IIIvib, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R^3 = OCH_3$, M^+, m/e 397
IIIviiib, $R^1 = R^2 = CH_3$, $R^3 = OCH_3$, M^+, m/e 339
IIIixb, $R^1 = Ph$, $R^2 = H$, $R^3 = H$, M^+, m/e 357
IIIxb, $R^1 = Ph$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 387
IIIxib, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 387
IIIxiii, $R^1 = 4\text{-}CH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 371
IIIxivb, $R^1 = 4\text{-}CH_3Ph$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 401
IIIxvb, $R^1 = 4\text{-}BrPh$, $R^2 = R^3 = H$, M^+, m/e 437
IIIxviib, $R^1 = 4\text{-}ClPh$, $R^2 = R^3 = H$, M^+, m/e 391
IIIxviiib, $R^1 = 4\text{-}ClPh$, $R^2 = H$, $R^3 = OCH_3$, M^+, m/e 421
IIIxixb, $R^1 = Ph$, $R^2 = CH_3$, $R^3 = H$, M^+, m/e 386
IIIxxb, $R^1 = R^2 = -(CH_2)_3-$, $R^3 = H$, M^+, m/e 321
IIIxxbi, $R^1 = R^2 = -(CH_2)_3-$, $R^3 = OCH_3$, M^+, m/e 351
IIIxxiib, $R^1 = R^2 = -(CH_2)_5-$, $R^3 = H$, M^+, m/e 349
IIIxxiiib, $R^1 = R^2 = -(CH_2)_5-$, $R^3 = OCH_3$, M^+, m/e 379
IIIxxxivb, $R^1 = R^2 = -(-CH=CH-CH=CH-)-$, $R^3 = H$, M^+, m/e 275
IIIxxxib, $R_1 = R_2 = -(-CH=C(OCH_3)-C(OCH_3)=CH-)-$, $R^3 = OCH_3$, M^+, m/e 365

IIIic, $R^1 = R^2 = -(CH_2)_4-$, $R^3 = H$, M^+, m/e 307
IIIiiic, $R^1 = CH_3$, $R^2 = COOCH_3$, $R^3 = H$, M^+, m/e 325
IIIvc, $R^1 = CH_3$, $R^2 = COOC_2H_5$, M^+, m/e 339
IIIvic, $R^1 = CH_3$, $R^2 = COOC_2H_5$, $R_3 = OCH_3$, M^+, m/e 369
IIIviiic, $R^1 = R^2 = CH_3$, $R_3 = OCH_3$, M^+, m/e 311
IIIxic, $R^1 = 4\text{-}OCH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 359
IIIxiiic, $R^1 = 4\text{-}CH_3Ph$, $R^2 = R^3 = H$, M^+, m/e 343
IIIxixc, $R^1 = Ph$, $R^2 = CH_3$, $R^3 = H$, M^+, m/e 343
IIIxxc, $R^1 = R^2 = -(CH_2)_3-$, $R_3 = H$, M^+, m/e 393
IIIxxiic, $R^1 = R^2 = -(CH_2)_5-$, $R_3 = H$, M^+, m/e 321

Scheme-24: General fragmentation pattern of condensed pyrimidinylmethylthiobenzimidazoles (**III*i-xxxv***) synthesized

Specimen IR spectrum of condesed derivatives of substituted 2-chloromethylthieno-pyrimidines and 2-mercaptobenzimidazoles.

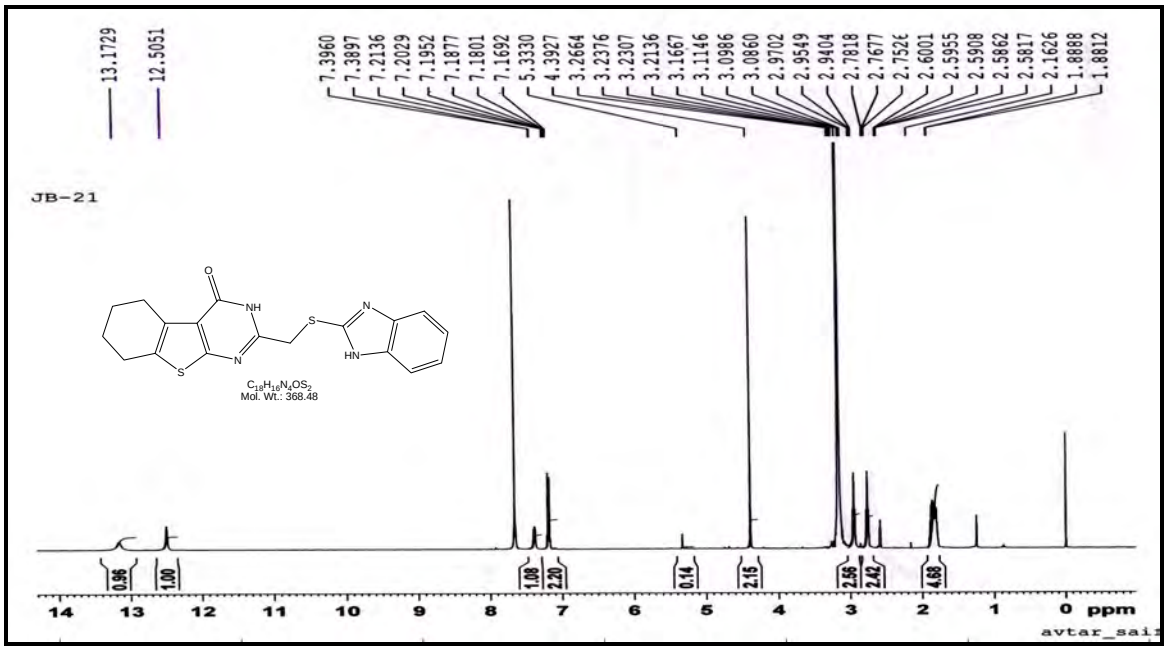
1. IR spectrum of 2-(1*H*-benzimidazol-2-yl)methylthio-5,6,7,8-tetrahydrobenzo(*b*)-thieno[2,3-*d*]pyrimidin-4-(3*H*)-one **III*i***



IR (KBr) cm^{-1}: 3247(γ_{NH}), 2939(γ_{C-H}), 1680(γ_{CONH}), 743(γ_{C-S}).

Specimen ^{1}H NMR spectrum of condesed derivatives of substituted 2-chloromethyl-thienopyrimidines and 2-mercaptobenzimidazoles.

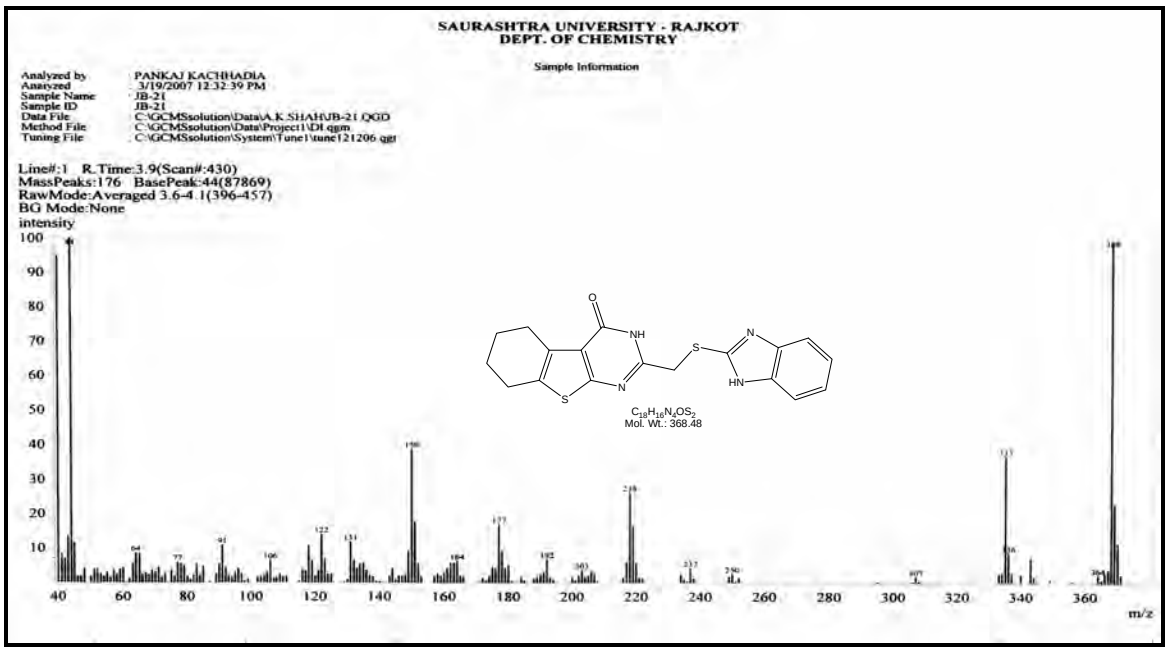
2. ^{1}HNMR spectrum of 2-(1*H*-benzimidazol-2-yl)methylthio-5,6,7,8-tetrahydrobenzo-(*b*)thieno[2,3-*d*]pyrimidin-4-(3*H*)-one **(III*i*)**



^{1}HNMR (DMSO-d_6)δppm: 1.83-1.88 (4H, m, C*H*$_2$ at 6 & 7), 2.76 (2H, t, C*H*$_2$ at 5, *J* = 5.64), 2.95 (2H, t, C*H*$_2$ at 8, *J* = 5.80), 4.39 (2H, s, CH$_2$ at SC*H*$_2$), 7.39-7.16 (4H, m, Ar-*H*), 12.50 (1H, s, N*H*), 13.17 (1H, s, N*H*).

Specimen Mass spectrum of condesed derivatives of substituted 2-chloro-methyl-thienopyrimidines and 2-mercaptobenzimidazoles.

3. Mass spectrum of 2-(1*H*-benzimidazol-2-yl)methylthio-5,6,7,8-tetrahydrobenzo(*b*)-thieno[2,3-*d*]pyrimidin-4-(3*H*)-one **(III*i*)**



MS m/e: 368(M$^+$), 335, 307, 150.

3.5.3 Spectral discussion of 5,6-disubstituted-2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (IV*i-xxxv)*

The selective oxidised products of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one are colorless to buff white colored solid, with the exception of **IV*xxi*** which is slightly orange colored solid with much lower melting points then the corresponding thio derivatives. However, melting point of some of the product is higher than the thio derivatives. These compounds are soluble in mixture of MDC/methanol and chloroform/methanol, practically insoluble in ethanol, methanol and hexane (Table-10).

The Infrared (IR) spectra:

IR spectra of all the oxidized (sulfinyl) compounds reveal characteristic $\gamma_{(S=O)}$ bands due to stretching vibrations in the regions of 1070-1030 cm^{-1}. These stretching vibrations are prominent in all spectrums. Additionally, stretching vibrations characteristic of $\gamma_{(C-S)}$ were observed in all the spectrums in the range of 700-600 cm^{-1}. Besides these, the usual bands are observed in the IR spectra of all the compounds, characteristic of $\gamma_{(C-H),}$ around 3030 to 2890 cm^{-1}, as well as bands corresponding to $\gamma_{(NH)}$ and $\gamma_{(CONH)}$ between 1680-1575 cm^{-1} (Table-10).

The ^{1}H NMR spectra:

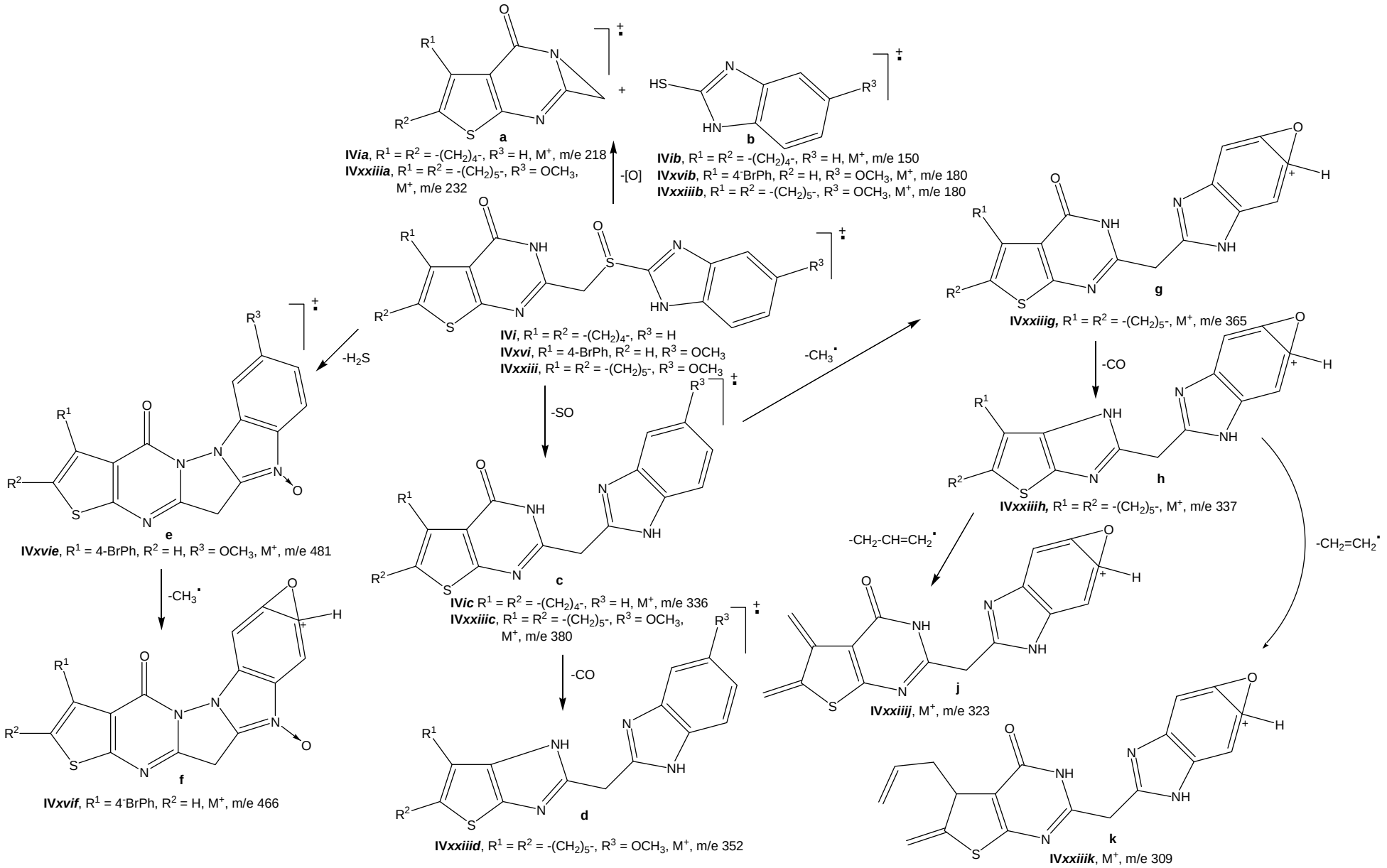
The ^{1}H NMR spectra of oxidized (sulfinyl) derivatives were taken in DMSO-d_6. These compounds showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons of the sulfinylmethyl linkage appear as a singlet at around 5.5-5.6 ppm, while the same protons were observed in thio derivatives in the range of 4.35 to 4.40 ppm. This downfield shifting of these protons is possibly due to attachment of additional electronegative atom (oxygen) to the neighboring sulfur atom. The NH protons present in these compounds at the 3 position of the pyrimidine ring and on the benzimidazole ring were observed much downfield, falling in the range of 12-14 ppm. However, in some of the compounds the NH protons are not seen in the spectra probably due to very faint or broad pecks. All the aromatic protons present in the molecules were observed as a multiplet at 7-8 ppm (Table-10).

The Mass spectra:

The fragmentation pattern of the condensed sulfinyl derivatives under electron impact has been studied. Though, the electron impact is a standard procedure for the ionizations of molecules in mass spectroscopy, this technique sometime have disadvantage, when it results in the disappearance of the molecular ion peak so that the molecular weight of the analyte cannot be established.[44] In the mass spectrum of sulfiny derivatives, the molecular ion peak was not observed. This is may be due the very labile nature of the oxygen atom attached to the sulfur atom. The fragmentation pattern of these molecules is almost same as observed for corresponding thio derivatives (Table-10).

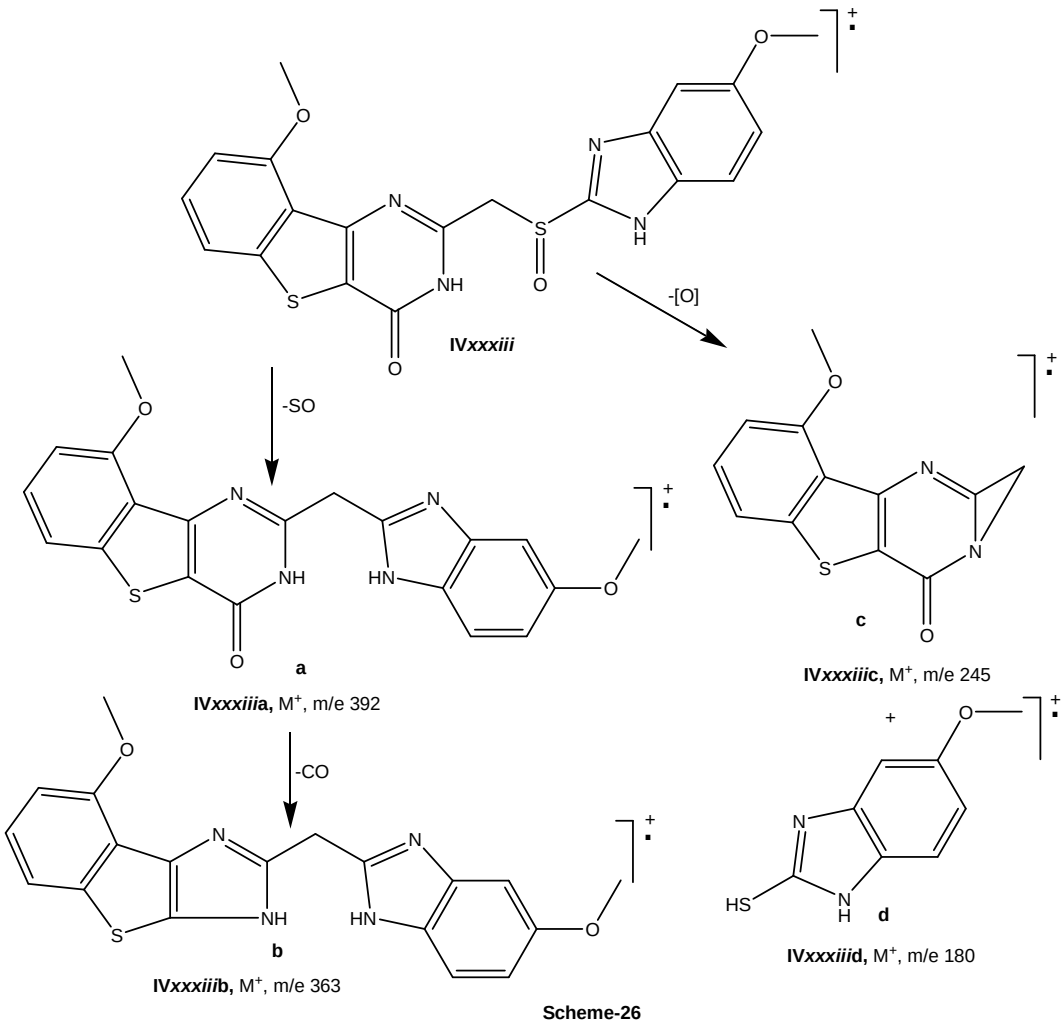
The common fragmentation pattern is depicted in Scheme-25. This decomposition of molecular ion involves loss of SO to give the daughter ion (**c**). Subsequently, loss of

neutral CO from the daughter ion (**c**) gives the fragment (**d**) and loses of methyl radical gives fragment (**g**). Fragment (**g**) loses neutral CO molecule to give fragment (**h**). This fragment subsequently gives fragment (**j**) and (**k**) by the lose of propylene and ethylene ion, respectively. In some of the spectra, lose of H_2S from the molecular ion has also been detected to give the fragment (**e**), which further loses methyl radical to give fragment (**f**). Breaking of carbon/sulfur bond in the molecular ion and subsequent lose of oxygen radical gives two daughter ions (**a**) and (**b**). The fragmentation pattern of these compounds compliance with the assigned structure to the compounds (Scheme-25).



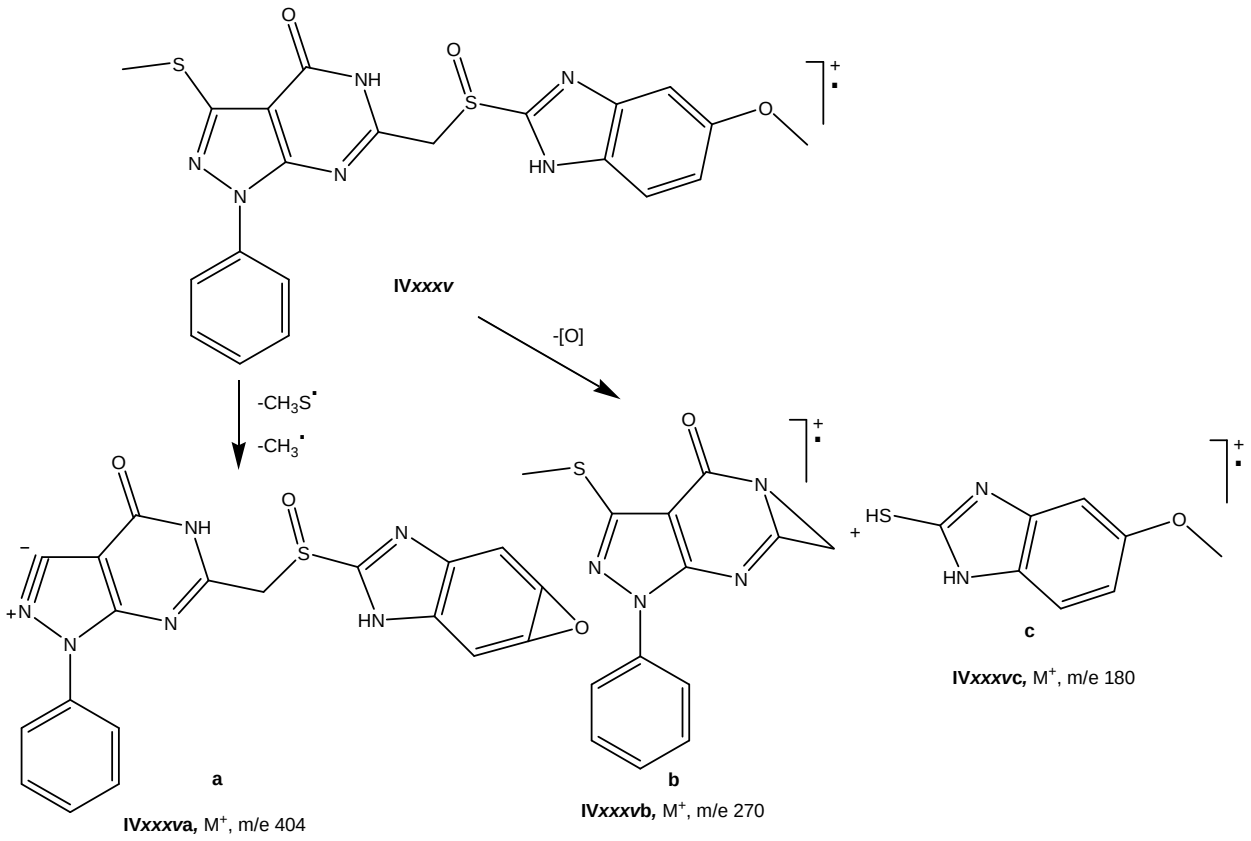
Scheme-25 General fragmentation pattern of condensedpyrimidinylmethylsulfinylbenzimidazoles (**IV*i-xxxv***) synthesized

The fragmentation pattern of the compound **IV*xxxiii*** is depicted in Scheme-26. Decomposition of molecular ion involves neutral loss of SO to give the daughter ion (**a**) at m/e 392. The daughter ion (**a**) further loses neutral CO molecule to give fragment (**b**) at m/e 363. Molecular ion also loses single oxygen atom to yield daughter ion (**c**) at m/e 245. The prominent daughter ion (**d**) at m/e 180 is attributed to the starting material, 2-mercapto-5-methoxybenzimidazole as a radical cation. The fragmentation pattern compliance with the structure assigned to the compound (Table-10).



Scheme-26

The fragmentation pattern of the compound **IV*xxxv*** is depicted in Scheme-27. Decomposition of molecular ion involves loss of thiomethyl and methyl radicals to give the daughter ion (**a**) at m/e 404. The molecular ion also ejects an oxygen atom to give daughter ion (**b**) at m/e 270. The prominent daughter ion (**c**) at m/e 180 is attributed to the starting material, 2-mercapto-5-methoxybenzimidazole as a radical cation. The fragmentation pattern compliance with the structure assigned to the compound (Scheme-27) (Table-10).



IV*xxxva,* M^+, m/e 404

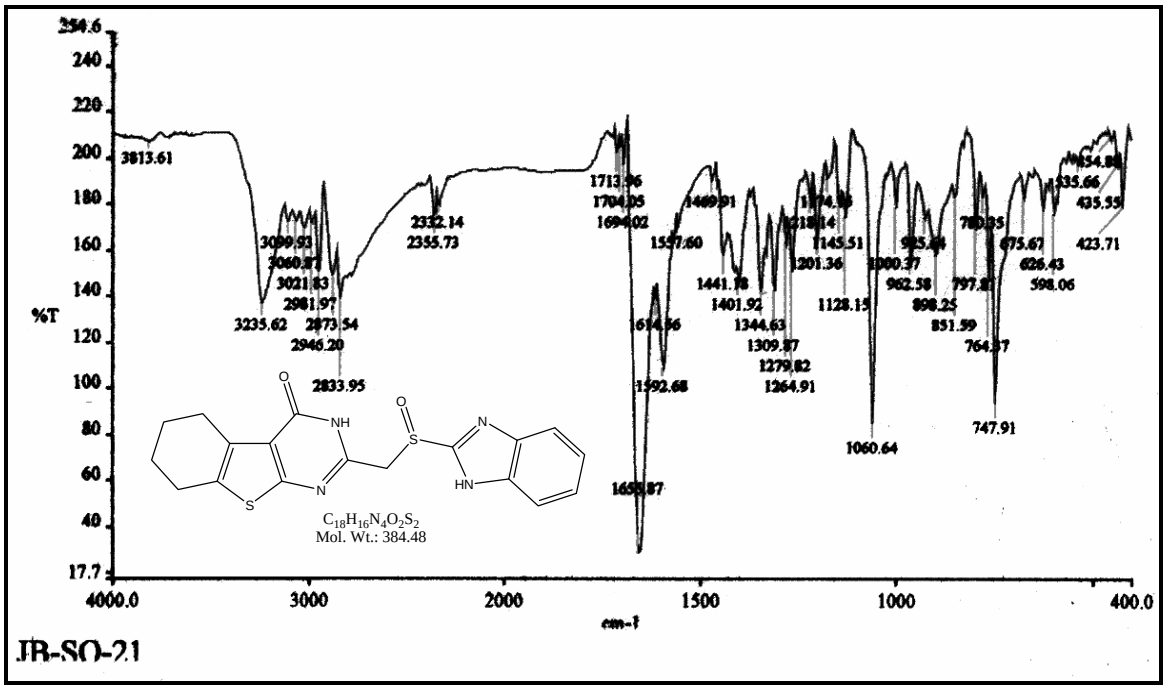
IV*xxxvb,* M^+, m/e 270

IV*xxxvc,* M^+, m/e 180

Scheme-27

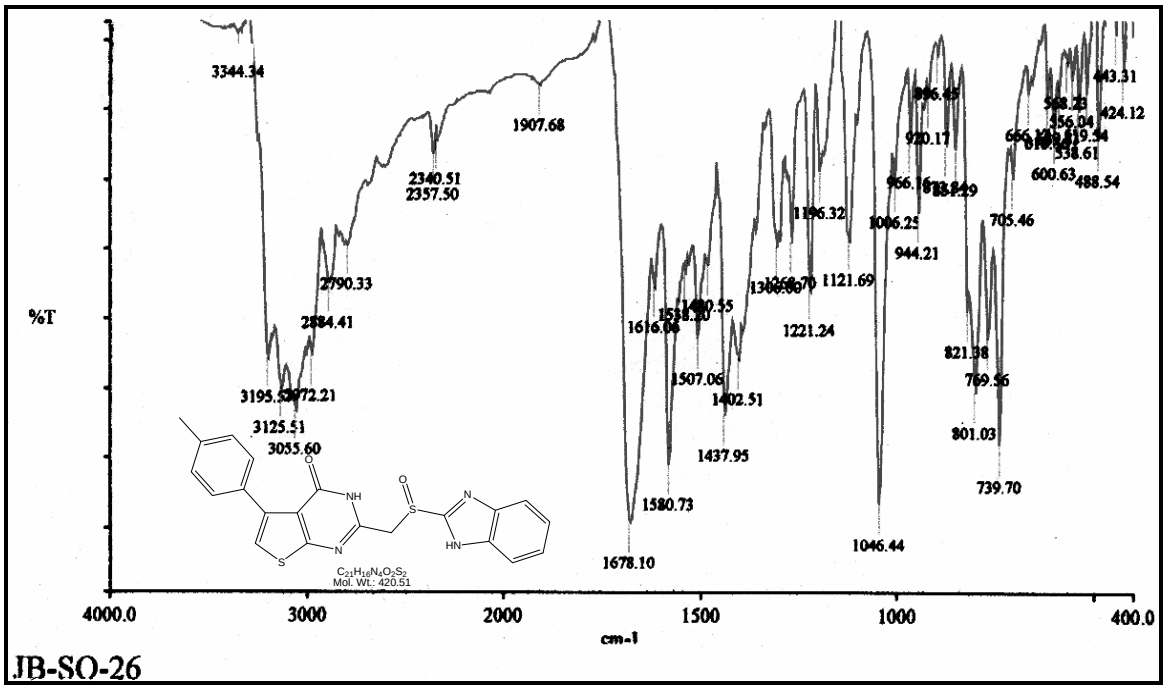
Specimen IR spectrum of some 5,6-disubstituted 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)-methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones:

1. IR spectrum of 2-(1*H*-benzimidazol-2-yl)methylsulfinyl-5,6,7,8-tetrahydrobenzo(*b*)-sulfinyl[2,3-*d*]pyrimidin-4-(3*H*)-one **(Iv*i*)**



IR (KBr) cm^{-1}: 3235(γ_{NH}), 2946(γ_{C-H}), 1655(γ_{CONH}). 1060($\gamma_{S=O}$), 747(γ_{C-S}).

2. IR spectrum of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-(4-methylphenyl)thieno-[2,3-*d*]pyrimidin-4(3*H*)-one **(Iv*xiii*)**



IR (KBr) cm^{-1}: 3195(γ_{NH}), 3055(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 739(γ_{C-S}).

3.6 Biological Evaluation of PPI's

3.6.1 Acute toxicity study (LD_{50} determination)

The acute oral toxicity study of newly synthesized derivatives was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) guideline 423. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401.

Principle of the test:

The principle of the test based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, *i.e.*

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

Description of the method:

The acute toxic class method/OECD423[45] set out in this guideline is a stepwise procedure with the use of three animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The acute toxic class method is based on biometric evaluations[46] with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD_{50} data obtained from the literature, both nationally and internationally.

The preferred rodent species is the rat, although other rodent species may be used. Normally females are used (OECD, 2000). Females were nulliparous and non-pregnant. The animals were randomly selected, marked to permit individual identification, and kept

in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substances were administered in a single dose by gavage using a stomach tube. Animals were fasted prior to dosing, following fasting period, the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3-4 h in rats.

The literature survey showed that LD_{50} value of omeprazole and related derivatives were calculated starting from the higher level dose *i.e* 2000 mg/kg of body weight or even higher dosages.[47] Hence, limit test for newly synthesized derivatives was conducted at the highest starting dose level 3000 mg/kg of body weight.

Observations:

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 h. In all cases, no death was observed during the whole study period. Additional observations like changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern were also observed during the period. Attention was also given to observation of tremors and convulsions. A dose of 30 mg/kg of the body weight was selected for the pylorus ligation in rat model.

3.6.2 Various methods for the evaluation of PPI's

1. Studies on Isolated Guinea Pig Mucosa[48]

Isolated guinea pig mucosa is mounted on a plastic funnel with the mucosal surface facing the tube lumen. Each preparation is immersed in an organ bath containing 40 ml of serosal solution having the different compositions.

Measurement of H^+ secretion: This is performed by continuous titration using a radiometer (Copenhagen, Denmark) pH-stat (pHM 82, TTT 80) and Autoburette (ABU 80).

Measurement of K^+ secretion: K^+ content of mucosal solution is determined on a flame-emission photometer.

Experiments with simultaneous measurements of K^+ and H^+ secretion: Histamine is added to serosal solutions followed by sample solutions and secretion rates are calculated.

2. Effect of H^+/K^+ ATPase inhibitors on serum gastrin levels[49]

Female wistar rats are treated with the H^+/K^+ATPase inhibitors to cause gastric inhibition. Blood samples are collected and gastrin is determined by radio-immunoassay using a commercially available kit. At the end of the study of 10 weeks, the animals are studied for their gastric acid output using pylorus ligation (Shay technique).

3. Pylorus Ligation in rats (Shay *et al.*)[49,50]

A simple and reliable method for production of gastric ulceration in the rat based on the ligature of the pylorus has been published by Shay *et al.*[51] The ulceration is caused by accumulation of acidic gastric juice in the stomach. The intensity of ulceration is expressed in terms of ulcer index.

Of all the different methods and models discussed above, the "Pylorus Ligation in rats method", reported by Shay *et al.*[51] appeared more acceptable for preliminary test and this is a more practical method with respect to the availability of equipment and infrastructure with us. The detailed procedure is as follows:

3.6.3 Adopted procedure:

The antiulcer activity was evaluated in wistar rats of either sex (200-250 gm). The animals were divided in thirty five groups of 5 rats each. The thirty five groups were as follows;

Group I : Treated with 1% Acacia (0.4 ml/kg, *p.o,*): Control Group.
Group II : Treated with Omeprazole (30 mg/kg, *p.o,*): Standard
Group III : Treated with Compound **IV*i*** (30 mg/kg, *p.o,*)
Group IV : Treated with Compound **IV*ii*** (30 mg/kg, *p.o,*)
Group V : Treated with Compound **IV*iii*** (30 mg/kg, *p.o,*)
Group VI : Treated with Compound **IV*iv*** (30 mg/kg, *p.o,*)
Group VII : Treated with Compound **IV*v*** (30 mg/kg, *p.o,*)
Group VIII : Treated with Compound **IV*vi*** (30 mg/kg, *p.o,*)
Group IX : Treated with Compound **IV*vii*** (30 mg/kg, *p.o,*)

Group X : Treated with Compound **IV*viii*** (30 mg/kg, *p.o*,)
Group XI : Treated with Compound **IV*ix*** (30 mg/kg, *p.o*,)
Group XII : Treated with Compound **IV*x*** (30 mg/kg, *p.o*,)
Group XIII : Treated with Compound **IV*xi*** (30 mg/kg, *p.o*,)
Group XIV : Treated with Compound **IV*xii*** (30 mg/kg, *p.o*,)
Group XV : Treated with Compound **IV*xiii*** (30 mg/kg, *p.o*,)
Group XVI : Treated with Compound **IV*xiv*** (30 mg/kg, *p.o*,)
Group XVII : Treated with Compound **IV*xv*** (30 mg/kg, *p.o*,)
Group XVIII : Treated with Compound **IV*xvi*** (30 mg/kg, *p.o*,)
Group XIX : Treated with Compound **IV*xvii*** (30 mg/kg, *p.o*,)
Group XX : Treated with Compound **IV*xviii*** (30 mg/kg, *p.o*,)
Group XXI : Treated with Compound **IV*xix*** (30 mg/kg, *p.o*,)
Group XXII : Treated with Compound **IV*xx*** (30 mg/kg, *p.o*,)
Group XXIII : Treated with Compound **IV*xxi*** (30 mg/kg, *p.o*,)
Group XXIV : Treated with Compound **IV*xxii*** (30 mg/kg, *p.o*,)
Group XXV : Treated with Compound **IV*xxiii*** (30 mg/kg, *p.o*,)
Group XXVI : Treated with Compound **IV*xxiv*** (30 mg/kg, *p.o*,)
Group XXVII : Treated with Compound **IV*xxv*** (30 mg/kg, *p.o*,)
Group XXVIII: Treated with Compound **IV*xxviii*** (30 mg/kg, *p.o*,)
Group XXIX : Treated with Compound **IV*xxix*** (30 mg/kg, *p.o*,)
Group XXX : Treated with Compound **IV*xxx*** (30 mg/kg, *p.o*,)
Group XXXI : Treated with Compound **IV*xxxi*** (30 mg/kg, *p.o*,)
Group XXXII : Treated with Compound **IV*xxxii*** (30 mg/kg, *p.o*,)
Group XXXIII: Treated with Compound **IV*xxxiii*** (30 mg/kg, *p.o*,)
Group XXXIV: Treated with Compound **IV*xxxiv*** (30 mg/kg, *p.o*,)
Group XXXV : Treated with Compound **IV*xxxv*** (30 mg/kg, *p.o*,)

Materials & Methods:

Wistar rats (each weighing 200-250 gm) of either sex was used for this experimental study. The animals were housed in standard metal cages and provided with food and water. Food was withdrawn 24 hrs before the study. However, water was allowed *ad libitum*.

Drug Preparation & Treatment:

Omeprazole (30 mg/kg) & test compounds were suspended in 1% suspension of acacia in distilled water and administered by oral route.

The animals were fasted for 24 hrs prior the experiment, but had free access to water. After the fasting period, the animals were given the drug samples *p.o,* 1 hr. prior the ligation. Thereafter, the rats were anaesthetized with anesthetic ether. After an hour, each of the rats was secured on the operating table. An incision of 1 cm length in the abdomen just below the sternum was made. The stomach was exposed. A thread was passed around the pyloric sphincter and a light knot was applied to it taking due care that no blood vessel was tied along with the knot. After this, the incision was closed by stitching the abdominal wall by a thread. The underlying skin was cleaned of any bleeding *etc.* An antiseptic cream was applied over the wound. Thereafter, the animal was kept in a separate cage and allowed to recover.

After 24 hrs, these animals were sacrificed and the stomach of each of the animals was isolated and cut open through its greater curvature. The gastric contents were carefully removed.

Following parameters were studied

1. Volume of gastric juice secreted: The volume of gastric juice was measured and centrifuged at 1000 rpm for 10 min.
2. Determination of total acidity of the gastric juice: From the supernatant, aliquots (1 ml of each) were taken for the determination of total acidity.
3. The ulcer index: gastric mucosa was also examined for ulcers.
4. The pH of the gastric secretion was measured by digital pH meter (Equipt-Tronics Digital pH meter, Model EQ-610).

Determination of total acidity: An aliquot of 1 ml of gastric juice was taken in a 50 ml conical flask then dilute with distill water to make volume 10 ml and 2 drops of phenolphthalein indicator was added to it. It was further titrated with 0.01 N NaOH until a permanent pale pink color was developed.

The volume of alkali consumed was noted. The total acidity is expressed as mEq./lt/gm by the following formula:

Total Acidity = Vol. of NaOH consumed x N x 100 / 0.1 (mEq/100gm)

Where;

N = Normality of NaOH

The Ulcer Score: The gastric mucosa was examined for ulcers by magnifying lens and the ulcer scored according to its severity in comparison with that of the Ulcer in the standard group. Ulcer score was recorded as follows;

0 = Normal, no Ulcer

0.5 = Red coloration

1 = Spot ulcer

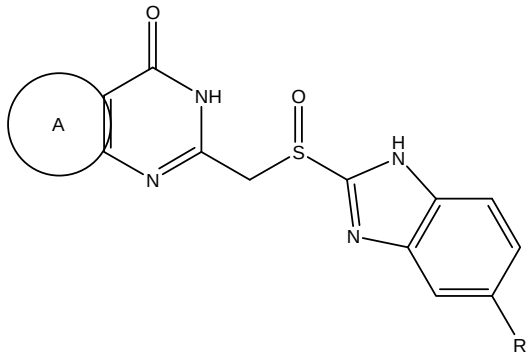
1.5 = Haemorrhagic breaks

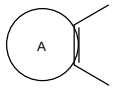
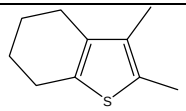
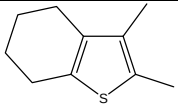
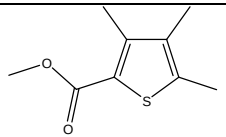
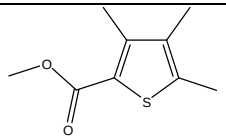
2 = Ulcer $\geq$ 3 but $\leq$ 5

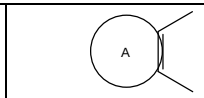
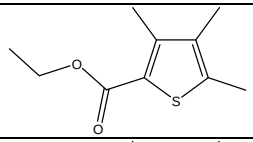
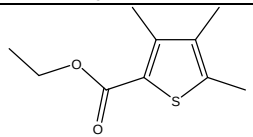
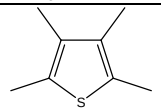
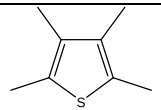
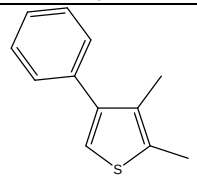
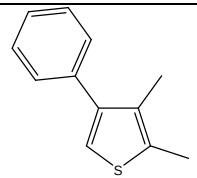
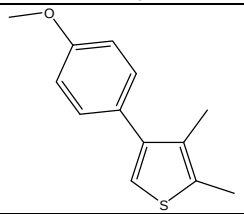
Mean ulcer score for each animal was expressed as ulcer index.[52]

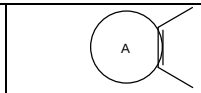
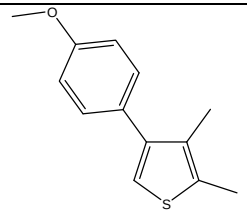
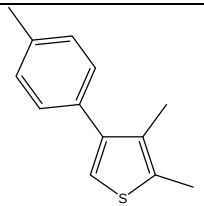
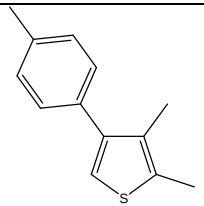
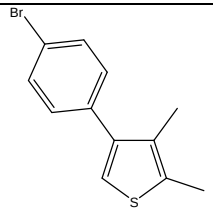
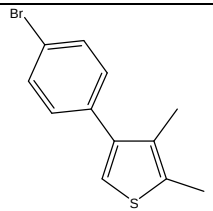
Statistical Analysis of Data: Results are expressed as mean±SEM. The statistical difference between the mean volume of gastric juice, mean total acidity, pH of gastric secretion and mean ulcer score of the treated group were calculated by using the Student's 't' test (Table-11).

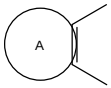
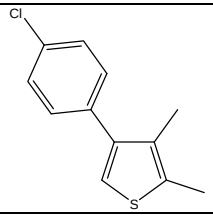
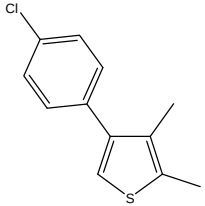
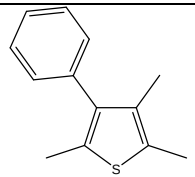
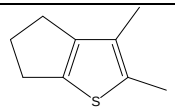
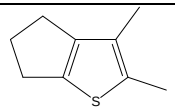
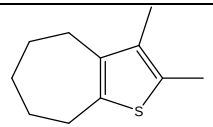
Table 11. Effect of newly synthesized proton pump inhibitors on gastric secretion and antiulcer activity in Shay rat model

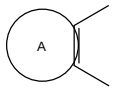
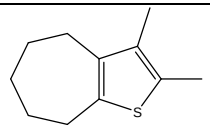
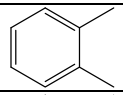
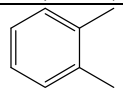
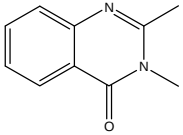
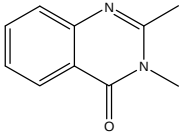
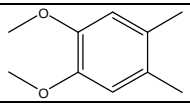
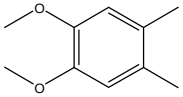
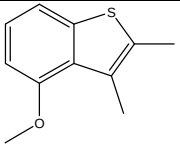


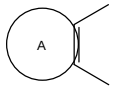
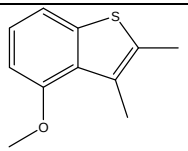
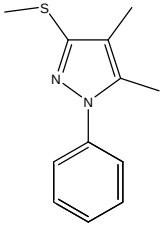
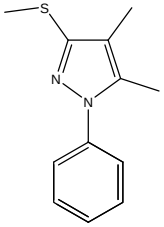
S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
1.	--	--	Group 1: Control group	2.40±0.11	63.60±1.20	10.16±0.52	2.60±0.24
2.	--	--	Group 2: Omeprazole (30)	7.11±0.27***	31.20±3.39***	5.72±0.43***	1.20±0.2**
3.		H	Group 3: **IV*i*** (30)	6.70±0.44***	21.60±2.40***	5.78±0.28***	0.51±0.15***
4.		OCH_3	Group 4: **IV*ii***(30)	2.89±0.31	60.40±8.83	11.72±1.34	2.50±0.15
5.		H	Group 5: **IV*iii*** (30)	3.22±0.29*	56.40±3.20	12.0±1.0	1.75±0.63
6.		OCH_3	Group 6: **IV*iv*** (30)	2.67±0.16	68.40±4.41	9.88±.057	1.5±0.91

S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
7.		H	Group 7: **IV*v*** (30)	3.42±0.19**	56.40±2.50**	8.72±0.43*	1.20±0.23**
8.		OCH_3	Group 8: **IV*vi*** (30)	2.75±0.23	69.20±5.56	9.62±0.21	2.60±0.24
9.		H	Group 9: **IV*vii*** (30)	6.70±0.19***	24.00±1.14***	7.08±0.21***	0.20±0.2***
10.		OCH_3	Group 10: **IV*viii*** (30)	3.71±0.13***	43.00±2.47***	6.08±0.45***	0.40±0.24***
11.		H	Group 11: **IV*ix*** (30)	3.48±0.40**	58.40±10.73**	7.95±1.35**	1.20±0.12**
12.		OCH_3	Group 12: **IV*x*** (30)	6.12±0.08***	38.20±2.28***	5.92±0.25***	1.20±0.12***
13.		H	Group 13: **IV*xi*** (30)	5.72±0.46***	45.00±2.85***	7.65±0.58**	1.25±0.11***

S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
14.		OCH_3	Group 14: **IV*xii*** (30)	2.93±0.16	66.50±8.26	9.20±0.57	2.60±0.24
15.		H	Group 15: **IV*xiii*** (30)	2.73±0.11	63.80±5.57	8.50±0.74	2.70±0.12
16.		OCH_3	Group 16: **IV*xiv*** (30)	3.38±0.90*	57.80±3.65*	5.36±1.05**	1.80±0.12*
17.		H	Group 17: **IV*xv*** (30)	3.31±0.15**	55.20±1.74**	7.24±0.38**	1.0±0.20***
18.		OCH_3	Group 18: **IV*xvi*** (30)	3.30±0.20**	56.40±1.34**	7.18±0.22**	1.12±0.22**

S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
19.		H	Group 19: **IV*xvii*** (30)	3.67±0.36***	52.00±3.67*	5.44±0.20***	0.60±0.1***
20.		OCH_3	Group 20: **IV*xviii*** (30)	3.11±0.11**	61.20±3.87*	7.68±0.27*	0.40±0.1***
21.		H	Group 21: **IV*xix*** (30)	3.68±0.19***	54.40±1.78**	8.08±0.36*	0.62±0.31***
22.		H	Group 22: **IV*xx*** (30)	2.39±0.06	64.4±1.03	10.68±0.48	2.5±0.20
23.		OCH_3	Group 23: **IV*xxi*** (30)	4.55±0.40***	47.6±0.67***	6.92±0.36**	0.87±0.27***
24.		H	Group 24: **IV*xxii*** (30)	2.86±0.33	62.40±1.99	8.68±0.42	2.25±0.4

S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
25.		OCH_3	Group 25: **IV*xxiii*** (30)	6.99±0.11***	23.60±1.03***	5.25±0.15***	0.5±0.12***
26.		H	Group 26: **IV*xxiv*** (30)	2.74±0.19	68.60±1.20	11.54±0.43	2.62±0.37
27.		OCH_3	Group 27: **IV*xxv*** (30)	3.66±0.19***	51.20±1.62***	7.66±0.39**	1.0±0.20***
28.		H	Group 28: **IV*xxviii*** (30)	5.15±0.57**	37.20±3.15***	6.28±0.79**	0.62±0.12***
29.		OCH_3	Group 29: **IV*xxix*** (30)	3.54±0.22**	51.20±1.6**	7.32±0.43**	0.62±0.12***
30.		H	Group 30: **IV*xxx*** (30)	3.76±0.79	60.60±2.85	8.12±0.49*	3.12±0.12
31.		OCH_3	Group 31: **IV*xxxi*** (30)	5.56±0.70**	35.60±3.37**	4.92±0.35***	1.9±0.20*
32.		H	Group 32: **IV*xxxii*** (30)	5.36±0.87**	39.20±5.16**	5.40±0.78***	1.3±0.32**

S. No.		R	Treatment group (mg/kg b.w.)	pH of Gastric juice	Acidity	Total vol. in Stomach (ml)	Ulcer score
33.		OCH_3	Group 33: **IV*xxxiii*** (30)	3.39±0.27*	58.20±5.95*	7.26±0.65**	2.75±0.14
34.		H	Group 34: **IV*xxxiv*** (30)	5.79±0.63***	43.20±3.24***	5.48±0.21***	1.5±0.20**
35.		OCH_3	Group 35: **IV*xxxv*** (30)	5.08±0.56**	45.80±2.38***	6.50±0.66**	1.0±0.20**

n = 5, values are expressed as mean ±SEM

***= $p<0.001$ compared to control group (Student's t test)

**= $p<0.01$ compared to control group (Student's t test)

*= $p<0.05$ compared to control group (Student's t test)

Figure-1. Effect of newly synthesized compounds on pH of gastric secretion in Shay rat model

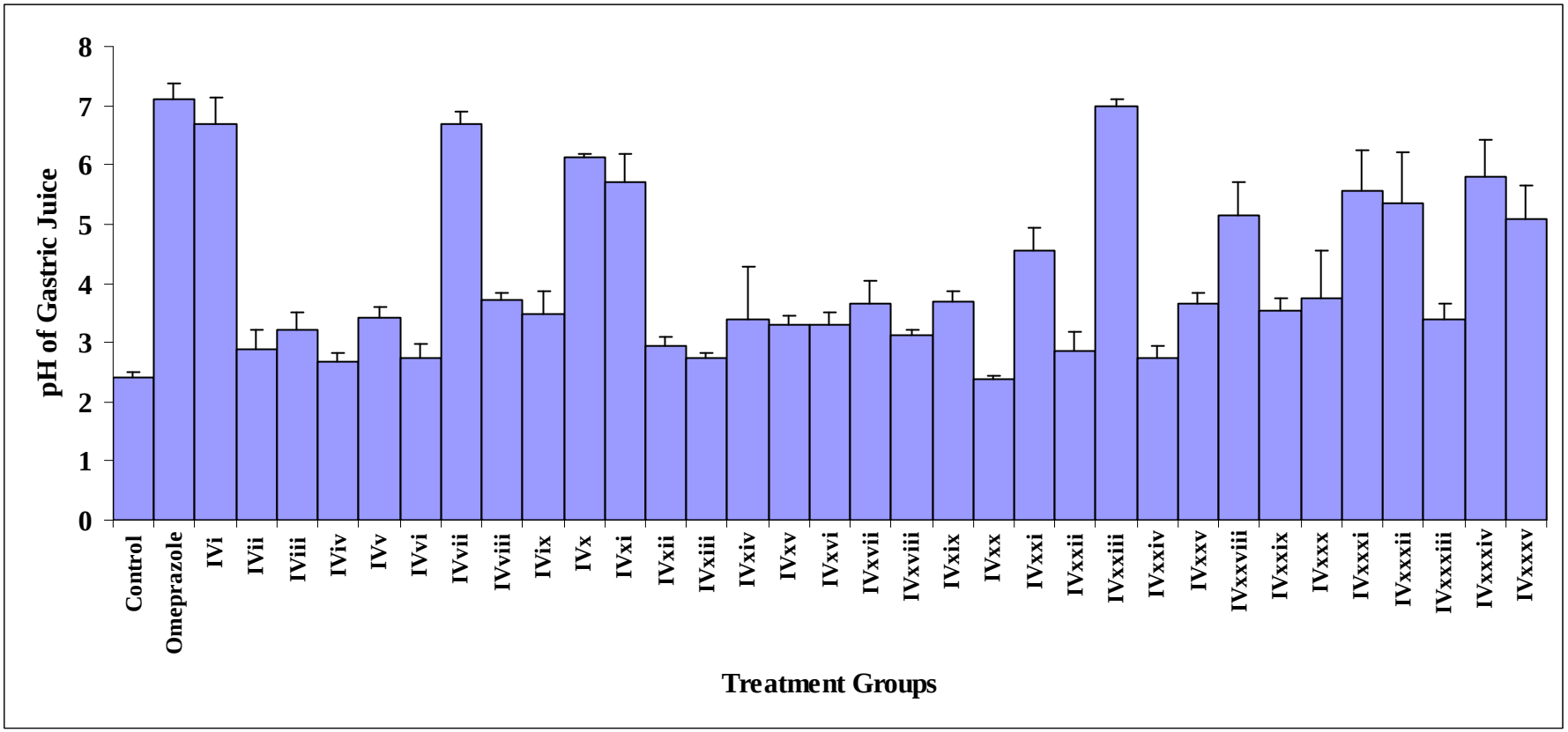


Figure-2. Effect of newly synthesized compounds on total acidity of gastric secretion in Shay rat model

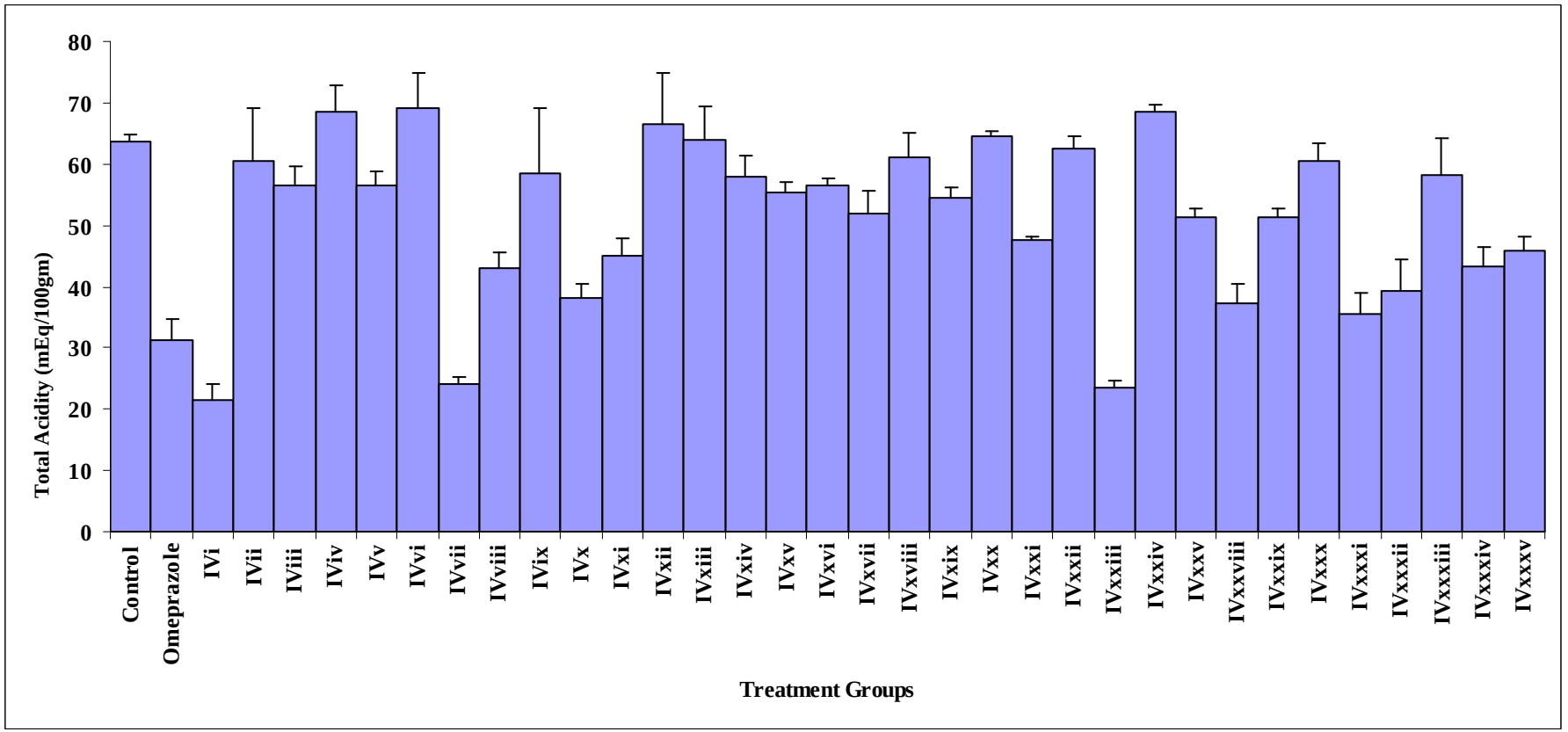


Figure-3. Effect of newly synthesized compounds on total volume of gastric secretion in Shay rat model

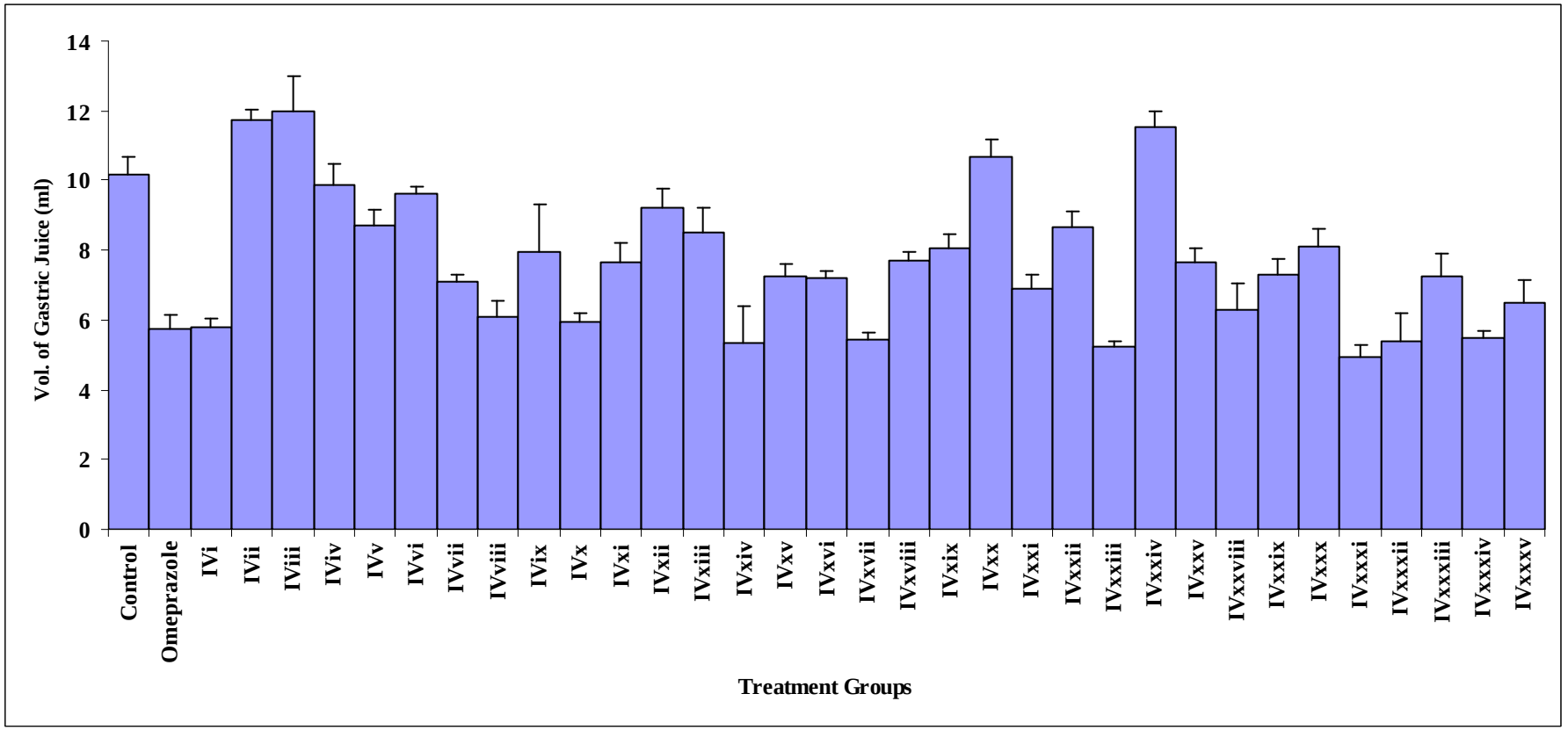


Figure-4. Effect of newly synthesized compounds on ulcer score in Shay rat model

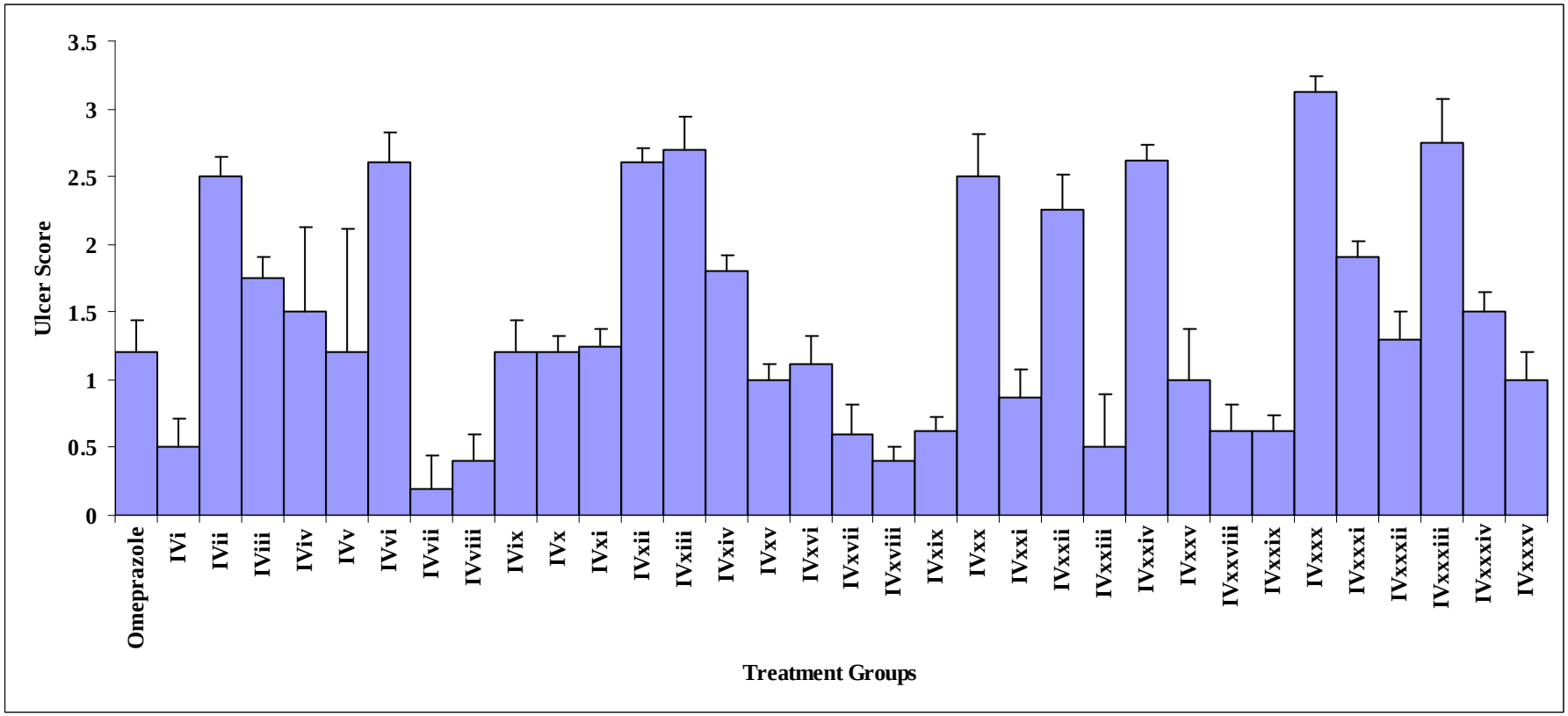


Figure 5. Stomach of rat in pylorus ligation model for antiulcer activity: control group.

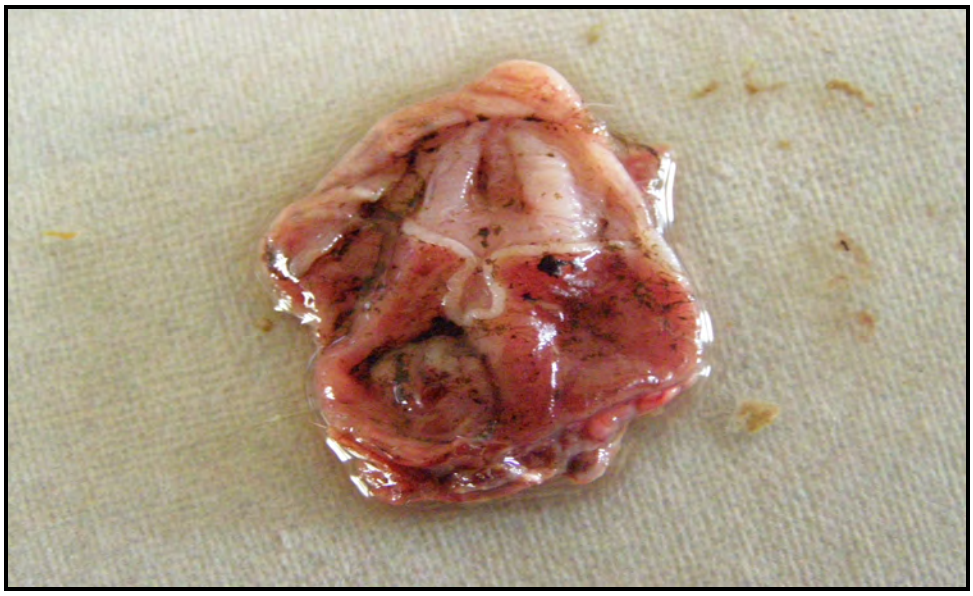


Figure 6. Stomach of rat in pylorus ligation model for antiulcer activity: Standard (Omerazole) group.

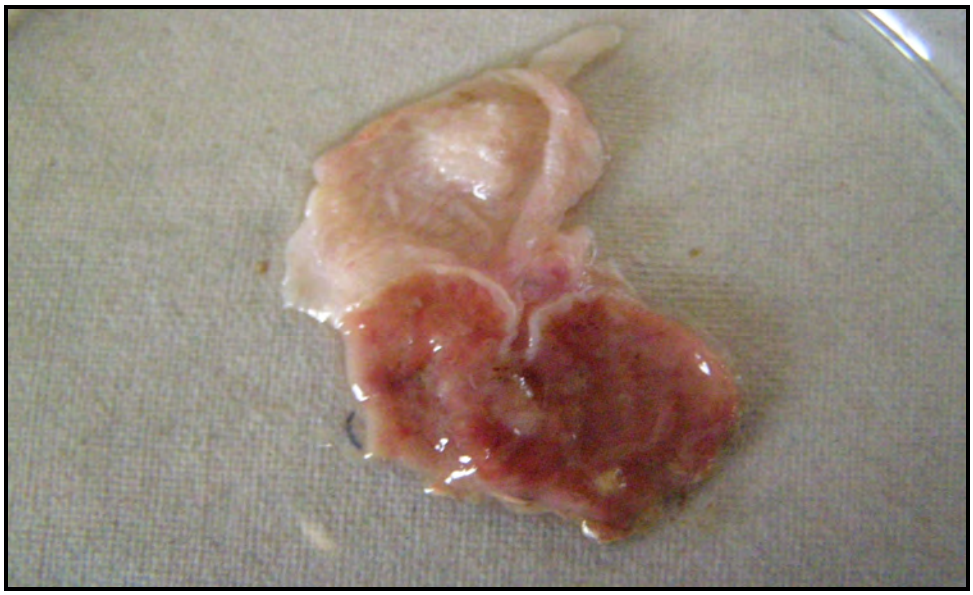


Figure-7. Stomach of rat in pylorus ligation model for antiulcer activity: IV*i*

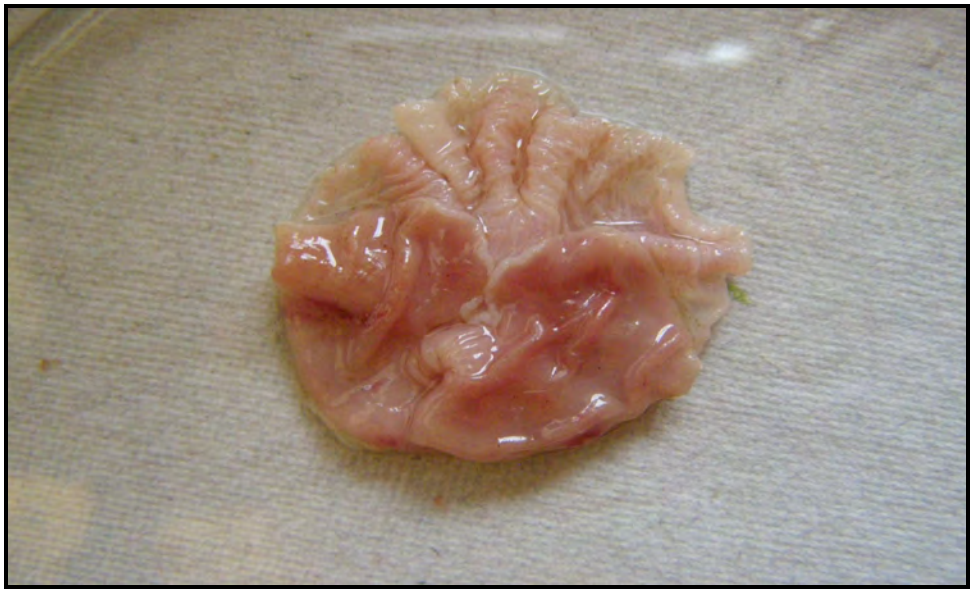


Figure 8. Stomach of rat in pylorus ligation model for antiulcer activity: IV*vii*

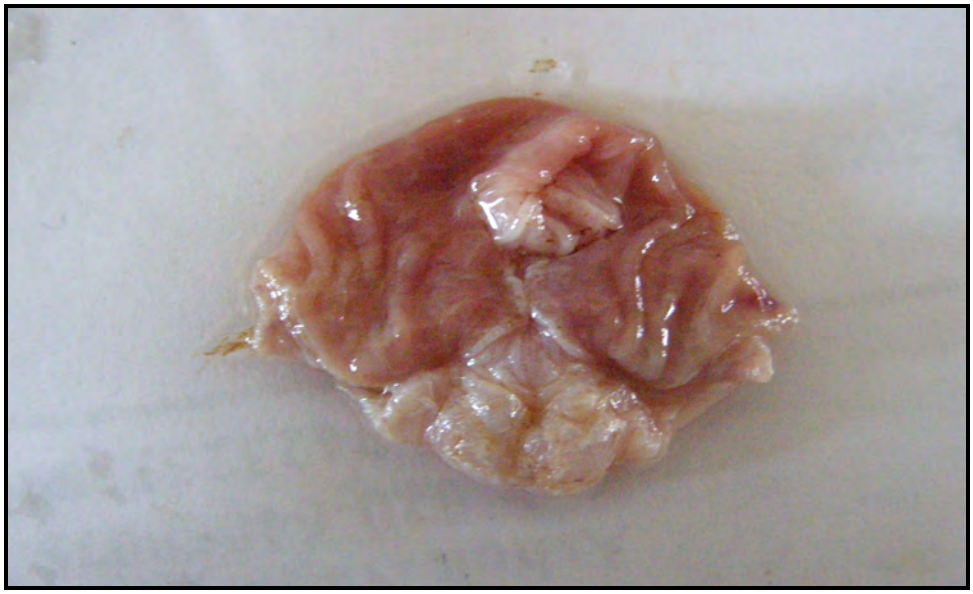


Figure 9. Stomach of rat in pylorus ligation model for antiulcer activity: IV*x*

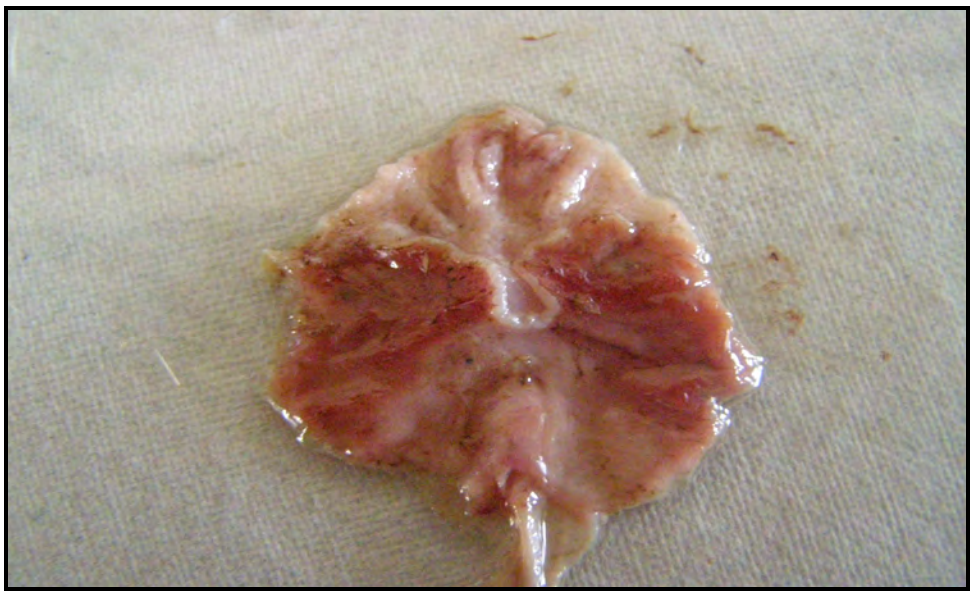
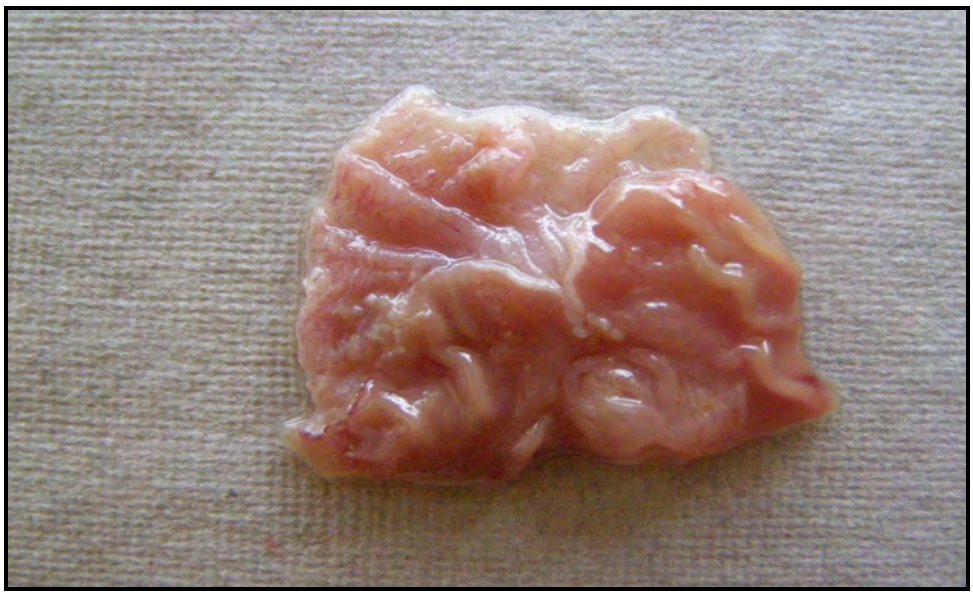


Figure-10. Stomach of rat in pylorus ligation model for antiulcer activity: IVxx*iii*



3.7 Results and discussion on biological activity and mechanism of action of the synthesized compounds.

The etiology of the ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms.[53] To regain the balance, different therapeutic agent are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucosal production, stabilizing the surface epithelial cells, interfering with the prostaglandins synthesis or inhibiting the activity of H^+/K^+ATPase pump responsible for the final secretion of the acid in the stomach.[54]

The cause of gastric ulcer after pylorus ligation is believed to be the stress-induced increase in gastric hydrochloric acid secretion/ or stasis of acid. According to Shay *et al.,*[51] the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.

In the present study, the results from pylorus ligated rats were expressed in terms of the pH of the gastric juice, total acidity, volume of gastric secretion and ulcer index values (Mean ± SEM) in control, standard (omeprazole) and test groups. LD_{50} values of all the test compounds were calculated according to OECD guidelines 423[55] and the dose of 30 mg/kg was selected as the treatment dose.

Omeprazole in a dose of 30 mg/kg produced significant ($p<0.001$) increase in pH (7.11±0.27), decrease in total acidity (31.20±3.39), volume of gastric secretion (5.72±0.43) and ulcer score (1.20±0.24) as compared to the control group. (pH (2.40±0.11), total acidity (63.60±1.20), volume of gastric secretion (10.16±0.52) and ulcer score (2.60±0.24) of the control group.

1. Effect of test compounds on pH of gastric secretion:

Some compounds in the test series showed comparable and significant ($p<0.001$) increase in pH of the gastric secretion. Compound **IV*xxiii*** showed the maximum increase in pH (6.99±0.11) of gastric secretion. Other compounds **IV*i***, **IV*vii*** and **IV*x*** were also significantly increase the pH (6.70±0.44, 6.70±0.19 and 6.12±0.08 respectively) of the gastric secretion in the pylorus ligated rats, (Omeprazole; 7.11±0.27).

2. Effect of test compounds on total acidity of gastric secretion:

Test compounds, **IV*i***, **IV*vii*** and **IV*xxiii*** produced significant ($p<0.001$) decrease in total acidity (21.60±2.4, 24.00±1.14 and 23.60±1.03, respectively). (Omeprazole; 31.20±3.39).

3. Effect of test compounds on total volume of gastric secretion:

Compounds, **IV*i*** (5.78±0.28), **IV*x*** (5.92±0.25), **IV*xiv*** (5.36±1.05), **IV*xvii*** (5.44±0.20), **IV*xxiii*** (5.25± 0.15*)*, **IV*xxxi*** (4.92±0.35), **IV*xxxii*** (5.40±0.78) and **IV*xxxiv*** (5.48±0.21) decreased significantly ($p<0.001$) the volume of gastric secretion as compared to control group whereas all other compounds did not produced significant decrease in volume of gastric secretion. (Omeprazole; 5.72±0.43)

4. Effect of test compounds on ulcer score values

Compounds **IV*i*** (0.51±0.15), **IV*vii*** (0.20±0.2), **IV*viii*** (0.40±0.24), **IV*xvii*** (0.60±0.1), **IV*xviii*** (0.40±0.1), **IV*xix*** (0.62±0.31), **IV*xxiii*** (0.5±0.12), **IV*xxviii*** (0.62±0.12) and **IV*xxix*** (0.62±0.12) produced significant ($p<0.001$) decrease in ulcer score values as compared to control values where as all other compounds did not produce significant decrease in ulcer score values. (Omeprazole; 1.20±0.2)

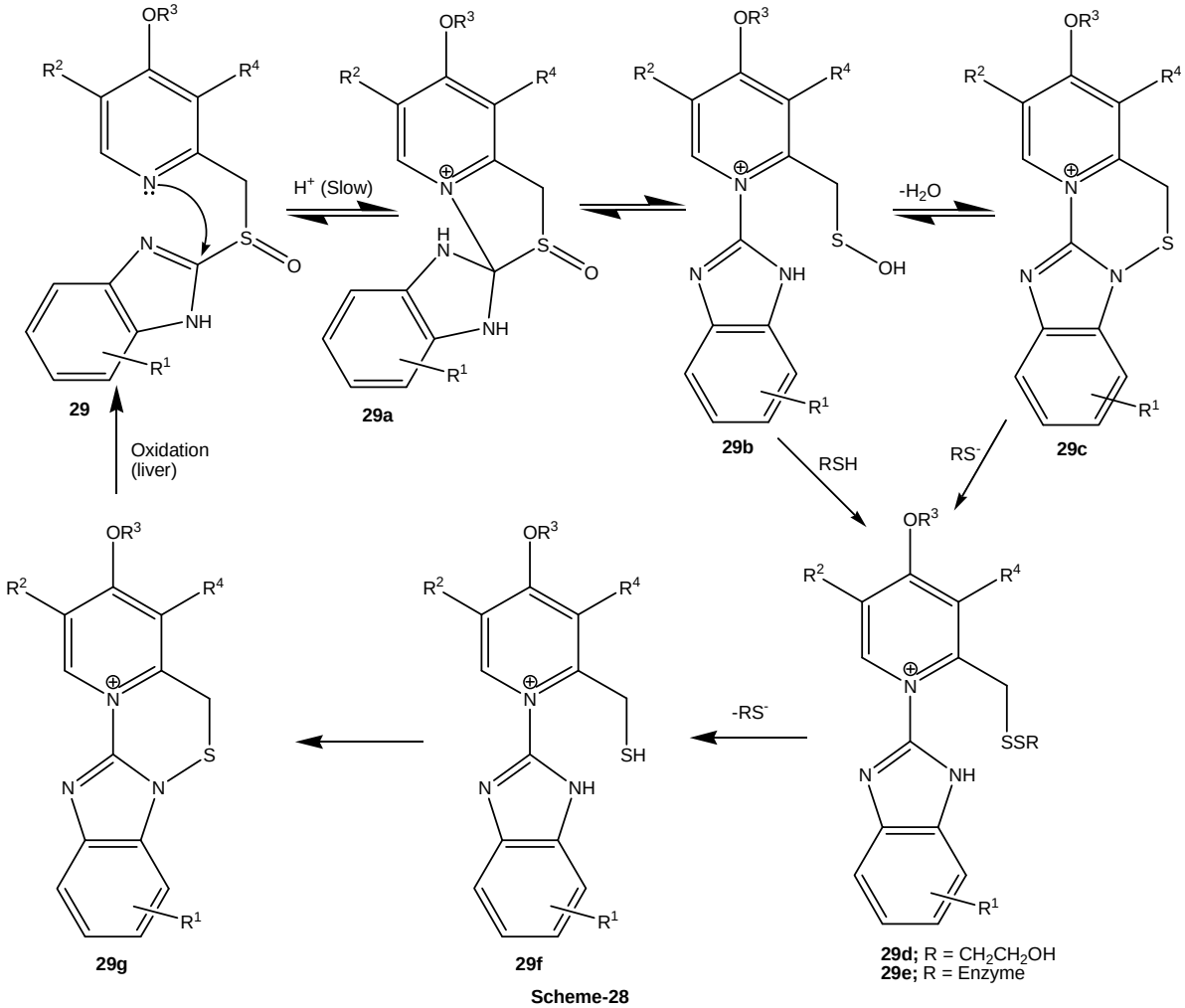
From the results, as shown in Table-6, Figure 1-4, compound **IV*i***, **IV*vii,* IV*x*** and **IV*xxiii*** are the most potentially promising compounds among all the screened compounds. The pH of the gastric acid secretion with these compounds was much close to the standard (omeprazole) group. The total acidity for these compounds is even lower than that of omeprazole. Also, the total volume of the stomach content (gastric juice) in these compounds was almost same as of the standard in contrast to the other test groups.

This suggests that these test compounds potentially inhibited the secretion of H^+ from the stomach as well as reduced the total volume of gastric content as compared to the control group which is in turn reflected by the ulcer score. The ulcer score for these compounds was much lower than the standard, confirming the potent activity of these compounds almost comparable to standard (Omeprazole 30 mg/kg).

Other screened compounds did not show significant improvement in the pH of the gastric juice. But, despite of low pH and high total acidity value, some of the tested compounds (**IV*viii***, **IV*xi***, **IV*xv***, **IV*xvii***, **IV*xviii***, **IV*xix***, **IV*xxi***, **IV*xxv***, **IV*xxviii***, and **IV*xxix***) have the ability to protect the stomach from the ulceration as indicated by the low values of ulcer scores, may be because of some other mechanism of protecting gastric mucosa.

Suggested Proton Pump Inhibitor mechanism of the test compounds:

In normal irreversible PPI's, the pyridinylmethylsulfinylbenzimidazoles (*e.g.*, Omeprazole, pentaprazole, rabiprazole *etc.*) the mechanism of action is depicted as below:

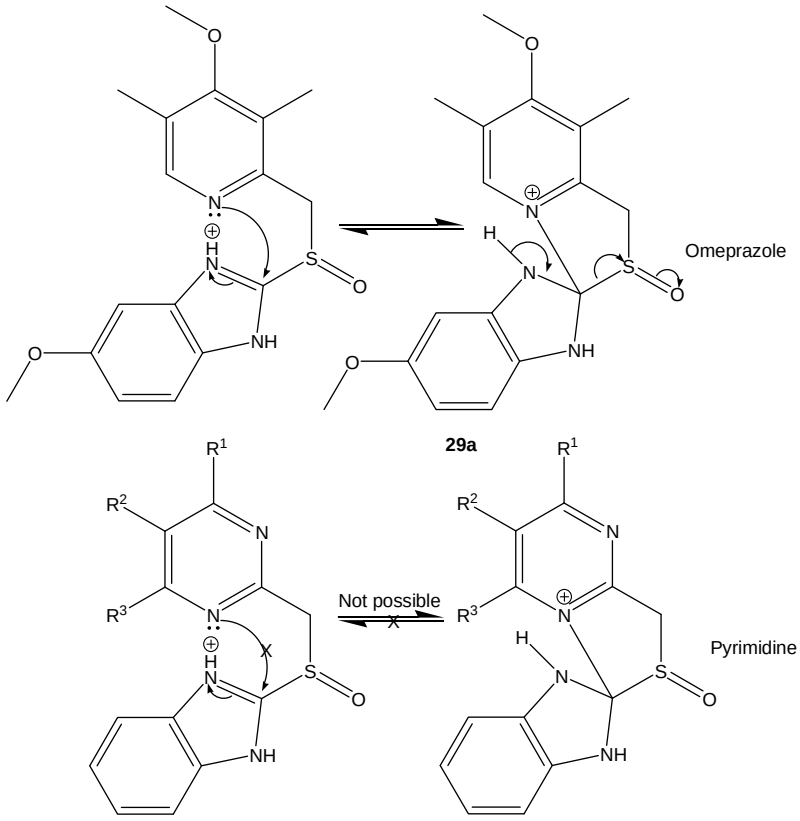


Scheme-28

Initial acid catalyzed transformation of pyridinylmethylsulfinyl benzimidazoles **29** to the sulfenamide **29c** isomers is outlined in scheme-28. The reaction is reversible and goes *via* a spiro intermediate, **29a** and the sulfenic acid **29b**. The reversibility was firmly proved

by kinetic measurements in both directions for example starting from **29** and **29c**. The formation of the spiro intermediate **29a** *via* Smile's rearrangement is a rate limiting step supported by kinetic measurements. The rate constant obtained for omeprazole analogs is strongly dependent on substituents in the pyridine ring, indicating that a positive charge is created in the pyridine nitrogen atom in the rate-limiting step. The spiro intermediate **29a** is dihyrobenzimidazole with a pronounced tendency to undergo aromatization, thus forming the sulfenic acid **29b** by a C-S bond cleavage. This sulfenic acid further loses a molecule of H_2O to form a sulfenamide, **29c**. This sulfenamide **29c** represents the active enzyme inhibitor and binds covalently to the sulfhydryl groups of the cysteines (Cys-813 and 822) of the proton pump. The recovery of enzyme's activity requires *de novo* synthesis of the enzyme which is consistent with the long duration of action of drug.[56] This blocking and deactivating of the H^+/K^+-ATPase enzyme results in irreversible inactivation of the proton pump and thus, affects its normal acid production and secretion. This leads to most of side effects associated with these PPI's usage.

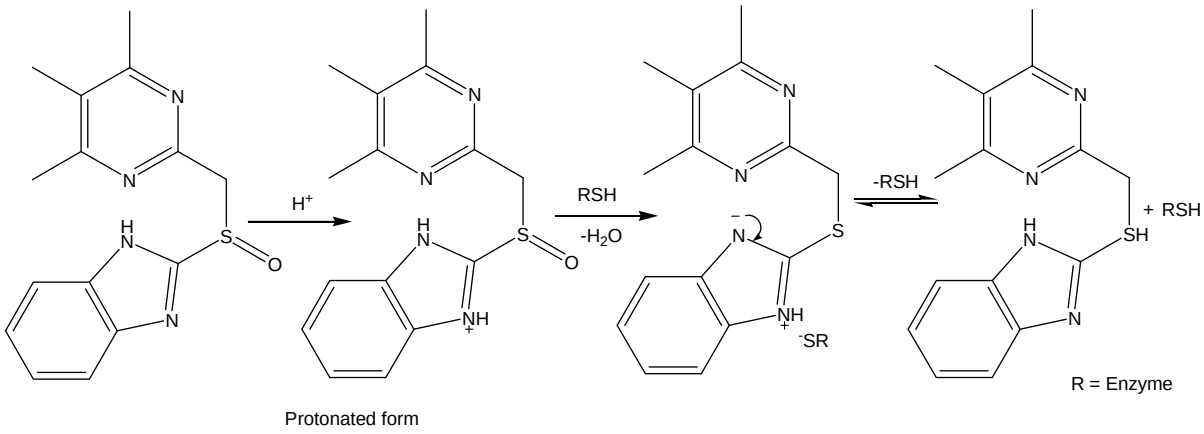
However, the pyrimidine ring is less basic than the pyridine ring and therefore thought in the first step the imidazole gets protonated, it can't form spiro intermediate **29a** as the pyrimidine N_1 nitrogen has no electron available for donation to the nucleophilic centre; the C_2 of the imidazole ring and therefore can't form the spiro intermediate analogous to **29a** *via* Smiles rearrangement (Scheme-29).



Scheme-29

However, it may abstract the proton of the sulfhydryl group of the cysteine 813 and cysteine 822 of the H^+/K^+-ATPase (proton pump) at its imidazolyl nitrogen. Thus, temporarily deprotonating the amino acid residue of the H^+/K^+-ATPase and makes it ineffective (Scheme-30).

However, in the presence of the SH group of the Cysteines in its protonated form, the molecules can still loose the molecules of H_2O and form an ionic bonding with the cysteine as depicted in scheme-30. But this is quite reversible and thus the effect is short lived.



Scheme-30

Further, a comparison of minimized 3D structures of these active compounds (**IV*i***, **IV*vii,*** **IV*x*** and **IV*xxiii***) with the standard PPI, therapeutically used drug, omeprazole, in three different orientation (Figure-11*a-c*), point out the fact that the mechanism of action of these compounds may be slightingly different from the irreversible PPI's (omeprazole and it's congeners). Because, the 3D structure of omeprazole has a distinctly different orientation compared to pyrimidymethylsulfinylbenzimidazoles synthesized by us. This is an indirect support to the proposed mechanism given by us.

However, further specific studies are needed to establish the mechanism involved in the antiulcer action of these compounds.

Figure-11*a*. Comparision of minimized 3D structures of the most active compounds (**IV*i***, **IV*vii,*** **IV*x*** and **IV*xxiii***) with the standard PPI's, omeprazole.

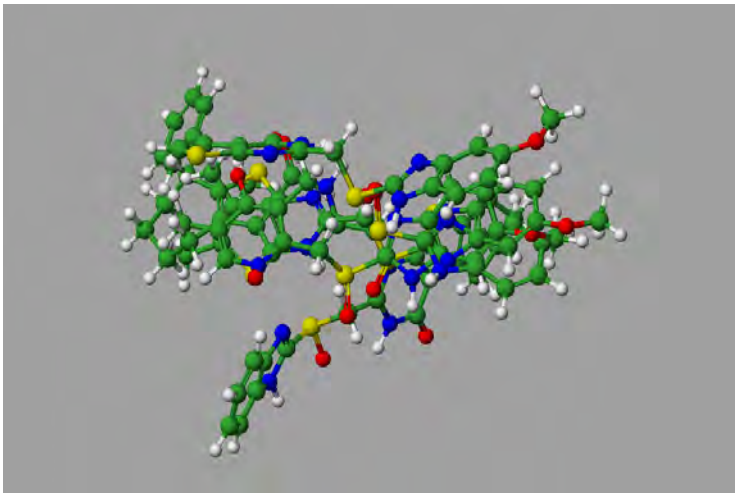


Figure-11*b*. Comparision of minimized 3D structures of the most active compounds (**IV*i***, **IV*vii*, IV*x*** and **IV*xxiii***) with the standard PPI's, omeprazole.

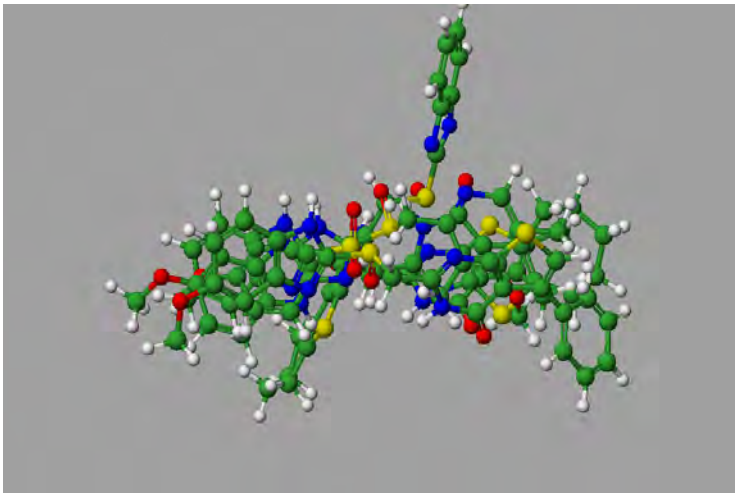
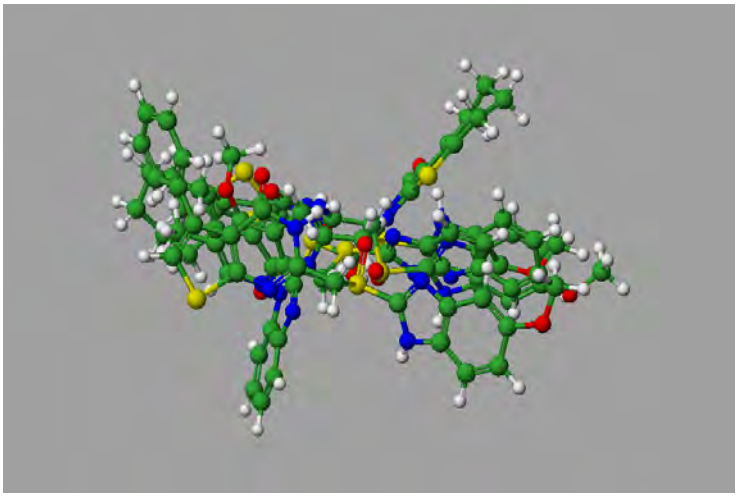


Figure-11*c*. Comparision of minimized 3D structures of the most active compounds (**IV*i***, **IV*vii*, IV*x*** and **IV*xxiii***) with the standard PPI's, omeprazole.



3.8. QSAR Studies

In order to deduce the correlation between physicochemical parameters and biological activity of present series of molecules, multiple regression analysis was used to generate different 2D-QSAR models (equations) by calculating various descriptors available within the software used for these molecules and correlating them with the observed biological activity. Such equations help in giving the insight in the optimal physiochemical properties required by molecules for lead activity, and also in predicting their mechanism of action, sometimes.

Methodology and Protocols

Chemical data

Softwares

All QSAR studies were carried out using the QSAR plus module of Molecular Designing Suite (MDS) of Vlife Sciences (Pune, India), running on Microsoft windows. Structures of the molecules were constructed in ChemDraw® Ultra, version 8.0, (CambridgeSoft Corporation).

Molecules

In the present study a set of 33 molecules belonging to pyrimidylmethylsulfinyl benzimidazole class of compounds synthesized by us and which exhibited antiulcer activity was used. These molecules were divided into two groups based on the type of benzimidazole used. Series A: 5-*H* benzimidazole derivatives (17 compounds) and Series B: 5-methoxybenzimidazole derivatives (16 molecules). All these molecules were constructed in ChemDraw® Ultra, version 8.0, and imported in MDS directly.

These molecular structures were subsequently minimized using MMFF force field using rms gradient 0.001 and other parameters as default values. Structures of some most active molecules with minimized energy are given as under,

Figure-12: Optimized structure of compound 2-(1*H*-benzimidazol-2-yl)methylsulfinyl-5,6,7,8-tetrahydro-benzo-(*b*)sulfinyl[2,3-*d*]pyrimidin-4-(3*H*)-one **(IV*i*)**

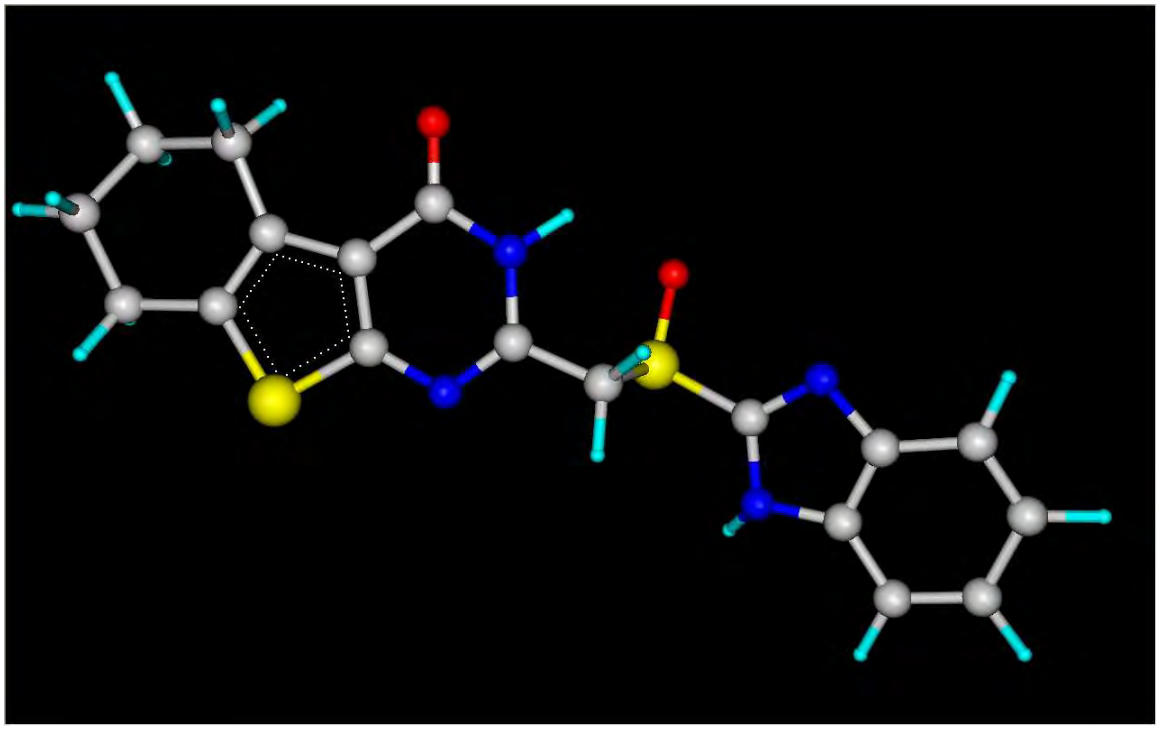


Figure-13: Optimized structure of compound 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl) 5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(IV*vii*)**

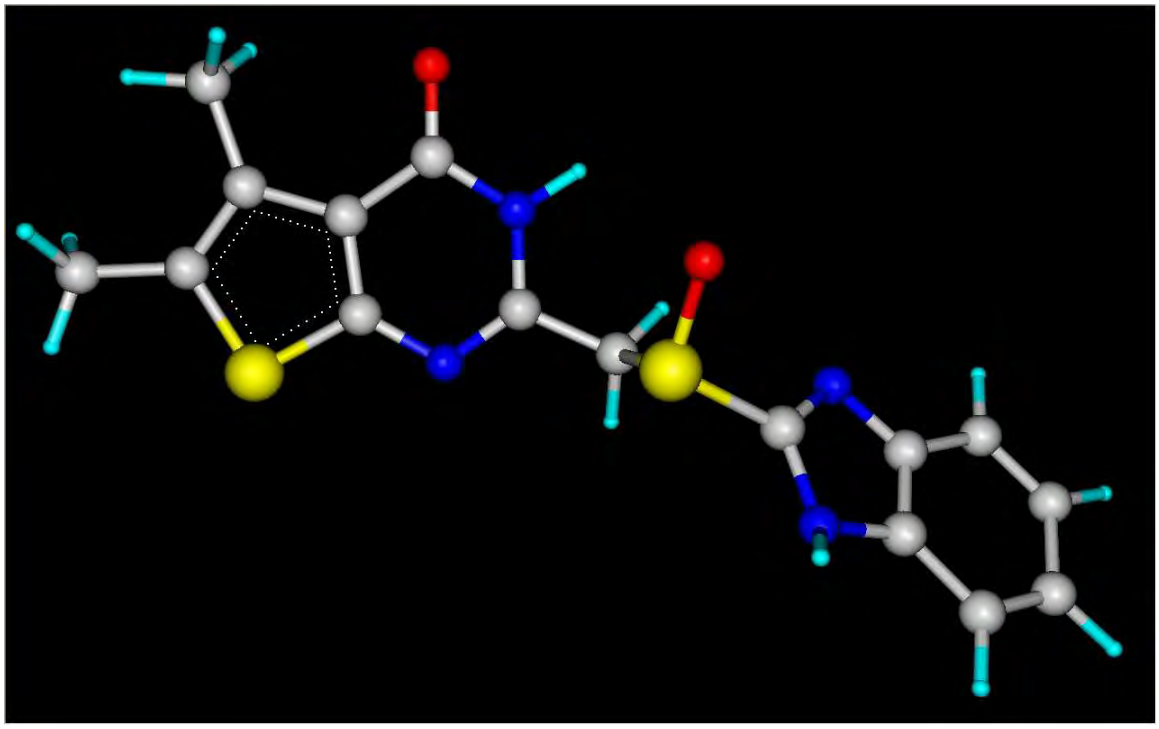


Figure-14: Optimized structure of compound 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-phenyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(IV*x*)**

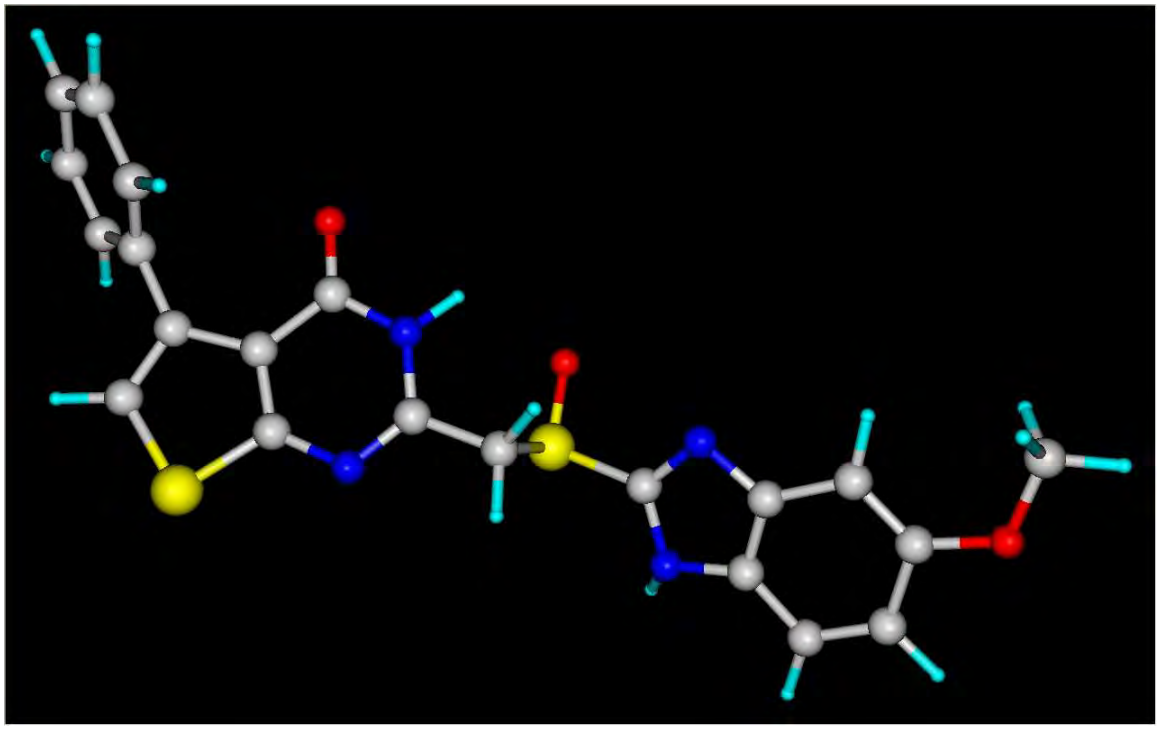


Figure-15: Optimized structure of compound 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one **(IV*xxiii*)**

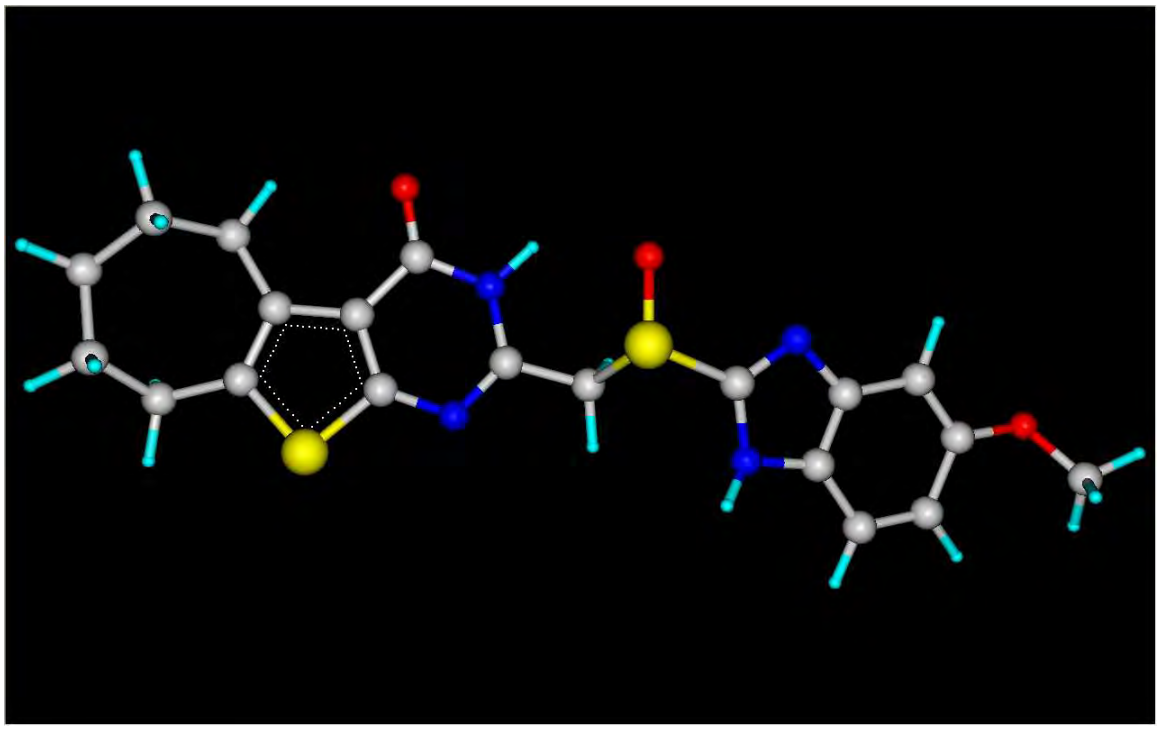
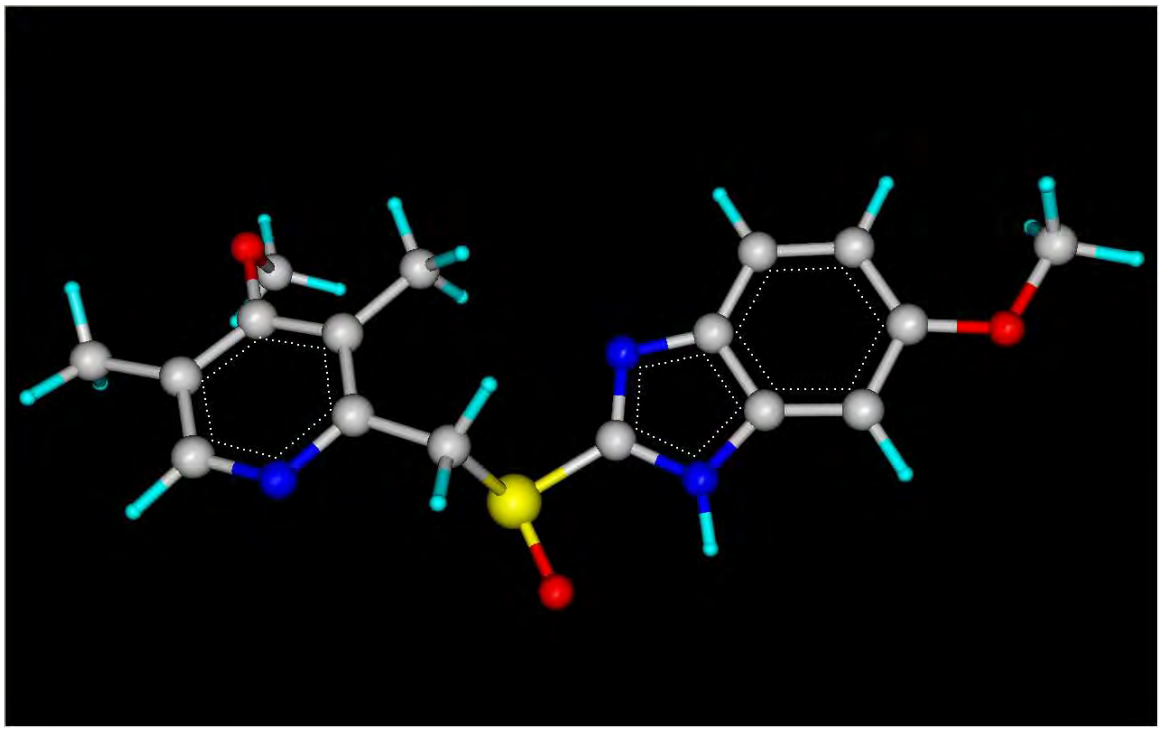


Figure-16: Optimized structure of Omeprazole.



Biological activity

Biological activity of the molecules was evaluated in four different headings namely, pH of the gastric secretion, total acidity of the gastric contents, volume of the gastric juice secreted and ulcer score. For this study, total acidity in the form of log(1/C), was used for this study. However, it was found that pH of the gastric secretion and total acidity were found intercorrelated with each other thus, only total acidity was taken in the present study.

Calculation of descriptors

Different types of descriptors were calculated for each of the energy minimized molecule in the study table using default settings of Molecular Designing Suite (MDS Vlife). These descriptors represented the properties; electronic, spatial, structural, thermodynamic, and molecular shape analysis (MSA). A complete list of descriptors used in the study includes 239 descriptors, based on the physiochemical properties of these molecules, and are divided in total 23 subclasses.

Physicochemical Descriptors Class: (Total 239 descriptors)

Physicochemical Descriptors are based on the physicochemical properties of the molecules. Subclasses of the physicochemical descriptors are as follows:

1. *Individual*
2. *Retention Index (chi)*
3. *Atomic valence connectivity index (chiv)*
4. *Path Count*
5. *Chi Chain*
6. *Chiv Chain*
7. *Chain Path Count*
8. *Cluster*
9. *Path Cluster*
10. *Kappa*
11. *Element Count*
12. *Dipole Moment*
13. *Distance Based Topological*
14. *Estate numbers*
15. *Estate Contributions*
16. *Polar Surface Area*
17. Electrostatic
18. Information Theory Index
19. Semi Empirical
20. Hydrophobicity XlogpA
21. Hydrophobicity XlogpK
22. Hydrophobicity SlogpA
23. Hydrophobicity SlogpK

However, for the present study the first 16 of the subclasses were only considered.

In addition to this, Alignment Independent Descriptors (AI) can also calculated in the MDS, which are more than 700 in number. Alignment Independent descriptors are calculated as discussed by Baumann.[57] For calculation of AI descriptors every atom in the molecule was assigned at least one and at most three attributes. The first attribute is 'Tattribute' that thoroughly characterizes the topology of the molecule. The second attribute is the atom type. The atom symbol is used here. The third attribute is assigned to atoms taking part in a double or triple bond. After all atoms have been assigned their respective attributes, selective distance count statistics for all combinations of different

attributes are computed.[57] A selective distance count statistic 'XY2' (*e.g.* 'TOPO2N3) counts all the fragments between start atom with attribute 'X' (*e.g.* '2' double bonded atom) and end atom with attribute 'Y' (*e.g.* 'N') separated by the graph distance 3. The graph distance can be defined as the smallest number of atoms along the path connecting two atoms in molecular structure. In this study to calculate AI descriptors, we have used following attributes: 2 (double bonded atom), C, N, O, S, Cl, and Br and the distance range of 0 to 7.

Table-12: List of the physicochemical descriptors available in the software.

S. No.	Descriptor	S. No.	Descriptor	S. No.	Descriptor
1	Mol.Wt	44	chiV3Cluster	87	SdsCHcount
2	Volume	45	3ClusterCount	88	SaaCHcount
3	H-AcceptorCount	46	chi4pathCluster	89	SsssCHcount
4	H-DonorCount	47	chiV4pathCluster	90	SddCcount
5	RotatableBondCount	48	4pathClusterCount	91	StsCcount
6	XlogP	49	kappa1	92	SdssCcount
7	slogp	50	kappa2	93	SaasCcount
8	clogP	51	kappa3	94	SaaaCcount
9	logP	52	k1alpha	95	SssssCcount
10	smr	53	k2alpha	96	SsNH3count
11	polarizabilityAHC	54	k3alpha	97	SsNH2count
12	polarizabilityAHP	55	HydrogensCount	98	SssNH2count
13	chi0	56	CarbonsCount	99	SdNHcount
14	chi1	57	SulfursCount	100	SssNHcount
15	chi2	58	OxygensCount	101	SaaNHcount
16	chi3	59	NitrogensCount	102	StNcount
17	chi4	60	ChlorinesCount	103	SsssNHcount
18	chi5	61	BrominesCount	104	SdsNcount
19	chiV0	62	XcompDipole	105	SaaNcount
20	chiV1	63	YcompDipole	106	SsssNcount
21	chiV2	64	ZcompDipole	107	SddsN(nitro)count
22	chiV3	65	DipoleMoment	108	SaasN(Noxide)count
23	chiV4	66	Quadrupole1	109	SssssN(onium)count
24	chiV5	67	Quadrupole2	110	SsOHcount
25	0PathCount	68	Quadrupole3	111	SdOcount
26	1PathCount	69	DistTopo	112	SssOcount
27	2PathCount	70	ConnectivityIndex	113	SaaOcount
28	3PathCount	71	WienerIndex	114	SsPH2count
29	4PathCount	72	RadiusOfGyration	115	SssPHcount
30	5PathCount	73	MomInertiaX	116	SsssPcount
31	chi3chain	74	MomInertiaY	117	SdsssPcount
32	chi4chain	75	MomInertiaZ	118	SssssPcount
33	chi5chain	76	BalabanIndexJ	119	SsSHcount
34	chi6chain	77	BalabanB	120	SdScount
35	chiV3chain	78	BalabanC	121	SssScount
36	chiV4chain	79	BalabanQ	122	SaaScount
37	chiV5chain	80	BalabanCdash	123	SdssS(sulfone)count
38	chiV6chain	81	BalabanQdash	124	SddssS(sulfate)count
39	3ChainCount	82	HosoyaIndex	125	SsClcount
40	4ChainCount	83	SsCH3count	126	SsBrcount
41	5ChainCount	84	SdCH2count	127	SsIcount
42	6ChainCount	85	SssCH2count	128	SsFcount
43	chi3Cluster	86	StCHcount	129	SsCH3E-index

S. No.	Descriptor	S. No.	Descriptor	S. No.	Descriptor
130	SdCH2E-index	153	15SddsN(nitro)E-index	176	SsOHE-index
131	SssCH2E-index	154	SaasN(Noxide)E-index	177	SdOE-index
132	StCHE-index	155	SssssN(onium)E-index	178	SssOE-index
133	SdsCHE-index	156	SsOHE-index	179	SaaOE-index
134	SaaCHE-index	157	SdOE-index	180	SsPH2E-index
135	SsssCHE-index	158	SssOE-index	181	SssPHE-index
136	SddCE-index	159	SaaOE-index	182	SssPE-index
137	StsCE-index	160	SsPH2E-index	183	SdsssPE-index
138	SdssCE-index	161	SssPHE-index	184	SssssPE-index
139	SaasCE-index	162	SsssPE-index	185	SsSHE-index
140	SaaaCE-index	163	SdsssPE-index	186	SdSE-index
141	SssssCE-index	164	SssssPE-index	187	SssSE-index
142	SsNH3E-index	165	SsSHE-index	188	SaaSE-index
143	SsNH2E-index	166	SdSE-index	189	SdssS(sulfone)E-index
144	SssNH2E-index	167	SssSE-index	190	SddssS(sulfate)E-index
145	dNHE-index	168	SaaSE-index	191	SsClE-index
146	SssNHE-index	169	SdssS(sulfone)E-index	192	SsBrE-index
147	SaaNHE-index	170	SddssS(sulfate)E-index	193	SsIE-index
148	StNE-index	171	SsClE-index	194	SsFE-index
149	SsssNHE-index	172	SsBrE-index	195	PolarSurfaceArea ExcludingPandS
150	SdsNEindex	173	SddsN(nitro)E-index	196	PolarSurfaceArea IncludingPandS
151	SaaNE-index	174	SaasN(Noxide)E-index		
152	SsssNE-index	175	SssssN(onium)E-index		

Variable Selection Method:

As stated earlier there are a large number molecular descriptors and alignment independent descriptors available in the software for building a QSAR model. The software automatically selects the best of these as independent variables. The dependant variable on the other hand was the biological activity expressed as log(1/C), where C = observed Total Acidity of the gastric secretion, Volume of the gastric secretion and Ulcer Score. Accordingly, three major sets of regression analyses were performed, based on these three activity expressions.

For model validation the dataset is required to be divided into training set (for building the QSAR model) and test set (for examining its predictive ability). For any QSAR model, it is of crucial importance that the training set selected to calibrate the model exhibits a well balanced distribution and contains representative molecules. In the present study Manual Selection method was used to select the molecules in the training set.

Evaluation of the statistical Model: (QSAR relationship equation)

There are various statistical measures available for evaluation of the significance of the model; following are the most commonly used:

n = number of molecules (>20 molecules)

k = number of descriptors in the model (statistically n = 5 per descriptor in a model is significant)

r^2 = coefficient of determination (>0.7)

r = coefficient of regression

q^2 = cross-validated r^2 (total variance in the internal predictive ability, respective to the training set)

F-test = Fishers-test for statistical significance of the model (higher is better, for same set of descriptors and compounds). This depends on the degree of freedom (Φ), where, Φ = n-m-1 = number of variables-number of data points-1. Higher Φ, is better. As thumb rule per variable five data points (here compounds), should be used minimally.

Generation of QSAR models

QSAR analysis is an area of computational research, which builds models of biological activity using physico-chemical properties of a series of compounds. The underlying assumption is that the variations of biological activity within a series can be correlated with changes in measured or computed molecular features of the molecules. In the present study, QSAR model generation was performed by multiple regression analysis technique. The application of the multiple regression analysis allows the construction of good quality predictive models. Multiple regression analysis was performed by applying stepwise-forward variable selection method using 0.5 as cross correlation limit, F-test cut off value as 2 and term selection criteria as r^2. The number of terms in the equation was fixed to 5 including the constant in the training set.

Results and Discussion:

For better insight the compounds were divided into two main series based on the benzimidazole part of the molecule

A) 5-*H*-Benzimidazole Series

B) 5-Methoxybenzimidazole

The MDS generates different descriptors belonging to different categories like conformational, electronic, shape, spatial, thermodynamic, *etc*. Interpretation of QSAR models with more terms becomes difficult for QSAR. Moreover all the terms may not be relevant. Multiple regression was run several time taking different calculated descriptors to obtain the equation with high coefficient of determination (r^2).

Further, the biological activity has been expressed in by different expressions *viz.* pH of Gastric Secretion, Total Acidity, Volume of Gastric Secretion and Ulcer Score of which pH of Gastric Secretion and Total Acidity are inter correlated therefore, only total acidity was selected for the present study. Thus, we had three different expressions of the biological activity. The biological activity for all the three expressions was taken as the log values of its inverted figures.

Thus, to summarize, the QSAR study was performed for two series of compounds, in which three main sets of biological activities per series was used.

A. 5-*H* benzimidazole Series:

1. Total Acidity:

Description of the descriptors used in the final equation:

1. **Quadrupole2**

   This descriptor signifies magnitude of second tensor of quadrupole moments and is directly proportional to the activity.

2. **T_C_O_2**

   This is the count of number of carbon atoms (single, double or triple bonded) separated from any oxygen atom (single or double bonded) by 2 bond distance in a molecule and is inversely proportion to the activity.

3. **chi4pathCluster**

   This descriptor signifies molecular connectivity index of 4^{th} order pathcluster and is directly proportional to activity.

Of these the best equation was given as under:

Acidity log(1/C) = + 0.0044(± 0.0000) **Quadrupole2** - 0.0792(± 0.0121) **T_C_O_2**
+ 0.1386(± 0.0547) **chi4pathCluster** -1.9411……..(**Equation-1**)

n = 17, Degree of freedom = 13, r = 0.880, r^2 = 0.7751, q^2 = 0.6818, F test = 14.9367, s = 0.0738, t = 2.16 (95%).

The resultant equation was evaluated on the basis of good r (coefficient of regression), (r^2) coefficient of determination, high cross-validated q^2 for internal productivity and other statistical terms like higher F value.

The observed and predicted biological activities of the molecules under study are given in table-13.

Table-13: Observed and predicted biological activities of the molecules under study.

Compound No.	Observed Activity	Predicted Activity	Residuals
IV*i*	-1.334	-1.31505	0.018954
IV*iii*	-1.751	-1.69053	0.060468
IV*v*	-1.751	-1.70559	0.045415
IV*vii*	-1.38	-1.5545	-0.1745
IV*ix*	-1.766	-1.80303	-0.03703
IV*xi*	-1.653	-1.636	0.016996
IV*xiii*	-1.805	-1.78416	0.020839
IV*xv*	-1.741	-1.73562	0.005376
IV*xvii*	-1.716	-1.68073	0.035275
IV*xix*	-1.736	-1.76821	-0.03221
IV*xx*	-1.809	-1.80641	0.002587
IV*xxii*	-1.795	-1.69803	0.096974
IV*xxiv*	-1.836	-1.82279	0.013208
IV*xxviii*	-1.571	-1.59371	-0.02271
IV*xxx*	-1.782	-1.78252	-0.00052
IV*xxxii*	-1.593	-1.72621	-0.13321
IV*xxxiv*	-1.635	-1.55096	0.084041

Regression analysis of these molecules with the lipophilic parameters, logP has also been carried out and the equation is given as under; and is not very significant.

Acidity log(1/C) = - 0.0703(± 0.0398) **logP** -1.5503

n = 17, Degree of freedom = 15, r =0.4149, r^2 = 0.1722, q^2 = -0.0157, F test = 3.1208.

2. Volume of gastric secretion:

Description of the descriptors used in the final equation:

1. Quadrupole2

This descriptor signifies magnitude of second tensor of quadrupole moments which is directly proportional to the activity.

2. T_2_O_2

This is the count of number of double bounded atoms (*i.e.* any double bonded atom, T_2) separated from Oxygen atom by 2 bonds in a molecule and is inversely proportional to the activity.

3. chi4pathCluster

This descriptor signifies molecular connectivity index of 4th order pathcluster and is directly proportional to the activity.

Of these the best equation was given as under:

Volume log(1/C) = + 0.0044(± 0.0000) **Quadrupole2** - 0.0792(± 0.0121) **T_2_O_2**
+ 0.1385(± 0.0548) **chi4pathCluster** -2.0199……. (**Equation-2**)

n = 17, Degree of freedom = 13, r = 0.880, r^2 = 0.7752, q^2 = 0.6818, F test = 14.9402, s = 0.0768, t =2.16 (95%).

The resultant equation was evaluated on the basis of good r (coefficient of regression), (r^2) coefficient of determination, high cross-validated r^2 for internal productivity and other statistical terms like higher F-value

The observed and predicted biological activities of the molecules used in the study are given in table-14.

Table-14: Observed and predicted biological activities of training set of molecules.

Compound No.	Observed Activity	Predictive activity	Residuals
IV*i*	-1.334	-1.31497	-0.01903
IV*iii*	-1.751	-1.69058	-0.06042
IV*v*	-1.751	-1.70558	-0.04542
IV*vii*	-1.38	-1.55453	0.174531
IV*ix*	-1.766	-1.80312	0.037119
IV*xi*	-1.653	-1.63602	-0.01698
IV*xiii*	-1.805	-1.78431	-0.02069
IV*xv*	-1.742	-1.73576	-0.00624
IV*xvii*	-1.716	-1.68084	-0.03516
IV*xix*	-1.736	-1.76835	0.032351
IV*xx*	-1.809	-1.8065	-0.0025
IV*xxii*	-1.795	-1.69808	-0.09692
IV*xxiv*	-1.836	-1.82276	-0.01324
IV*xxvii*	-1.571	-1.59378	0.022784
IV*xxx*	-1.782	-1.78242	0.000419
IV*xxxii*	-1.593	-1.72625	0.133254
IV*xxxiv*	-1.635	-1.55104	-0.08396

Regression analysis of these molecules with the lipophilic parameter logP has also been carried out and the equation is given as under; which is not significant.

Volume log(1/C) = - 0.0704(± 0.0398) **logP**-1.5501

n = 17, Degree of freedom = 15, r = 0.4154, r^2 *= 0.1726,* q^2 *= -0.0153, F test = 3.129.*

3. Ulcer Score:

Description of the descriptors used in the final equation:

1. chiV6chain:

This descriptor signifies atomic valence connectivity index for six membered ring and is directly proportional to the activity.

2. chi3Cluster

This descriptor signifies simple 3rd order cluster chi index in a compound and is directly proportional to the activity.

3. XcompDipole

This descriptor signifies the x component of the dipole moment (external coordinates) and is inversely proportional to the activity.

For the best equation Compound **IV*vii*** and **IV*xiii*** were removed as outliers and the best equation was given as under:

Ulcer score log(1/C) = +11.5236(±1.5660)**chiV6chain** + 1.0933(±0.0853)**chi3Cluster**
- 0.0592(±0.0009) **XcompDipole** -2.8581…….(**Eqation-3**)

n = 15, Degree of freedom = 11, r = 0.9311, r^2 = 0.8671, q^2 = 0.7599, F test = 23.9156, s = 0.2303, t =2.201 (95%).

The observed and predicted biological activities of the molecules used in the study are given in table-15.

Table-15: Observed and predicted biological activities of training set of molecules.

Compound	Observed Activity	Predicted Activity	Residuals
IV*i*	0.292	0.249781	-0.04222
IV*iii*	-0.243	-0.12875	0.114248
IV*v*	-0.079	-0.25869	-0.17969
IV*ix*	-0.079	-0.1125	-0.0335
IV*xi*	-0.097	-0.07537	0.021628
IV*xv*	0	0.141555	0.141555
IV*xvii*	0.222	0.126106	-0.09589
IV*xix*	0.208	0.13629	-0.07171
IV*xx*	-0.398	-0.48522	-0.08722
IV*xxii*	-0.352	-0.32535	0.026648
IV*xxiv*	-0.418	-0.38635	0.031655
IV*xxviii*	0.208	0.136855	-0.07115
IV*xxx*	-0.494	-0.43894	0.05506
IV*xxxii*	-0.114	-0.01292	0.101084
IV*xxxiv*	-0.176	-0.08657	0.089427

Regression analysis of these molecules with the lipophilic parameter logP has also been carried out and the equation is given as under; which is not very significant.

Ulcer Score log(1/C) = + 0.0452(± 0.0615) **XlogP** -0.2637
n = 17, Degree of freedom = 15, r =0.1865, r^2 = 0.0348, q^2 = -0.2394, F test = 0.5403.

B. 5-Methoxybenzimidazole Series:

1. Total Acidity:

Description of the descriptors used in the final equation:

1. T_2_O_6

This is the count of number of double bounded atoms (i.e. any double bonded atom, T_2) separated from Oxygen atom by 6 bonds in a molecule and is inversely proportional to the activity.

2. XcompDipole

This descriptor signifies the *x* component of the dipole moment (external coordinates) and is directly proportional to the activity.

3. DipoleMoment

This descriptor signifies dipole moment calculated from the partial charges of the molecule and is directly proportional to the activity.

Compound **IV*ii*** was taken out from the study taking as outlier and the best equation was given as under:

Acidity log(1/C) = - 0.0469(± 0.0128) **T_2_O_6** + 0.0391(± 0.0164) **XcompDipole**
+ 0.0183(± 0.0110) **DipoleMoment** -1.3513..... (**Equation-4**)

n = 15, Degree of freedom = 11, r = 0.819, r^2 = 0.6712, q^2 = 0.3759, F test = 7.4847, s = 0.777, t = 2.201 (95%).

The resultant equation was evaluated on the basis of good r (coefficient of regression), (r^2) coefficient of determination, high cross-validated r^2 for internal productivity and other statistical terms like higher F value

The observed and predicted biological activities of the molecules under study are given in table-16.

Table-16: Observed and predicted biological activities of the molecules under study.

Compound No.	Observed Activity	Predicted Activity	Residuals
IV*iv*	-1.835	-1.85122	0.01622
IV*vi*	-1.84	-1.85958	0.019576
IV*viii*	-1.633	-1.66489	0.031885
IV*x*	-1.582	-1.73331	0.151309
IV*xii*	-1.822	-1.75702	-0.06498
IV*xiv*	-1.761	-1.73812	-0.02288
IV*xvi*	-1.75	-1.70894	-0.04106
IV*xviii*	-1.786	-1.69871	-0.0873
IV*xxi*	-1.678	-1.57946	-0.09854
IV*xxiii*	-1.372	-1.50115	0.129148
IV*xxv*	-1.709	-1.65017	-0.05883
IV*xxix*	-1.709	-1.69189	-0.01711
IV*xxxi*	-1.551	-1.56647	0.015468
IV*xxxiii*	-1.765	-1.81873	0.053725
IV*xxxv*	-1.661	-1.63431	-0.02669

Regression analysis of these molecules with the lipophilic parameter, logP has also been carried out and the equation is given as under; and not very significant.

Acidity log(1/C) = - 0.0315(± 0.0403) **logP**-1.6491

n = 16,Degree of freedom = 14, r = 0.2046, r^2 *= 0.0419,* q^2 *= -0.2264, F test = 0.6126.*

2. Volume of Gastric Secretion:

Description of the descriptors used in the final equation:

1. SaaNE-index:

An Electro topological state index for number of nitrogen atom connected with two aromatic bonds and is directly proportional to the activity.

2. chi3Cluster:

This descriptor signifies simple 3rd order cluster chi index in a compound and is inversely proportional to the activity.

3. XcompDipole:

This descriptor signifies the *x* component of the dipole moment (external coordinates) and is directly proportional to the activity.

Of these, the best equation was given as under:

Volume log(1/C) = + 0.3554(± 0.0221) **SaaNE-index** - 0.4466(± 0.1681) **chi3Cluster**
+ 0.0439(± 0.0139) **XcompDipole** -2.3894…….. (**Equation-5**)

n = 16, Degree of freedom = 12, r = 0.9793, r^2 = 0.9591, q^2 = 0.6276, F test = 93.7008, s = 0.1181, t = 2.179 (95%).

The resultant equation was evaluated on the basis of good r (coefficient of regression), (r^2) coefficient of determination, high cross-validated r^2 for internal productivity and other statistical terms like higher F value.

The observed and predicted biological activities of the molecules under study are given in table-17.

Table-17: Observed and predicted biological activities the molecules under study.

Compound	Observed Activity	Predictive Activity	Residuals
IV*ii*	-1.781	-1.64482	0.13618
IV*iv*	-1.835	-1.76983	0.065166
IV*vi*	-1.84	-1.77703	0.062968
IV*viii*	-1.633	-1.67031	-0.08831
IV*x*	-1.582	-1.7035	0.119503
IV*xii*	-1.823	-1.7585	-0.1255
IV*xiv*	-1.762	-1.80356	-0.04156
IV*xvi*	-1.751	-1.78582	-0.03482
IV*xviii*	-1.787	-1.77591	0.011091
IV*xxi*	-1.678	-1.62873	0.049266
IV*xxiii*	-1.373	-1.56862	-0.19562
IV*xxv*	-1.709	-1.62632	0.082684
IV*xxix*	-1.709	-1.666	0.043002
IV*xxxi*	-1.551	-1.58987	-0.03887
IV*xxxiii*	-1.765	-1.81295	-0.04795
IV*xxxv*	-0.861	0.002715	0.002715

Regression analysis of these molecules with the lipophilic parameter logP has also been carried out and the equation is given as under; and is not very significant.

Volume log(1/C) = - 0.0909(± 0.0778) **logP** -1.4993

n = 16, Degree of freedom = 14, r = 0.2981, r^2 = 0.0889, q^2 = -0.1806, F test = 1.3656.

3. Ulcer score

Description of the descriptors used in the final equation:

1. Quadrupole2:

This descriptor signifies magnitude of second tensor of quadrupole moments and is inversely proportional to the activity.

2. chiV6chain:

This descriptor signifies atomic valence connectivity index for six membered ring and is inversely proportional to the activity.

3. T_O_S_3:

This is the count of number of oxygen atoms (single double or triple bonded) separated from sulfur atom by 3 bond distance in a molecule and is inversely proportional the activity.

Compound **IV*xviii*** has been removed as an outlier from the series. Of these the best equation was given as under:

Ulcer score log(1/C) = - 0.0067(± 0.0000) **Quadrupole2** -9.6483(± 1.8345) **chiV6chain** - 0.1924(± 0.0029) **T_O_S_3** + 0.4548….. …… (**Equation-6**)

n = 15, Degree of freedom = 11, r = 0.9106, r^2 *= 0.8292,* q^2 *= 0.6485, F test = 17.8008, s = 0.1993, t = 2.201 (95%).*

The resultant equation was evaluated on the basis of good r (coefficient of regression), (r^2) coefficient of determination, high cross-validated r^2 for internal productivity and other statistical terms like higher F value.

The observed and predicted biological activities of the molecules under study are given in table-18.

Table-18: Observed and predicted biological activities of the molecules under study.

Compound	Observed Activity	Predicted Activity	Residuals
IV*ii*	-0.398	-0.41755	-0.01955
IV*iv*	-0.176	-0.36548	-0.18948
IV*vi*	-0.415	-0.31857	0.096429
IV*viii*	0.398	0.209533	-0.18847
IV*x*	-0.079	-0.19428	-0.11528
IV*xii*	-0.415	-0.41383	0.00117
IV*xiv*	-0.255	-0.18184	0.073165
IV*xvi*	-0.049	-0.09709	-0.04809
IV*xxi*	0.06	0.174431	0.114431
IV*xxiii*	0.301	0.350227	0.049227
IV*xxv*	0	0.097442	0.097442
IV*xxix*	0.208	0.142233	-0.06577
IV*xxxi*	-0.279	-0.21341	0.065595
IV*xxxiii*	-0.439	-0.2529	0.186102
IV*xxxv*	0	-0.05693	-0.05693

Regression analysis of these molecules with the lipophilic parameter logP has also been carried out and the equation is given as under; which is not very significant.

Ulcer Score log(1/C) = + 0.1239(± 0.0906) **logP** -0.2800

n = 16, Degree of freedom = 14, r =0.3432, r^2 *= 0.1178,* q^2 *= -0.1596, F test = 1.8699.*

Table-19a: Properties of the most active and most inactive molecules used in the study and their comparison with currently used PPI's.

Comp. No.	Quadrupole2	T_C_O_2	chi4path Cluster	T_2_O_2	chiV6chain	chi3Cluster	XcompDipole	T_2_O_6	Dipole Moment	SaaNE-index	T_O_S_3
Omeprazole	-0.503	6	4.339	5.000	0.040	1.671	1.244	6	2.995	8.939382	0.000
Esomeprazole	-15.812	6	4.339	5.000	0.040	1.671	1.276	6	2.893	8.939382	0.000
Lansoprazole	1.584	8	3.888	7.000	0.043	1.817	2.205	7	2.804	8.546975	0.000
Pentaprazole	-11.284	8	3.888	7.000	0.043	1.817	2.594	7	2.804	8.546975	0.000
Rabiprazole	-38.656	6	3.855	3.000	0.044	1.467	-3.178	7	6.051	9.019930	0.000
IV*i*	58.752	3	4.356	2.000	0.104	1.756	0.175	--	--	--	--
IV*vii*	-2.166	3	4.573	--	--	--	--	--	--	--	--
IV*iii*	-11.035	5	5.018	--	--	--	--	--	--	--	--
IV*xiii*	-61.437	3	4.808	--	--	--	--	--	--	--	--
IV*xx*	-52.304	3	4.356	--	--	--	--	--	--	--	--
IV*xvii*	-38.059	--	4.808	2.000	0.070	2.045	0.983	--	--	--	---
IV*xxxii*	-14.619	--	4.875	4.000	--	--	--	--	--	--	--
IV*xxiv*	-31.911	--	4.859	2.000	--	--	--	--	--	--	--
IV*xix*	--	--	--	--	0.074	1.967	0.149	--	--	--	--
IV*xxiv*	--	--	--	--	0.070	1.478	-0.832	--	--	--	--
IV*xxx*	--	--	--	--	0.063	1.812	4.863	--	--	--	--
IV*x*	--	--	--	--	--	1.960	-0.101	9	2.417	4.499	--
IV*xxxiii*	-12.414	--	--	--	0.062	--	-1.96	11	6.853	--	1.000
IV*vi*	--	--	--	--	--	--	0.482	12	1.963	--	--
IV*xii*	--	--	--	--	--	--	1.187	11	3.497	--	--
IV*xiv*	--	--	--	--	--	2.249	-0.253	9	2.479	4.506	--
IV*ii*	--	--	--	--	--	1.960	0.399	--	--	4.509	--
IV*iv*	10.279	--	--	--	0.038	2.222	0.719	--	--	4.447	2.000
IV*viii*	-18.163	--	--	--	0.038	--	--	--	--	--	0.000
IV*xxiii*	-39.210	--	--	--	0.038	--	--	--	--	--	0.000

Discussion:

From these results it is can be seen that multiple regression analysis method allows building statistically significant model. It is evident from all the six equations (models) developed. It is the electronic and stearic parameters that govern the activity of the proton pump inhibitors used in the present study.

For 5-*H*-benzimidazole series of compounds, total acidity and volume of gastric secretion is directly influenced by the two common electronic and steric parameters *i.e.* quadrupole2 and chi4pathCluster. This suggests that substitutions that increase the contribution of quadrupole2 and chi4pathCluster may lead to increase in the activity. For total acidity, the third descriptor, T_C_O_2 is inversely proportional to the activity that means substitution with oxygen atom separated from any carbon atom (single or double bonded) by two bond distance in a molecule may lead to negative effect on the activity. Similarly, for volume of gastric secretion, descriptor T_2_O_2 is inversely proportional to the activity that means, substitution with oxygen atom separated from any double bonded atom by two bond distances in a molecule may lead to negative effect on the activity. For ulcer score, steric parameter (chiV6chain and chi3cluster) directly influences the activity, while the electronic parameter XcompDipole, is influencing the activity, inversely as indicated by its minus sign.

In 5-methoxybenzimidazole series, descriptor XcompDipole (electronic parameter) is directly influencing the total acidity and volume of the gastric secretion. This suggests that substitution that leads to positive contribution in XcompDipole, may lead to increase in activity.

For total acidity, dipole moment is contributing positively in the activity of the molecule while descriptor T_2_O_6 that is substitution on oxygen atom separated from any double bonded atom by six bond distance shall lead to negative effect on the activity.

For volume of gastric secretion, SaaNE-index (any nitrogen atom connected with two aromatic bonds) plays important role in activity as it directly influences the activity. While increases in the value of chi3Cluster leads to negative effects on the activity. For ulcer score, both electronic (Quadrupole2) and stearic (chiV6chain) parameters influence the activity in the indirect manner, that means substitutions that increases the contributions of these parameters may lead to decrease in the activity. The third descriptor, T_O_S_3 that is presence of substitution on sulfur atom separated from any oxygen atom (single or double bonded) by three bond distance may lead to decrease in the activity.

Table-19b. List of QSAR equations developed for the 5-*H* benzimidazole and 5-methoxybenzimidazole series of compounds under study.

S. No.	Total Acidity	Volume of Gastric Secretion	Ulcer Score
For *H*-Benzimidazole series			
1.	**Acidity log(1/C) = +0.0044(±0.0000)Quadrupole2 -0.0792(± 0.0121)T_C_O_2 +0.1386(±0.0547)chi4pathCluster -1.9411** and Acidity log(1/C) = -0.0703(± 0.0398)logP -1.5503	**Volume log(1/C) = +0.0044(± 0.0000)Quadrupole2 -0.0792(±0.0121)T_2_O_2+0.1385(±0.0548) +chi4pathCluster -2.019** and Volume log(1/C) = -0.0704(± 0.0398) logP-1.5501	**Ulcer score log(1/C) = +11.5236(±1.5660)chiV6chain +1.0933(±0.0853)chi3Cluster -0.0592(±0.0009) XcompDipole-2.8581** and Ulcer Score log(1/C) = + 0.0452(± 0.0615)XlogP -0.2637
For 5-Methoxybenzimidazole series			
2.	**Acidity log(1/C) = -0.0469(± 0.0128)T_2_O_6 +0.0391(± 0.0164)XcompDipole +0.0183(± 0.0110)DipoleMoment -1.3513** and Acidity log(1/C) = -0.0315(± 0.0403)logP-1.6491	**Volume log(1/C) = +0.3554(± 0.0221)SaaNE-index -0.4466(± 0.1681)chi3Cluster + 0.0439(± 0.0139)XcompDipole-2.3894** and Volume log(1/C) = -0.0909(± 0.0778)logP -1.4993	**Ulcer score log(1/C) = -0.0067(±0.0000)Quadrupole2 -9.6483(± 1.8345)chiV6chain -0.1924(± 0.0029)T_O_S_3+0.4548** and Ulcer Score log(1/C) = +0.1239(± 0.0906)logP -0.2800

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4. Experimental

4. Experimental

All the chemicals used in the synthesis were of laboratory grade. The melting points were determined in open capillary method on Veego (VMP-D) electronic apparatus and are uncorrected.

The IR spectra of synthesized compounds were recorded on Shimadzu 8400-S FT-IR, as well as, Perkin Elmer BX_2 FT-IR Spectrophotometer in potassium bromide discs.

^{1}H NMR spectra were recorded on a Bruker AC 400 MHz FT-NMR spectrometer using TMS (Tetramethyl silane) as an internal standard and DMSO-d_6 as a solvent at SAIF, Punjab University, Chandigarh.

Mass spectra were obtained by Electron Impact method on (GCMS-QP2010 spectrometer) using 70 eV ionizing beam and using direct insertion probe.

To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated silica plates (Merck Silicagel F_{254}) using hexane-ethyl acetate-glacial acetic acid as the solvent systems and the spots were visualized by exposure to iodine vapors or under ultra violet (UV) light at 254 nm and 360 nm.

4.1 Synthesis of Starting Materials

4.1.1 Synthesis of chloroacetonitrile (CAS # 107142)[1,2]:

a. Synthesis of chloroacetamide

In a 2 liter round-bottomed flask, fitted with a mechanical stirrer and surrounded by an ice-bath was placed 215 gm (1.75 mole) of ethyl chloroacetate. To the vigorously stirred cold ester, 200 ml of chilled aq. ammonia (sp. gr 0.9) was added. The solution was stirred in the cold for further 30 min; thereafter another 200 ml portion of aq. ammonia was added and the stirring was continued for about 15 min. The mixture was then allowed to stand for 30 min at 0-5^{o}C, filtered under suction and washed with 25 ml portion of cold water to remove ammonium chloride. The yield of air-dried material, melting at 118-

119^{o}C was 128-138 gm (78-84% of the theoretical amount). The crude product was used as such in further step.

b. Synthesis of chloroacetonitrile

In a 3 litre round-bottomed flask fitted with an efficient mechanical stirrer, a reflux condenser and a thermometer were placed 170 gm (1.2 mole) of phosphorous pentoxide, 187 gm (2 mole) of chloroacetamide and 800 ml of dry technical trimethylbenzene. The mixture was refluxed gently with vigorous stirring for 1 hr. The reaction mixture was then allowed to cool to about 100^{o}C with continuous stirring and the reflux condenser was replaced with a distilling adapter fitted with a thermometer and a water-cooled condenser. The crude product and part of solvent were distilled at atmospheric pressure. The yield of crude product boiling at 124-128^{o}C was 121-131 gm (80-87%). To obtain pure product, the crude chloroacetonitrile was mixed with 10 g of phosphorous pentoxide and redistilled through an efficient packed fractionating column. The yield of the pure chloroacetonitrile distilling at 123-124^{o}C was 93-106 gm (62-70%).

4.1.2 Synthesis of Thiophene *o*-aminoesters (I*i*-*xii*)[3,4]

1. Synthesis of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)thiophene(I*i*) (Method A)

Cyclohexanone (9.8 gm; 0.1 mole), powdered sulfur (3.2 gm; 0.1mole), ethyl cyanoacetate (11.7 gm; 0.1 mole) and ethanol (20 ml) were mixed and stirred together at room temperature. To this well stirred mixture, diethylamine (9.14 gm; 0.125 mole) was added dropwise in 0.5 hrs and stirring continued for another 3 hrs at ambient temperature. The reaction mixture was allowed to attain room temperature and thereafter kept in refrigerator overnight. The solid separated was filtered at suction and washed with 20 ml chilled 50% aq. methanol. The product (18.0 gm; 80% yield) having m.p 110-112^{o}C (112-115^{o}C)[5] was characterized as 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)-thiophene (**I*i*).**

Mol. Formula : $C_{11}H_{15}NO_2S$; Mol. Wt. 225

IR (KBr) cm^{-1} : 3414, 3306(γ_{NH}), 3165, 3074, 2988(γ_{C-H}), 1725(γ_{COOEt}).

UV(MeOH) : 311 nm

2. Synthesis of 2-amino-3-carbethoxy-5-cabemethoxy-4-methylthiophene (I*ii*) (Method A)

Methyl acetoacetate (11.6 gm; 0.1 mole), sulfur (3.2 gm; 0.1 mole) and ethyl cyanoacetate (11.7 gm; 0.1 mole) were reacted in ethanol (20 ml) in presence of diethylamine (9.14 gm; 0.125 mole) as per the procedure described for compound (**I*i*).** The product (17 gm; 70% yield) having m.p. 80-82^{o}C was characterized as 2-amino-3-carbethoxy-5-cabemethoxy-4-methylthiophene **(I*ii*).**

Mol. Formula : $C_{10}H_{13}NO_4S$; Mol. Wt. 243.2
IR (KBr) cm^{-1} : 3430, 3311(γ_{NH}), 3170, 3070, 2979(γ_{C-H}), 1724(γ_{COOEt}).
UV(MeOH) : 312 nm

3. Synthesis of 2-amino-3,4-dicarbethoxy-5-methylthiophene (I*iii*) (Method A)

Ethyl acetoacetate (13.0 gm; 0.1 mole), sulfur (3.2 gm; 0.1 mole) and ethyl cyanoacetate (11.7 gm; 0.1 mole) were reacted in ethanol (20 ml) in the presence of diethylamine (9.14 gm; 0.125 mole) as per the procedure described for compound (**I*i*).** The product (13.0 gm; 50.5% yield) having m.p. 103-105^{o}C (108-109^{o}C)[5] was characterized as 2-amino-3,4-dicarbethoxy-5-methylthiophene **(I*iii*).**

Mol. Formula : $C_{11}H_{15}NO_4S$; Mol. Wt. 257.3
IR (KBr) cm^{-1} : 3408, 3294(γ_{NH}), 2988(γ_{C-H}), 1722(γ_{COOEt}).
UV(MeOH) : 314.4 nm

4. Synthesis of 2-amino-3-carbethoxy-4,5-dimethylthiophene (I*iv*) (Method A)

2-Butanone (ethylmethylketone) (7.21 gm; 0.1 mole), sulfur (3.2 gm; 0.1 mole) and ethyl cyanoacetate (11.7 gm; 0.1 mole) were reacted in ethanol (20 ml) in the presence of diethylamine (10.0 gm; 0.125 mole) as per the procedure described for compound (**I*i*).** The product (10 gm; 50% yield) having m.p. 92-93^{o}C (91-93^{o}C)[5] was characterized as 2-amino-3-carbethoxy-4,5-dimethylthiophene **(I*iv*).**

Mol. Formula : $C_9H_{13}NO_2S$; Mol. Wt. 199.2
IR (KBr) cm-1: 3425, 3312(γ_{NH}), 3155, 2984(γ_{C-H}), 1724(γ_{COOEt}).
UV(MeOH) : 310.4 nm

5. Synthesis of ethyl 2-amino-4-phenylthiophene-3-carboxylate (I*v*) (Method B)

Step-I

Acetophenone (12.0 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole), glacial acetic acid (4.8 gm; 0.08 mole), anhydrous ammonium acetate (1.54 gm; 0.02 mole) and dry benzene (50 ml) were refluxed in a round bottomed flask, fitted with a Dean-Stark condenser until the total water removed in the side arm was slightly excess than the calculated value. Benzene was distilled out thereafter and reaction mixture was dissolved in dichloromethane (50 ml) and given washings of aq. $NaHCO_3$ (20 ml; 10% w/v solution), aq. NaCl (20 ml; 10% w/v solution) and water (20 ml). The organic layer was separated, dried (Na_2SO_4) and dichloromethane was distilled out. The solid product alkylidene ethyl cyanoacetate obtained (24.2 gm) was used as such for the second step, without purification.

Step-II

The alkylidene ethyl cyanoacetate was thereafter dissolved in methanol (50 ml) and sulfur (2.6 gm; 0.08 mole) was added, the reaction mixture was then stirred and maintained at 50-60^oC. Then, diethylamine (7.39 gm; 0.1 mole) was added dropwise over 30 min. at a temperature around 60^oC. The reaction mixture was stirred further at 50^oC for 6-8 hrs & cooled overnight. The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (9.0 gm; 75% yield) melting at 95-97^oC, (97-99^oC)[5], was characterized as ethyl 2-amino-4-phenylthiophene-3-carboxylate **(I*v*).**

Mol. Formula : $C_{13}H_{13}NO_2S$; Mol. Wt. 247.3
IR (KBr) cm^{-1} : 3435, 3320(γ_{NH}), 3163, 2980(γ_{C-H}), 1722(γ_{COOEt}).
UV(MeOH) : 298.4 nm

6. Synthesis of ethyl 2-amino-4-(4-methoxyphenyl)thiophene-3-carboxylate (I*vi*) (Method B)

This compound was prepared in two steps by reacting 4-methoxyacetophenone (15.0 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (11 gm; 73.3% yield) melting at 205-208^oC, (208-210^oC)[5] was characterized as ethyl 2-amino-4-(4-methoxyphenyl)thiophene-3-carboxylate (**I*vi***).

Mol. Formula : $C_{14}H_{15}NO_3S$; Mol. Wt. 277.3

IR (KBr) cm^{-1} : 3440, 3315(γ_{NH}), 3160, 2983(γ_{C-H}), 1724(γ_{COOEt}).

UV(MeOH) : 270.6 nm

7. Synthesis of ethyl 2-amino-4-(4-methylphenyl)thiophene-3-carboxylate (I*vii*) (Method B)

This compound was prepared in two steps by reacting 4-methylacetophenone (13.4 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (12 gm; 89% yield) melting at 102-104^oC (102-104^oC)[5] was characterized as ethyl 2-amino-4-(4-methylphenyl)thiophene-3-carboxylate (**I*vii***).

Mol. Formula : $C_{14}H_{15}NO_2S$; Mol. Wt. 261.3

IR (KBr) cm^{-1} : 3432, 3319(γ_{NH}), 3173, 2985(γ_{C-H}), 1727(γ_{COOEt}).

UV(MeOH) : 292.4 nm

8. Synthesis of ethyl 2-amino-4-(4-bromophenyl)thiophene-3-carboxylate (I*viii*) (Method B)

This compound was prepared in two steps by reacting 4-bromoacetophenone (19.7 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (15 gm; 76% yield) melting at 78-80^oC was characterized as ethyl 2-amino-4-(4-bromophenyl)thiophene-3-carboxylate **(I*viii*)**.

Mol. Formula : $C_{13}H_{12}BrNO_2S$; Mol. Wt. 326.2

IR (KBr) cm^{-1} : 3433, 3323(γ_{NH}), 3162, 2985(γ_{C-H}), 1719(γ_{COOEt}).

UV(MeOH) : 309 nm

9. Synthesis of ethyl 2-amino-4-(4-chlorophenyl)thiophene-3-carboxylate (I*ix*) (Method B)

This compound was prepared in two steps by reacting 4-chloroacetophenone (15.4 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per

the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (12 gm; 80% yield) m.p. 102-104^{o}C (102-104^{o}C)[5] was characterized as ethyl 2-amino-4-(4-chlorophenyl)-thiophene-3-carboxylate **(I*ix*)**.

Mol. Formula : $C_{13}H_{12}ClNO_2S$; Mol. Wt. 281.7
IR (KBr) cm^{-1} : 3450, 3333(γ_{NH}), 3109, 2890(γ_{C-H}), 1721(γ_{COOEt}).
UV(MeOH) : 286.2 nm

10. Synthesis of ethyl 2-amino-5-methyl-4-phenylthiophene-3-carboxylate (I*x*) (Method B)

This compound was prepared in two steps by reacting propiophenone (14.4 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (11.0 gm; 76% yield) of m.p. 91-93^{o}C (91-93^{o}C)[5] was characterized as ethyl 2-amino-5-methyl-4-phenylthiophene-3-carboxylate **(I*x*)**.

Mol. Formula : $C_{14}H_{15}NO_2S$; Mol. Wt. 261.3
IR (KBr) cm^{-1} : 3450, 3333(γ_{NH}), 3109, 2890(γ_{C-H}), 1723(γ_{COOEt}).
UV(MeOH) : 307 nm

11. Synthesis of ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (I*xi*) (Method B)

This compound was prepared in two steps by reacting cyclopentanone (8.4 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (5 gm; 59% yield) melting at 89-91^{o}C (91-93^{o}C)[5] was characterized as ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophene-3-carboxylate **(I*xi*)**.

Mol. Formula : $C_{10}H_{13}NO_2S$; Mol. Wt. 211.2
IR (KBr) cm^{-1} : 3450, 3333(γ_{NH}), 3109, 2890(γ_{C-H}), 1728(γ_{COOEt}).

UV(MeOH) : 312 nm

12. Synthesis of ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (I*xii*) (Method B)

This compound was prepared in two steps by reacting cycloheptanone (11.2 gm; 0.1 mole), ethyl cyanoacetate (11.3 gm; 0.1 mole) and sulfur (2.6 gm; 0.08 mole) as per the procedure described for compound (**I*v***). The crystalline product separated was filtered, washed with 50% aq. ethanol and dried. The product, (8.0 gm; 71% yield) of m.p. 88-90^{o}C (88-90^{o}C)[6] was characterized as ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta-[*b*]thiophene-3-carboxylate **(I*xii*)**.

Mol. Formula : $C_{12}H_{17}NO_2S$; Mol. Wt. 239.1
IR (KBr) cm^{-1} : 3453, 3337(γ_{NH}), 3112, 2895(γ_{C-H}), 1723(γ_{COOEt}).
UV(MeOH) : 310 nm

4.2 Synthesis of starting materials (I*xiii-xvii*) of other condensed pyrimidines (I*xiii-xvii*)

13. Synthesis of 3-amino-2-carbethoxy-4, 6-dimethylthieno[2,3-*b*]pyridine (I*xiii*)

a. Synthesis of thiocyanoacetamide

To a solution of malononitrile (6.6 gm; 1 mole) in ethanol (20 ml); triethylamine (0.5 ml) was added and the mixture was stirred while H_2S gas was bubbled into the solution for 3-4 hrs at room temperature. Three crops of crystals were isolated and recrystallization from ethanol to yield 7.84 gm (80%) of a crystalline product. m.p. 114-115^{o}C (116-117^{o}C)[7].

Mol. Formula : $C_3H_4N_2S$; Mol. wt. 100

b. Synthesis of 4, 6-dimethyl-3-cyano-2-mercaptopyridine

To a suspension of thiocyanoacetamide (9.8 gm; 0.1 mole) in ethanol (100 ml), was added acetylacetone (10 gm; 0.1 mole) followed by triethylamine (1 ml) dropwise, while stirring the solution. Thereafter, the solution was allowed to stand at room temperature for 1 hr. The solid separated out was filtered washed with cold ethanol and dried.

Recrystallization from ethanol yielded 15.4 gm (90%) of crystalline product, m.p. 259-260°C (262-264°C).[8]

Mol. Formula : $C_8H_8N_2S$; Mol. Wt. 164

c. Synthesis of 3-amino-2-carbethoxy-4,6-dimethylthieno[2,3-*b*]pyridine[8]

To a solution of sodium ethoxide, prepared by dissolving sodium (2.3 gm; 0.1 mole) in absolute ethanol (50 ml), 4, 6-dimethyl-3-cyano-2-mercaptopyridine (8.2 gm; 0.05 mole) was added, followed by dropwise addition of ethyl chloroacetate (6.2 gm; 0.05 mole) over 15 min. The reaction mixture was stirred at room temperature for 1.5 hrs and then allowed to stand at RT for 1.5 hrs and poured into ice-water (100 ml) mixture. The solid separated out was filtered, washed with water, dried and recrystallized from ethanol to yield 11.2 gm (90%) of crystalline product. m.p. 152-156°C (152-156°C)[8].

M.P.	: 152-156°C; Yield: 90%
Mol. Formula	: $C_{12}H_{14}N_2O_2S$; Mol. Wt. 250.3
IR (KBr) cm^{-1}	: 3435, 3332(γ_{NH}), 2979(γ_{C-H}), 1668($\gamma_{C=O}$).
^{1}H NMR ($CDCl_3$)δppm	: 1.38 (3H, t, $COOCH_2CH_3$, *J* = 5.1 & 6.9), 2.57 (3H, s, CH_3), 2.71 (3H, s, CH_3), 4.32 (2H, q, $COOCH_2CH_3$, *J* = 6.9 & 7.2), 6.14 (2H, s, NH_2), 6.82 (1H, s, Ar-*H*).
MS m/e	: 250(M^+), 222, 204, 176, 149, 132.

14. Synthesis of 3-amino-2-carbethoxyquinazolin-4-one[9] **(I*xiv*)**

a. Synthesis of anthranilic acid hydrazide:

Methyl anthranilate (0.065 mole; 10 ml) and hydrazine hydrate (0.2 mole; 9.57 ml) were taken in a 250 ml RBF attached with a reflux condenser. The reaction mixture was refluxed for 2 hrs. The reaction mixture was cooled, avoiding direct exposure to the sunlight. Solid crystals of anthranilic acid hydrazide separated out. The flask was chilled out overnight and the crystals were filtered, washed with isopropyl alcohol and dried to afford the product (10.0 gm).

M.P.	: 118-119°C (120-122°C)[9]; Yield: 90%
Mol. Formula	: $C_7H_9N_3O$; Mol. Wt.: 151.1

b. Synthesis of 3-amino-2-carbethoxyquinazolin-4-one (I*xiv*)

The mixture of anthranilic acid hydrazide (10.02 gm; 0.05 mole) and diethyl oxalate (19.5 gm; 0.133 mole) was heated under reflux with stirring in an oil bath at 180^{o}C for 6 hrs. The excess of diethyl oxalate was removed under vacuum to give a semi-solid which became crystalline on treatment with ethanol. Recrystallization from methylene dichloride gave 6.8 gm (44%) of colorless crystals of 3-amino-2-carbethoxyquinazolin-4-one **(I*xiv*)** (140-142^{o}C)[9].

M.P. : 137-139^{o}C; Yield: 44%

Mol. Formula : $C_{11}H_{11}N_3O_3$; Mol. Wt. 233.2

IR(KBr)cm^{-1} : 3476, 3334(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$), 1687(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 1.45 (3H, t, $COOCH_2\mathit{CH_3}$, *J* = 7.2), 4.50 (2H, q, $COO\mathit{CH_2}CH_3$, *J* = 6.9 & 7.2), 5.15 (2H, s, br, $\mathit{NH_2}$), 7.48-8.29 (4H, m, Ar-*H*).

MS m/e : 233(M^+), 218, 204, 161, 144.

15. Synthesis of methyl 3, 4-dimethoxy-6-amino-benzoate[10] **(I*xv*)**

a. Synthesis of 3, 4-dimethoxybenzaldehyde

The solution of KOH (30.3 gm in 49.5 ml water) was added dropwise into the melted vanillin (0.3 mol; 50.0 gm) with constant stirring. Simultaneously, dimethyl sulfate (52.8 gm; 0.39 mol) was added dropwise with continuous stirring till addition was complete. The mixture was transferred to porcelin dish and kept overnight, then washed with ice cold water and dried in a vacuum dessicator. The product (51.0 gm; 93.4%) melting at 43-44^{o}C (44^{o}C)[10] was characterized as 3, 4-dimethoxybenzaldehyde (veratraldehyde).

M.P. : 43-45^{o}C; Yield: 93.4%

Mol. Formula : $C_9H_{10}O_3$; Mol. Wt. 166

b. Synthesis of 3-4-dimethoxy-6-nitrobenzaldehyde

To the stirred solution of conc. HNO_3 (310 ml) in an ice bath (0-5^{o}C), powdered veratraldehyde (50.0 gm; 0.3 mole) was added over a period of 45 min. After the addition was complete, the reaction mixture was allowed to stand at 15^{o}C for half an hour in dark and then poured on an ice-water mixture (2 lit). The yellow voluminous precipitated solid was filtered, washed with ice cold water, dried and recrystallized from methanol to yield a yellow crystalline solid product. The product (40.0 gm; 62.6% yield) melting at 132-134^{o}C (134-136^{o}C)[10] was characterized as 3-4-dimethoxy,6-nitrobenzaldehyde.

M.P. : 132-134^{o}C; Yield: 62.6%

Mol. Formula : $C_9H_9NO_5$; Mol. Wt. 211

c. Synthesis of 3, 4-dimethoxy-6-nitrobenzoic acid

In a 250 ml conical flask, 2, 3-dimethoxy-6-nitrobenzaldehyde (0.01 mol) was taken in acetone (100 ml) and aq. solution of $KMnO_4$ (15 gm in 25 ml water) was charged dropwise over a period of 20 min in it. The solution was stirred at RT for another 2 hrs. The color of reaction mass changed from dark grey to violet at the end of the addition. The reaction mass was filtered through highflow bed and washed with hot water. The filtrate was concentrated to remove excess of acetone and acidify with conc. HCl. The precipitate formed were filtered, washed with cold water and dried under vacuum to give yellow solid (yield 70%) melting at 191-194^{o}C.

M.P. : 191-194^{o}C (191-194^{o}C)[10]; Yield: 70%

Mol. Formula : $C_9H_9O_6$; Mol. Wt. 213

IR(KBr)cm^{-1} : 1703(γ_{COOH}), 1529($\gamma_{C\text{-}NO2}$), 1282($\gamma_{Ar\text{-}O\text{-}CH3}$).

d. Synthesis of methyl 3, 4-dimethoxy-6-nitrobenzoate

Through a solution of 2, 3-dimethoxy-6-nitrobezoic acid (2.13 gm; 0.01 mol) in dry methanol (50 ml) dry HCl gas was bubbled over a period of 1-1.5 hrs. Yellow colored precipitates were observed after 2-3 hrs of refluxing. The reaction completion was monitored by TLC. The reaction mixture was concentrated to half the original volume and then quenched in ice-water mixture (100 ml). The solution was extracted with chloroform, washed with 10% w/v aq. $NaHCO_3$ solution, dried and concentrated to give the yellow colored solid, melting at 141-142^{o}C.

M. P. : 140-142^{o}C (141-142^{o}C)[10]; Yield: 88%

Mol. Formula : $C_{10}H_{11}NO_6$; Mol. Wt. 241

IR(KBr)cm^{-1} : 1726($\gamma_{Ar\text{-}O\text{-}OR}$), 1519($\gamma_{C\text{-}NO2}$), 1288($\gamma_{Ar\text{-}O\text{-}CH3}$).

e. Synthesis of methyl 3, 4-dimethoxy-6-aminobenzoate (**I*xv***)

This step involved the reduction of the nitro group of methyl 3, 4-dimethoxy-6-nitrobenzoate with the use of iron powder (activated 80#mesh) and catalytic amount of

conc. HCl in ethanol at 80°C for 8-9 hrs. The mixture of iron (8.0 gm; 0.62 mol), rectified spirit (100 ml) and HCl (3 ml) were stirred and refluxed for 0.5 hr. To this mixture, solution of 2-nitro-4, 5-dimethoxymethylbenzoate (5.0 gm; 0.019) in 50 ml of ethanol was added over 45 min. Then, the mixture was refluxed with stirring for 12-14 hrs. After completion of the reaction, reaction mixture was neutralized with sodium carbonate and filtered hot. The filtrate was concentrated to 1/5th of its original volume, cooled to room temperature and poured on ice cold water (100 ml). The solid obtained was filtered and washed with cold water followed by washing with potassium thiocyanate to remove iron impurities. (120-122°C).[10]

M.P. : 120-122°C; Yield: 47%
Mol. Formula : $C_{10}H_{13}NO_4$; Mol. Wt. 211
IR(KBr)cm^{-1} : 3476, 3373(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$)
MS m/e : 211(M^+), 196, 164, 136.

16. Synthesis of methyl 3-amino-4-methoxybenzo[*b*]thiophene 2-carboxylate (I*xvi*)

a. Synthesis of 2-nitro-6-methoxybenzonitrile[11]

m-Dinitrobenzene (50 gm; 0.3 mole) was dissolved in 750 ml of methanol in a RBF fitted with a mechanical stirrer. The temp was raised to 40°C on water bath & maintained, while a solution of KCN (23.0 gm; 0.30 mole) in 400 ml of water was added with stirring. The purple mixture was stirred for 2 hrs & then it was allowed to stand at RT for 2-3 days. The black ppt. were collected by suction on a buchner funnel & pressed as dry as possible. The filtrate was diluted with 6.0 lit of cold water & allowed to stand overnight. The brown sludge formed was filtered by suction. The combined precipitates were refluxed for 30 min each with 3x50 ml portions of chloroform. The combined chloroform extract was filtered while hot and concentrated by distillation. Then petroleum ether (b.p. 40-60°C) was added to the combined extracts till it became hazy. On chilling the solution, the 2-nitro-6-methoxy benzonitrile separated as red powder.

M.P. : 154-156°C (148-157°C)[11]; Yield: 30%
Mol. Formula : $C_8H_6N_2O_3$; Mol. Wt. 178.1
IR(KBr)cm^{-1} : 2962(γ_{C-H}), 2228($\gamma_{C\equiv N}$)

b. Synthesis of methyl thioglycolate[12]

A solution of thioglycolic acid (9.2 gm; 0.1 mole) in 50 ml of methanol was cooled in ice bath. Dry HCl gas was bubbled in this solution over 3-4 hrs. The reaction mixture was kept overnight, undisturbed at room temperature. Next day, the mixture was boiled on water bath for 2-3 hrs, cooled to room temperature, quenched with ice water (50 ml), extracted with chloroform and washed with $NaHCO_3$ (10% w/v) and water and dried (Na_2SO_4). On concentrating this organic extracts, gave yellow colored liquid.

B.P. : 120-124^oC (120-124^oC)[12]; Yield: 90%
Mol. Formula : $C_3H_6O_2S$; Mol. Wt. 106

c. Synthesis of methyl 3-amino-4-methoxybenzo[*b*]thiophene 2-carboxylate[13]

To a cold solution of 2-nitro-6-methoxybenzonitrile (53 gm; 0.30 mole) and methyl thioglycolate in 60 ml of dry dimethylformamide (31.8 gm; 0.30 mole), an aq. solution of KOH (3.0 gm in 15 ml of water) was added with continuous stirring. The solution was stirred at 0^oC for 3 hrs and then diluted with ice-water. The solid obtained was filtered, dried and recrystallized from ethanol-water mixture to yield colorless needles.

M.P. : 140-143^oC (147-148^oC)[13]; Yield: 80%
Mol. Formula : $C_{11}H_{11}NO_3S$; Mol. Wt. 237.2
IR(KBr)cm^{-1} : 3484, 3376(γ_{NH}), 2947(γ_{C-H}), 1670($\gamma_{C=O}$).
^1H NMR ($CDCl_3$)δppm : 3.90 (3H, s, CH_3 at COO*CH_3*), 3.95 (3H, s, CH_3 of O*CH_3*), 6.75 (2H, s, br, NH_2 at 3), 7.21-7.40 (3H, m, Ar-*H*).
MS m/e : 238(M+1), 237(M^+), 222, 206.

17. Synthesis of 5-amino-4-carboxamide-3-(methylthio)pyrazole[14] **(I*xvii*)**

a. Synthesis of ethyl-2,2-di-(methylthio)methylene cyanoacetamide

In an ice cold solution of KOH in 10 ml of water was added 30 ml dimethylformamide slowly with stirring and cooling. To this, cyanoacetamide and carbon disulphide were added with continuous stirring and cooling. Thereafter, the reaction mixture was cooled and stirred for 1 hr at 5-10^oC and again stirred for 1 hr at room temperature. The reaction mixture was further cooled to 0-5^oC and to this, dimethylsulphate was added dropwise while maintaining the temperature below 20^oC. Stirring was continued further for 2 hrs and thereafter the reaction mixture was allowed to stand overnight at room temperature.

Next day the reaction mass was poured on to ice-water mixture (100 ml) and the separated precipitates were filtered, washed with water and air dried.

M.P. : 72-74^{o}C (74-76^{o}C)[14]; Yield: 80%
Mol. Formula : $C_6H_8N_2OS_2$; Mol. Wt. 156

b. Synthesis of 5-amino-4-carboxamido-3-(methylthio)pyrazole

A mixture of ethyl di(methylthio)methylene cyanoacetamide (16.9 gm; 0.07 mole) and phenylhydrazine (10.8 gm; 0.1 mole) in ethanol (100 ml, 95%) was refluxed for 3-4 hrs. The excess of ethanol was removed by distillation under reduced pressure. The crystals obtained were recrystallized from cyclohexane.

M.P. : 146-150^{o}C; Yield: 56%
Mol. Formula : $C_{11}H_{12}N_4OS$; Mol. Wt. 248.3
IR(KBr)cm^{-1} : 3449, 3394(γ_{NH}), 3138(γ_{C-H}), 1662(γ_{CONH}).
MS m/e : 248(M^+), 231, 216, 198, 186, 157.

4.3 Synthesis of condensed 2-chloromethylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones (II*i-xviii*)

1. Reaction of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)thiophene with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*i*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)thiophene **(I*i*,** 13.52 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was allowed to stand at room temperature for 12 hrs. The reaction mixture was thereafter heated on a water bath for 2 to 3 hrs., cooled to room temperature and poured onto ice-water mixture (150-200 ml) and neutralized with strong aq. NH_4OH solution (50 %v/v). The solid separated was filtered, washed with water and dried. The crude product on recrystallization from dioxane yielded fine needles (12.62 gm; 82.7%), m.p. 273-276^{o}C (273-276^{o}C)[15], characterized as 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (**II*i***).

Mol. Formula : $C_{11}H_{11}ClN_2OS$; Mol. Wt. 254.5
IR(KBr) cm^{-1} : 3014(γ_{Ar-H}), 1662(γ_{CONH}), 754(γ_{C-Cl}).

NMR ($CDCl_3$)δppm : 1.62 (4H, s, CH_2 at 6 & 7), 2.77 (2H, s, CH_2 at 4), 3.02 (2H, s, CH_2 at 8), 4.55 (2H, s, CH_2 at 2), 10.60 (1H, s, N*H* at 3).

MS m/e : 255(M^+), 221, 149.

2. Reaction of 2-amino-3-carbethoxy-5-carbemethoxy-4-methylthiophene with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*ii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 2-amino-3-carbethoxy-5-carbemethoxy-4-methylthiophene **(I*ii*,** 16.3 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (14 gm; 86%), melting at 250-250°C, characterized as methyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate (**II*ii***).

Mol. Formula : $C_{10}H_9ClN_2O_3S$; Mol. Wt. 272.71

IR (KBr) cm^{-1} : 1724(γ_{COO-}), 1664(γ_{CONH}), 2863(γ_{ArH}), 1254(γ_{CH2}), 686(γ_{C-Cl}).

3. Reaction of diethyl 5-amino-3-methylthiophene 2,4-dicarboxylate with chloro-acetonitrile in the presence of dry hydrogen chloride gas (II*iii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of diethyl 5-amino-3-methylthiophene 2,4-dicarboxylate **(I*iii*,** 17.1 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction was woked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (15 gm; 87%), m.p 241-243°C (243-246°C)[3], characterized as ethyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine-6-carboxylate **(II*iii*)**.

Mol. Formula : $C_{11}H_{11}ClN_2O_3S$; Mol. Wt. 286.7

IR (KBr)/cm^{-1} : 2864(γ_{CH-}), 1725(γ_{COO-}), 1670(γ_{CONH}), 763(γ_{C-Cl}).

NMR ($CDCl_3$)δppm : 1.41 (3H, t, *J* = 7, CH_3), 2.95 (3H, s, CH_3), 4.38 (2H, quartlet, *J* = 7, CH_2), 4.57 (2H, s, CH_2), 10.62 (1H, s, N*H*),

MS m/e : 286(M^+).

4. Reaction of 2-amino-3-carbethoxy-4,5-dimethylthiophene with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*iv*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 2-amino-3-carbethoxy-4,5-dimethylthiophene **(I*iv*,** 11.9 gm; 0.06 mole) and chloroaceto-nitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (15 gm; 83%), m.p 252-254^{o}C (253-255^{o}C)[3], characterized as 2-(chloromethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*iv*)**.

Mol. Formula : $C_9H_9ClN_2OS$; Mol. Wt. 228.7

IR(KBr)/cm^{-1} : 2917(γ_{C-H}), 1662(γ_{CONH}), 1211(γ_{CH2}), 769(γ_{C-Cl}).

NMR ($CDCl_3$)δppm : 2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 4.51 (2H, s, C*H*$_2$), 10.03 (1H, s, br, N*H*).

MS m/e : 229(M^+).

5. Reaction of ethyl 2-amino-4-phenylthiophene 3-carboxylate with chloro-acetonitrile in the presence of dry hydrogen chloride gas (II*v*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-4-phenylthiophene 3-carboxylate **(I*v*,** 14.8 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and dried. The crude product on recrystallisation from dioxane yielded fine needles (18 gm; 80%), m.p 220-222^{o}C (221-223^{o}C)[3], characterized as 2-(chloromethyl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*v*)**.

Mol. Formula : $C_{13}H_9ClN_2OS$; Mol. Wt. 276.7

IR (KBr) cm^{-1} : 2855(γ_{C-H}), 1663(γ_{CONH}), 1294(γ_{CH2}), 1046 and 748(γ_{C-Cl}).

NMR ($CDCl_3$)δppm : 4.58 (2H, s, C*H*$_2$), 7.31-7.52 (5H, m, Ar-*H* and 1H at 6 position), 12.69 (1H, s, br, N*H*).

MS m/e : 276(M^+).

6. Reaction of ethyl 2-amino-4-(4-methoxyphenyl)thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*vi*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-4-(4-methoxyphenyl)thiophene 3-carboxylate (**II*vi*,** 16.2 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (14 gm; 86%), m.p 205-207^{o}C (208-210^{o}C)[3], characterized as 2-(chloromethyl)-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**II*vi*)**.

Mol. Formula : $C_{14}H_{11}ClN_2O_2S$; Mol Wt. 306.7
IR (KBr) cm^{-1} : 1672(γ_{CONH}), 3094(γ_{Ar-H}), 2945(γ_{C-H}), 715(γ_{C-Cl}).
NMR ($CDCl_3$)δppm : 3.84 (3H, s, Ar-O*CH*$_3$), 4.49 (2H, s, *CH*$_2$ at 2), 7.14-7.54 (5H, m, Ar-*H* at 6).

7. Reaction of ethyl 2-amino-4-(4-methylphenyl)thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*vii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-4-(4-methylphenyl)thiophene 3-carboxylate **(I*vii*,** 15.6 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and dried. The crude product on recrystallisation from dioxane yielded fine needles (12 gm; 77%), m.p 258-260^{o}C (260-262^{o}C)[4], characterized as 2-(chloromethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*vii*)**.

Mol. Formula : $C_{14}H_{11}ClN_2OS$; Mol. Wt. 290.7
IR (KBr) cm^{-1} : 3028(γ_{ArH}), 1651(γ_{CONH}), 762(γ_{C-Cl}).
NMR ($CDCl_3$)δppm : 2.39 (3H, s, C*H*$_3$), 4.53 (2H, s, CH_2), 7.13 (1H, s, C*H*), 7.19-7.46 (4H, m, Ar-*H*), 10.43 (1H,s, N*H*)
MS m/e : 290(M^+)

8. Reaction of ethyl 2-amino-4-(4-bromophenyl)thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*viii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-4-(4-bromophenyl)thiophene 3-carboxylate **(I*viii*,** 19.5 gm; 0.06 mole) and

chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was woked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (15 gm; 77%), m.p 247-249^{o}C and characterized as 5-(4-bromophenyl)-2-(chloromethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*viii*)**.

Mol. Formula : $C_{13}H_8BrClN_2OS$; Mol. Wt. 355.6
IR (KBr) cm^{-1} : 3049(γ_{ArH}), 1660(γ_{CONH}), 767($\gamma_{C\text{-}Cl}$).

9. Reaction of ethyl 2-amino-4-(4-chlorophenyl)thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*ix*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-4-(4-chlorophenyl)thiophene 3-carboxylate **(I*ix*,** 16.8 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (14 gm; 83%), m.p 233-234^{o}C (229-231^{o}C)[13], characterized as 2-(chloromethyl)-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*ix*)**.

Mol. Formula : $C_{13}H_8Cl_2N_2OS$; Mol. Wt. 311.1
IR (KBr) cm^{-1} : 3107($\gamma_{Ar\text{-}H}$), 1649(γ_{CONH}), 756($\gamma_{C\text{-}Cl}$)
NMR ($CDCl_3$)δppm : 4.55 (2H, s,CH_2 at 2), 7.40-7.55 (5H, m, Ar-*H* & *H* at 6).

10. Reaction of ethyl 2-amino-5-methyl-4-phenylthiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*x*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-5-methyl-4-phenylthiophene 3-carboxylate **(I*x*,** 15.6 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (12 gm; 77%), m.p 261-264^{o}C, (262-264^{o}C)[4] characterized as 2-(chloromethyl)-6-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*x*)**.

Mol. Formula : $C_{14}H_{11}ClN_2OS$; Mol. Wt. 290.7
IR (KBr) cm^{-1} : 3035(γ_{CH2}), 1658(γ_{CONH}), 628($\gamma_{C\text{-}Cl}$)

NMR ($CDCl_3$)δppm : 2.40 (3H, s, C*H*$_3$ at 6), 4.42 (2H, s, *CH*$_2$ at 2), 7.38-7.44 (5H, m, Ar-*H* & *H* at 5 & 6).

11. Reaction of ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*xi*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene 3-carboxylate **(I*xi*,** 12.6 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (9.0 gm; 72%), m.p 276-278^{o}C (278-280^{o}C)[3], characterized as 2-chloromethyl-3,5,6,7-tetrahydrocyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*xi*).**

Mol. Formula : $C_{10}H_9ClN_2OS$; Mol. Wt. 240.7
IR (KBr) cm^{-1} : 1678(γ_{CONH}), 3015(γ_{CH2}), 754, 686, 625(γ_{C-Cl}).

12. Reaction of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene 3-carboxylate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*xii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate **(I*xii*,** 14.3 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60ml) for 6-8 hrs. The reaction mixture was worked up as for **II*i***. The crude product on recrystallisation from dioxane yielded fine needles (10 gm; 70%), m.p 188-190^{o}C, characterized as 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **(II*xii*)**.

M.P. : 188-190^{o}C; Yield: 70%
Mol. Formula : $C_{12}H_{13}ClN_2OS$; Mol. Wt. 268.7
IR (KBr) cm^{-1} : 2924(γ_{C-H}), 1670(γ_{CONH}), 1471(γ_{C-H}), 736, 752(γ_{C-Cl})

13. Reaction of 3-amino-2-carbethoxy-4,6-dimethylthieno[2,3-*b*]pyridine with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*xiii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 3-amino-2-carbethoxy-4,6-dimethylthieno[2,3-*b*]pyridine **(I*xiii*,** 1.5 gm; 0.0066 mole) and

chloroacetonitrile (0.98 gm; 0.012 mole) in dry dioxane (20 ml) for 6 hrs. at temperature below 10°C. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and dried. The crude product on recrystallization from chloroform-methanol mixture yield fine needles characterized as 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triaza-fluoren-8-one **(II*xiii*)**.

M.P. : 275-277°C (273-275°C)[7]; Yield: 90%
Mol. Formula : $C_{12}H_{10}Cl\,N_3OS$; Mol. Wt. 279.7
IR (KBr) cm^{-1} : 3013(γ_{C-H}), 1675(γ_{CONH}), 746(γ_{C-Cl})
MS m/e : 281(M+1), 279(M^+), 244, 216.

14. Reaction of 3-amino-2-carbethoxyquinazolin-4-one with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*xiv*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of 3-amino-2-carbethoxyquinazolin-4-one **(I*xiv*,** 2 gm; 0.0085 mole) and chloroacetonitrile (1.30 gm; 0.017 mole) in dry dioxane (20 ml) for 10 hrs. at temperature below 10°C. The reaction mixture was then worked up as for **II*i***. The solid separated was filtered, washed with water and dried. The crude product on recrystallization from chloroform-methanol mixture yielded fine needles, characterized as 2-(chloromethyl)-3*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-dione **(II*xiv*)**.

M.P. : 240-243°C (242-244°C)[16]; Yield: 60%
Mol. Formula : $C_{11}H_7Cl\,N_4O_2$; Mol. Wt. 262.6
IR (KBr) cm^{-1} : 2896(γ_{C-H}), 1686(γ_{CONH}), 778(γ_{C-Cl}).

15. Reaction methyl 2-amino-4,5-dimethoxybenzoate with chloroacetonitrile in the presence of dry hydrogen chloride gas (II*xv*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of methyl 2-amino-4,5-dimethoxybenzoate **(I*xv*,** 13.5 gm; 0.06 mole) and chloroacetonitrile (6.8 gm; 0.09 mole) in dry dioxane (60 ml) for 6 hrs. at temperature below 10°C. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and dried. The crude product on recrystallization from dioxane yielded fine needles characterized as 2-(chloromethyl)-6,7-dimethoxyquinazolin-4(3*H*)-one **(II*xv*)**.

M.P. : 240-245°C (240-245°C)[12]; Yield: 70%

Mol. Formula : $C_{11}H_{11}Cl\,N_2O_3$; Mol Wt. 254.6

IR (KBr) cm^{-1} : 3012(γ_{Ar-H}), 2888(γ_{C-H}), 1666(γ_{CONH}), 792, 754(γ_{C-Cl})

MS m/e : 254(M^+), 239, 219

16. Reaction of 2-carbethoxy-3-amino-4-methoxybenzo(*b*)thiophene with chloro-acetonitrile in the presence of dry hydrogen chloride gas (II*xvi*)

A stream of dry HCl gas was bubbled through an ice-cold mixture of 2-carbethoxy-3-amino-4-methoxybenzo(*b*)thiophene **(I*xvi*,** 2 gm; 0.0079 mole) and chloroacetonitrile (1.18 gm; 0.015 mole) in dry dioxane (20 ml) for 6 hrs. at temperature below 10°C. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and air dried. The crude product on recrystallization from chloroform-methanol mixture yield fine needles, characterized as 2-chloromethyl-9-methoxy-3*H*-benzo-[4,5]thieno[3,2-*d*]pyrimidin-4-one **(II*xvi*)**.

M.P. : 265-267°C; Yield: 70%

Mol. Formula : $C_{12}H_9Cl\,N_2O_2S$; Mol Wt. 280.7

IR(KBr)cm^{-1} : 2978(γ_{C-H}), 1676(γ_{CONH}), 736(γ_{C-Cl})

17. Synthesis of 2-chloromethylquinazolin-4(3*H*)-one (II*xvii*)

A stream of dry hydrogen chloride gas was bubbled through an ice-cold mixture of methylanthranilate (9.1 gm; 0.06 mole) and chloroacetonitrile (6.7 gm; 0.09 mole) in dry dioxane (60 ml) for 6 hrs at temperature below 10°C. The reaction mixture was allowed to stand at RT for 12 hrs. The reaction mixture was worked up as for **II*i***. The solid separated was filtered, washed with water and air dried. The crude product on recrystallization from chloroform-methanol mixture yield white crystals characterized as 2-chloromethylquinazolin-4(3*H*)-one **(II*xvii*)**.

M.P. : 257-258°C (257-258°C)[17]; Yield: 72.0%

Molecular formula : $C_9H_7ClN_2O$; Mol. wt: 194.5

IR (KBr) cm^{-1}: : 1699(γ_{CONH})

18. Synthesis of 6-(chloromethyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one (II*xviii*)

Mixture of 4-methylthio-1-phenyl-pyrazolo-*o*-aminoamide **(II*xvii*,** 2.0 gm, 0.008 mole) and potassium carbonate (6.67 gm, 0.048 mole) were taken in dimethylformamide (15 ml) and the reaction mixture was cooled to 0-5^{o}C. Chloroacetylchloride (3.13 gm, 0.028 mole) was then added dropwise and reaction was continued to stirr for 2 hrs. Progress of the reaction was monitored by TLC for the formation of the intermediate acetylated derivative. At this point, 50 ml of water was added to the reaction mixture and reaction was stirred further at 0-5^{o}C for 4-6 hrs. Thereafter, it was allowed to stand overnight. Next day the reaction mixture was poured into ice water mixture (100 ml) and the product precipitated out as yellow solid was filtered off and air dried and on recrystallization from chloroform-methanol mixture, yielded fine crystals, characterized as 6-(chloromethyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(II*xviii*)**.

M.P.	: 275-277^{o}C; Yield: 90%
Mol. Formula	: $C_{13}H_{11}ClN_4OS$; Mol. Wt. 306.07
IR (KBr) cm^{-1}	: 2849(γ_{C-H}), 1676(γ_{CONH}), 765(γ_{C-Cl})
^{1}H NMR(DMSO-d$_6$)δppm	: 2.50 (3H, s, C*H*$_3$), 4.35 (2H, s, *CH*$_2$Cl), 7.12-7.91 (5H, m, Ar-*H*).

4.4 Condensation of the condensed 2-chloromethylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones (II*i-xviii*) with 2-mercaptobenzimidazoles (III*i-xxxv*)

1. Condensation of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]-pyrimidin-4(3*H*)-one with 2-mercaptobenzimidazole (III*i*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride (Triethyl benzyl ammonium chloride; PTC) was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-5,6,7,8-tetrahydrobenzo-(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*i*,** 2.54 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. The reaction mixture was stirred at room temperature for 6-8 hrs. After completion of reaction, the organic phase was separated and washed with cold water. The organic layer on evaporation under reduced pressure gave crude product. The crude product on

recrystallization from methanol-chloroform, afforded pale yellow crystals (1.90 gm; 51% yield), m.p 264-267^{o}C, characterized as 2-(1*H*-benzimidazol-2-yl)methylthio-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4-(3*H*)-one (**III*i*)**.

M.P. : 264-267^{o}C; Yield: 51%

Mol. Formula : $C_{18}H_{16}N_4OS_2$; Mol. Wt. 368

IR (KBr) cm^{-1} : 3247(γ_{NH}), 2939(γ_{C-H}), 1680(γ_{CONH}), 743(γ_{C-S}).

NMR (DMSO-d_6)δppm : 1.83-1.88 (4H, m, C*H*$_2$ at 6 & 7), 2.76 (2H, t, C*H*$_2$ at 5, *J* = 5.64), 2.95 (2H, t, C*H*$_2$ at 8, *J* = 5.80), 4.39 (2H, s, CH_2 at SC*H*$_2$), 7.39-7.16 (4H, m, Ar*H*), 12.50 (1H, s, N*H*), 13.17 (1H, s, N*H*).

MS m/e : 368(M^+), 335, 307, 150.

2. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5,6,7,8-tetrahydro[1]benzo-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*ii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*i*,** 2.54 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 210-215^{o}C; Yield: 60%.

Mol. Formula : $C_{19}H_{18}N_4O_2S_2$; Mol. Wt. 398.5.

IR (KBr) cm^{-1} : 3266(γ_{NH}), 2940(γ_{C-H}), 1670(γ_{CONH}), 643(γ_{C-S}).

NMR(DMSO-d_6)δppm : 1.83-1.88 (4H, m, C*H*$_2$ at 6 & 7), 2.75 (2H, t, C*H*$_2$ at 5, *J* = 5), 2.97 (2H, t, C*H*$_2$ at 8, *J* = 5), 3.87 (3H, s, OC*H*$_3$), 4.32 (2H, s, CH_2 at SC*H*$_2$), 6.81-7.25 (3H, m, Ar*H*), 7.57 (1H, s, N*H*), 12.21 (1H, s, N*H*).

MS m/e : 398(M^+), 365, 219, 180.

3. Synthesis of methyl 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (III*iii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of methyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(II*ii*,** 2.72 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 258-260^{o}C; Yield: 65%.

Mol. Formula : $C_{17}H_{14}N_4O_3S_2$; Mol. Wt. 386.4.

IR (KBr) cm^{-1} : 3282(γ_{NH}), 2956(γ_{C-H}), 1667(γ_{CONH}), 741(γ_{C-S})

NMR (DMSO-d_6)δppm : 2.90 (3H, s, C*H*$_3$ at 5), 3.87 (3H, s, C*H*$_3$ of C*H*$_3$OOC), 4.36 (2H, s, C*H*$_2$ at SCH_2), 7.17 (2H, q, *H* at imidazole, *J* = 3.2), 7.53 (2H, q, imidazole, *J* = 3.16).

MS m/e : 386(M^+), 353, 150.

4. Synthesis of methyl 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (III*iv*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of methyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(II*ii*,** 2.72 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 230-235^{o}C; Yield: 73%.

Mol. Formula : $C_{18}H_{16}N_4O_4S_2$; Mol. Wt. 416.4.

IR (KBr) cm^{-1} : 3339(γ_{NH}), 2943(γ_{C-H}), 1690(γ_{CONH}), 613(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.68 (3H, s, C*H*$_3$ at 5), 3.86 (3H, s, OC*H*$_3$), 3.88 (3H, s, C*H*$_3$-O-CO-), 4.65 (2H, s, CH_2 at SC*H*$_2$), 7.05 (1H, dd, C*H* at imidazole, *J* = 2.30 & 6.64), 7.15 (1H, d, C*H* at imidazole, *J* = 2.2), 7.54

(1H, d, C*H* at imidazole, *J* = 8.92), 12.47 (1H, br s, N*H*), 13.45 (1H, s, N*H*).

MS m/e : 416(M^+), 383, 210, 180.

5. Synthesis of ethyl 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (III*v*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of ethyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate (**II*iii***, 2.86 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 270-272^{o}C; Yield: 52%.

Mol. Formula : $C_{18}H_{16}N_4O_3S_2$; Mol. Wt. 400.

IR(KBr) cm^{-1} : 3237(γ_{NH}), 2945(γ_{C-H}), 1716(γ_{COOEt}), 1673(γ_{CONH}), 740(γ_{C-S}).

MS m/e : 400(M^+), 372, 296, 150.

6. Synthesis of ethyl 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (III*vi*)

2-Mercapto-5-methoxybenzimidazole (1.8 g; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of ethyl 2-(chloromethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(II*iii*,** 2.86 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for compound the **III*i*** to get the title compound.

M.P. : 257-260^{o}C; Yield: 65%.

Mol. Formula : $C_{19}H_{18}N_4O_4S_2$; Mol. Wt. 430.5.

IR (KBr) cm^{-1} : 3247(γ_{NH}), 2985(γ_{C-H}), 1690(γ_{CONH}), 650(γ_{C-S}).

NMR (DMSO-d_6)δppm : 1.38 (3H, t, CH_3 of C*H*$_3$-CH_2-O-, *J* = 6), 2.67 (3H, s, C*H*$_3$ at 5), 3.84 (3H, s, OC*H*$_3$), 4.20-4-38 (4H, m, CH_3-C*H*$_2$-O and SC*H*$_2$),

6.82 (1H, dd, C*H* at Imidazole *J* = 6.8 & 2.04); 7.03 (1H, s, C*H* at imidazole), 7.42 (1H, d, C*H* at imidazole), 10.84 (1H, br s, N*H*), 12.94 (1H, br s, N*H*).

MS m/e : 430(M^+), 397, 369, 180.

7. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-5,6-dimethylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one (III*vii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*iv*,** 2.28 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 272-275^{o}C; Yield: 48%.

Mol. Formula : $C_{16}H_{14}N_4OS_2$; Mol. Wt. 342.

IR (KBr) cm^{-1} : 3263(γ_{NH}), 2917(γ_{C-H}), 1669(γ_{CONH}), 605(γ_{C-S}).

8. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*viii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*iv*,** 2.28 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 130-134^{o}C; Yield: 65%.

Mol. Formula : $C_{17}H_{16}N_4O_2S_2$; Mol. Wt. 372.4.

IR (KBr) cm^{-1} : 3306(γ_{NH}), 2966(γ_{C-H}), 1683(γ_{CONH}), 601(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.38 (3H, s, C*H*$_3$ at 5), 2.47 (3H, s, C*H*$_3$ at 6), 3.80 (3H, s, OC*H*$_3$), 4.27 (2H, s, CH_2 at SC*H*$_2$), 6.77-6.80 (3H, m, Ar-*H*), 10.19 (1H, s, N*H*), 13.25 (1H, s, N*H*).

MS m/e : 368(M^+), 339, 180.

9. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-phenylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one (III*ix*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*v*,** 2.76 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 224-227^{o}C; Yield: 60%.

Mol. Formula : $C_{20}H_{14}N_4OS_2$; Mol. Wt. 390.4.

IR (KBr) cm^{-1} : 3044(γ_{C-H}), 1680(γ_{CONH}), 741(γ_{C-S}).

NMR (DMSO-d_6)δppm : 4.39 (2H, s, CH_2 at SC*H*$_2$), 7.10 (1H, s, *H* at 6), 7.16-7.54 (9H, m, Ar-*H*).

MS m/e : 390(M^+), 357, 272, 150.

10. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-phenyl-thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (III*x*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-phenylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(II*v*,** 2.76 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 165-170^{o}C; Yield: 65%.

Mol. Formula : $C_{21}H_{16}N_4O_2S_2$; Mol. Wt. 420.5.

IR (KBr) cm^{-1} : 3091(γ_{NH}), 2988(γ_{C-H}), 1685(γ_{CONH}), 620(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.82 (3H, s, OC*H*$_3$), 4.36 (2H, s, CH_2 at SC*H*$_2$), 6.81 (1H, dd, C*H* at Imidazole, *J* = 6.44 & 2.36), 7.11 (1H, s, C*H* at 6), 7.01-7.56 (7H, m, Ar-*H*).

MS m/e : 420(M^+), 387, 256, 180.

11. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-(4-methoxyphenyl)-thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (III*xi*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*vi*,** 3.0 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 244-248^oC; Yield: 70%.

Mol. Formula : $C_{21}H_{16}N_4O_2S_2$; Mol. Wt. 420.5.

IR (KBr) cm^{-1} : 3256(γ_{NH}), 2839(γ_{C-H}), 1663(γ_{CONH}), 746(γ_{C-S})

NMR (DMSO-d_6)δppm : 3.82 (3H, s, OCH_3), 4.43 (2H, s, C*H*$_2$ at SCH_2), 6.84 (2H, d, *H* at imidazole, *J* = 8.6), 7.08 (1H, s, *H* at 7), 7.17 (2H, q, *H* at imidazole, *J* = 3.16), 7.47-7.52 (4H, m, Ar-*H*), 12.40 (1H, s, N*H*), 13.25 (1H, s, N*H*).

MS m/e : 421(M^+), 387, 359, 159.

12. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-(4-methoxy-phenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*xii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-methoxyphenyl)thieno-[2,3-*d*]pyrimidin-4(3*H*)-one **(II*vi*,** 3.0 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 140-142^{o}C; Yield: 73%.

Mol. Formula : $C_{22}H_{18}N_4O_3S_2$; Mol. Wt. 450.5.

IR (KBr) cm^{-1} : 3242(γ_{NH}), 2941(γ_{C-H}), 1690(γ_{CONH}), 603(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.34 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 4.34 (2H, s, CH_2 at SC*H*$_2$), 7.06 (H, s, *H* at thiophene), 6.89-7.50 (7H, m, Ar-*H*), 12.35 (1H, br s, N*H*), 13.20 (1H, s, N*H*).

MS m/e : 450(M^+), 272, 180.

13. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-(4-methylphenyl)-thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (III*xiii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*vii*,** 2.90 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 260-262^{o}C; Yield: 73%.

Mol. Formula : $C_{21}H_{16}N_4OS_2$; Mol. Wt. 404.5.

IR (KBr) cm^{-1} : 3229(γ_{NH}), 3032(γ_{C-H}), 1685(γ_{CONH}), 743(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.36 (3H, s, C*H*$_3$), 4.42 (2H, s, C*H*$_2$ at SCH_2), 7.10 (1H, s, *H* at 6), 7.15-7.43 (8H, m, Ar-*H*), 12.54 (1H, s, N*H*), 13.37 (1H, s, N*H*).

MS m/e : 404(M^+), 371, 343, 150.

14. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-(4-methyl-phenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*xiv*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*vii*,** 2.90 gm; 0.01 mole) in methylene dichloride (25 ml), over

a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 245-247^{o}C; Yield: 65%.
Mol. Formula : $C_{22}H_{18}N_4O_3S_2$; Mol. Wt. 434.5.
IR (KBr) cm^{-1} : 3246(γ_{NH}), 2878(γ_{C-H}), 1659(γ_{CONH}), 627(γ_{C-S}).
MS m/e : 434(M^+), 401, 270, 180.

15. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-(4-bromophenyl)-thieno-[2,3-*d*]pyrimidin-4(3*H*)-one (III*xv*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 5-(4-bromophenyl)-2-(chloromethyl)thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(II*viii*,** 3.5 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 247-249^{o}C; Yield: 68%.
Mol. Formula : $C_{20}H_{13}BrN_4OS_2$; Mol. Wt. 469.3.
IR (KBr) cm^{-1} : 3235(γ_{NH}), 2940(γ_{C-H}), 1647(γ_{CONH}), 756(γ_{C-S}).
NMR (DMSO-d_6)δppm : 4.42 (2H, s, C*H*$_2$ at SCH$_2$), 7.16-7.20 (3H, m, *H* at imidazole), 7.43-7.56 (6H, m, Ar-*H*); 12.45 (1H, s, N*H*), 13.55 (1H, s, N*H*).
MS m/e : 470(M^+), 437, 150.

16. Synthesis of 5-(4-bromophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)-thio]-methyl}thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*xvi*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 5-(4-bromophenyl)-2-(chloromethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*viii*,** 3.5 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 135-141^{o}C; Yield: 67%.

Mol. Formula : $C_{21}H_{15}BrN_4O_2S_2$; Mol. Wt. 499.4.

IR (KBr) cm^{-1} : 3262(γ_{NH}), 2945(γ_{C-H}), 1690(γ_{CONH}), 666(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.82 (3H, s, OC*H*$_3$), 4.37 (2H, s, CH_2 at SC*H*$_2$), 6.80 (1H, dd, C*H* at imidazole *J* = 2.4 & 6.5), 6.99 (1H, s, C*H* at imidazole), 7.14 (1H, s, C*H* at imodazole), 7.38-7.51 (5H, m, 4Ar-*H* and 1*H* at 6 of thiophene), 12.50 (1H, br s, N*H*).

MS m/e : 500(M^+), 322, 180.

17. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-5-(4-chlorophenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*xvii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*ix*,** 3.11 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 227-230^{o}C; Yield: 58%.

Mol. Formula : $C_{20}H_{13}ClN_4OS_2$; Mol. Wt. 425.

IR (KBr) cm^{-1} : 3279(γ_{NH}), 1662(γ_{CONH}),743.18 (γ_{C-S}).

MS m/e : 424(M^+), 391, 363, 150.

18. Synthesis of 5-(4-chlorophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]-methyl}thieno[2,3-*d*]pyrimidin-4(3*H*)-one (III*xviii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*ix*,** 3.11 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 128-130^{o}C; Yield: 71%.

Mol. Formula : $C_{21}H_{15}ClN_4O_2S_2$; Mol. Wt. 454.9.

IR (KBr) cm^{-1} : 3194(γ_{NH}), 3090(γ_{C-H}), 1680(γ_{CONH}), 622(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.82 (3H, s, OC*H*$_3$), 4.38 (2H, s, CH_2 at SC*H*$_2$), 7.15 (1H, s, C*H* at 6), 6.80 (1H, dd, C*H* at imidazole, *J* = 2.4 & 6.36), 7.00 (1H, d, C*H* at imidazole, *J* = 2.16), 7.40 (1H, d, C*H* at imidazole, *J* = 8.76), 7.32-7.52 (4H, m, Ar-*H*).

MS m/e : 454(M^+), 421, 276, 180.

19. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-6-methyl-5-phenylthieno-[2,3-*d*]pyrimidin-4(3*H*)-one (III*xix*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-6-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*x*,** 2.9 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 263-265^{o}C; Yield: 51%.

Mol. formula : $C_{21}H_{16}N_4OS_2$; Mol. Wt. 404.

IR (KBr) cm^{-1} : 3243(γ_{NH}), 2937(γ_{C-H}), 1656(γ_{CONH}), 740(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.20 (3H, s, C*H*$_3$ at 6), 4.51 (2H, s, C*H*$_2$ at C*H*$_2$S), 7.12-7.61 (9H, m, Ar-*H*); 12.25 (1H, s, N*H*), 13.00 (1H, s, N*H*).

MS m/e : 404(M^+), 371, 343, 150.

20. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-3,5,6,7-tetrahydro-4*H*-cyclo-penta[4,5]thieno[2,3-*d*]pyrimidin-4-one (III*xx*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-3,5,6,7-tetrahydrocyclopenta[4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*xi*,** 2.40 gm; 0.01 mole) in methylene dichloride (25

ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 258-260^{o}C; Yield: 65%.

Mol. Formula : $C_{17}H_{14}N_4OS_2$; Mol. Wt. 354.4.

IR (KBr) cm^{-1} : 3247(γ_{NH}), 2943(γ_{C-H}), 1685(γ_{CONH}), 741(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.45 (2H, m, C*H*$_2$ at 6), 2.94 (4H, m, C*H*$_2$ at 5 and 7), 4.40 (2H, s, C*H*$_2$ at SCH_2), 7.19 (2H, m, *H* at imidazole), 7.51 (2H, m, *H* at imidazole), 12.90 (1H, s, N*H*), 13.45 (1H, s, N*H*).

MS m/e : 354(M^+), 321, 293, 205, 150.

21. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-3,5,6,7-tetra-hydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (III*xxi*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-3,5,6,7-tetrahydrocyclopenta-[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(II*xi*,** 2.40 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 162-165^{o}C; Yield: 65%.

Mol. Formula : $C_{18}H_{16}N_4O_2S_2$; Mol. Wt. 384.4.

IR (KBr) cm^{-1} : 3235(γ_{NH}), 2992(γ_{C-H}), 1668(γ_{CONH}), 665(γ_{C-S}).

MS m/e : 384(M^+), 351, 205, 180.

22. Synthesis of 2-[(1*H*-benzimidazol-2-ylthio)methyl]-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]-thieno[2,3-*d*]pyrimidin-4-one (III*xxii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **(II*xii*,** 2.68 gm; 0.01 mole) in methylene dichloride (25 ml), over a

period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 264-266^{o}C; Yield: 68%.

Mol. Formula : $C_{19}H_{18}N_4OS_2$; Mol. Wt. 382.5.

IR (KBr) cm^{-1} : 3212(γ_{NH}), 2916(γ_{C-H}), 1685(γ_{CONH}), 737(γ_{C-S}).

NMR (DMSO-d_6)δppm : 1.68 (6H, m, C*H*$_2$ at 6, 7, & 8), 1.88 (2H, t, C*H*$_2$ at 5, *J* = 3.36), 3.30 (2H, t, C*H*$_2$ at 9, *J* = 5.36), 4.36 (2H, s, C*H*$_2$ at SCH_2), 7.20 (2H, m, *H* at imidazole), 7.56 (2H, m, *H* at imidazole, *J* = 3.16), 12.50 (1H, s, N*H*), 13.40 (1H, s, N*H*).

MS m/e : 382(M^+), 349, 232, 150.

23. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-3,5,6,7,8,9-hexa-hydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (III*xxiii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **(II*xii*,** 2.68 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 226-230^{o}C; Yield: 70%.

Mol. Formula : $C_{20}H_{20}N_4O_2S_2$; Mol. Wt. 412.5.

IR (KBr) cm^{-1} : 3190(γ_{NH}), 2918(γ_{C-H}), 1685(γ_{CONH}), 750(γ_{C-S}).

NMR (DMSO-d_6)δppm : 1.66 (4H, m, C*H*$_2$ at 6 & 7), 1.87 (2H, m, C*H*$_2$ at 5), 2.85 (2H, m, C*H*$_2$ at 8), 3.29(2H, m, C*H*$_2$ at 9), 3.84 (3H, s, C*H*$_3$ at OCH_2), 4.34 (2H, s, SC*H*$_2$), 6.79-7.43 (3H, m, Ar-*H*), 12.30 (1H, s, N*H*), 13.21 (1H, s, N*H*).

MS m/e : 412(M^+), 379, 232, 180.

24. Synthesis of 2-(1*H*-benzimidazol-2-yl)methylthioquinazolin-4-(3*H*)-one (III*xxiv*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA

chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethylquinazoline-4(3*H*)-one **(II*xiii*,** 1.94 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 196-198^{o}C; Yield: 62%.
Mol. Formula : $C_{16}H_{12}N_4OS$; Mol. Wt. 308
IR (KBr) cm^{-1} : 3145(γ_{NH}), 1670(γ_{CONH}), 742(γ_{C-S})
NMR (DMSO-d_6)δppm : 4.53 (2H, s, C*H*$_2$ at C*H*$_2$S), 7.11-8.09 (8H, m, Ar-*H*).
MS m/e. : 308(M^+), 275, 247, 163.

25. Synthesis of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-quinazolin-4(3*H*)-one (III*xxv*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethylquinazoline-4(3*H*)-one **(II*xiii*,** 1.94 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 177-179^{o}C; Yield: 71%
Mol. Formula : $C_{17}H_{14}N_4O_2S$; Mol. Wt. 338.4
IR (KBr) cm^{-1} : 3061(γ_{NH}), 1675(γ_{CONH}), 772(γ_{C-S}).

26. Synthesis of 6-(1*H*-benzoimidazol-2-ylsulfanylmethyl)-2,4-dimethyl-7*H*-9-thia-1,5,7-triazafluoren-8-one (III*xxvi*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triazafluoren-8-one **(II*xiv*,** 2.79 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 120-122^{o}C; Yield: 70%

Mol. Formula : $C_{19}H_{15}N_5OS_2$; Mol. Wt. 393.4

IR (KBr) cm^{-1} : 2922(γ_{C-H}), 1685(γ_{CONH}), 1570(γ_{C-C}) 738(γ_{C-S})

27. Synthesis of 6-(5-methoxy-1*H*-benzoimidazol-2-ylsulfanylmethyl)-2,4-dimethyl-7*H*-9-thia-1,5,7-triazafluoren-8-one (III*xxvii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triaza-fluoren-8-one **(II*xiv*,** 2.79 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 178-180^{o}C; Yield: 75%

Mol. Formula : $C_{20}H_{17}N_5O_2S_2$; Mol. Wt. 423.5

IR (KBr) cm^{-1} : 2922(γ_{C-H}), 1654(γ_{CONH}), 1570(γ_{C-C}), 785(γ_{C-S})

MS m/e : 423(M^+), 390, 362, 180.

28. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3*H*-[1,2,4]triazino[6,1-*b*]-quinazoline-4,10-dione (III*xxviii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-3*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-dione **(II*xv*,** 2.62 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 180-182^{o}C; Yield: 65%

Mol. Formula : $C_{18}H_{12}N_6O_2S$; Mol. Wt. 376.39

IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1670(γ_{CONH}), 1606(γ_{C-C}), 742(γ_{C-S})

29. Synthesis of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3*H*-[1,2,4]-triazino[6,1-*b*]quinazolin-4,10-dione (III*xxix*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-3*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-dione **(II*xv*,** 2.62 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 154-156^{o}C; Yield: 72%
Mol. Formula : $C_{19}H_{14}N_6O_3S$; Mol. Wt. 406.4
IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1672(γ_{CONH}), 1607(γ_{C-C}), 774(γ_{C-S})

30. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-6,7-dimethoxy-quinazolin-4(3*H*)-one (III*xxx*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-6,7-dimethoxyquinazolin-4(3*H*)-one **(II*xvi*,** 2.54 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 200-202^{o}C; Yield: 56%
Mol. Formula : $C_{18}H_{16}N_4O_3S$; Mol. Wt. 368.4
IR (KBr) cm^{-1} : 3201(γ_{NH}), 1662(γ_{CONH}), 1608(γ_{C-C}), 742(γ_{C-S})

31. Synthesis of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-6,7-dimethoxy-quinazolin-4(3*H*)-one (III*xxxi*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-(chloromethyl)-6,7-dimethoxyquinazolin-4(3*H*)-one **(II*xvi*,** 2.54 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of

15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 115-118^{o}C; Yield: 68%
Mol. Formula : $C_{19}H_{18}N_4O_4S$; Mol. Wt. 398.4
IR (KBr) cm^{-1} : 2926(γ_{C-H}), 1647(γ_{CONH}), 785(γ_{C-S})
MS m/e : 398(M^+), 365, 220, 180.

32. Synthesis of 9-methoxy-2-(1*H*-benzoimidazol-2-ylsulfanylmethyl)-3*H*-benzo[4,5]-thieno[3,2-*d*]pyrimidin-4-one (III*xxii*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **(II*xvii*,** 2.8 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 155-158^{o}C; Yield: 55%
Mol. Formula : $C_{19}H_{14}N_4O_2S_2$; Mol. Wt. 394.4
IR (KBr) cm^{-1} : 2916(γ_{C-H}), 1663(γ_{CONH}), 736(γ_{C-S})

33. Synthesis of 9-methoxy-2-(5-methoxy-1*H*-benzoimidazol-2-ylsulfanyl-methyl)-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (III*xxiii*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 2-chloromethyl-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **(II*xvii*,** 2.8 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 115-120^{o}C; Yield: 45%
Mol. Formula : $C_{20}H_{16}N_4O_3S_2$; Mol. Wt. 424.5

IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1669(γ_{CONH}), 738(γ_{C-S})

34. Synthesis of 6-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (III*xxxiv*)

2-Mercaptobenzimidazole (1.5 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 6-(chloromethyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(II*xviii*,** 3.0 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 240-242^{o}C; Yield: 65%

Mol. Formula : $C_{20}H_{16}N_6OS_2$; Mol. Wt. 420.5

IR (KBr) cm^{-1} : 2922(γ_{C-H}), 1691(γ_{CONH}), 739(γ_{C-S})

35. Synthesis of 6-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (III*xxxv*)

2-Mercapto-5-methoxybenzimidazole (1.8 gm; 0.01 mole) was dissolved in 25 ml of 10% sodium hydroxide solution by stirring at room temperature. To this clear solution pinch of TEBA chloride was added and stirring was further continued for 10 min. To this well stirred solution, added clear solution of 6-(chloromethyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(II*xviii*,** 3.0 gm; 0.01 mole) in methylene dichloride (25 ml), over a period of 15-20 min. Reaction was worked up as described for the compound **III*i*** to get the title compound.

M.P. : 82-85^{o}C; Yield: 50%

Mol. Formula : $C_{21}H_{18}N_6O_2S_2$; Mol. Wt. 450.5

IR (KBr) cm^{-1} : 3123(γ_{C-H}), 1685(γ_{CONH}), 1589(γ_{C-C}), 758(γ_{C-S}).

MS m/e : 450(M^+), 417, 272, 180.

4.5 Oxidation of condensed 2-(1*H*-benzimidazole-2-yl)methylthiopyrimidin-4(3*H*)-ones using *meta*-chloro perbenzoic acid (*m*-CPBA) to obtain corresponding sulfinyl derivatives (IV*i-xxxv*)

1. Synthesis of 2-(1*H*-benzimidazol-2-yl)methylsulfinyl-5,6,7,8-tetrahydro-benzo-(*b*)sulfinyl[2,3-*d*]pyrimidin-4-(3*H*)-one using *m*-CPBA (IV*i*)

2-(1*H*-Benzimidazol-2-yl)methylthio-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-(3*H*)-one (**III*i***, 1.98 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. The reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added with stirring and continued the stirring for 30-45 mins. Progress of the reaction was monitored using precoated TLC using benzene: methanol::4.5:0.5 as a solvent system. After completion of the reaction, the reaction was stopped by the addition of 10% sodium bicarbonate. The organic layer was separated and washed with 10% NaCl and water (50 ml each). The organic layer was dried with anhydrous sodium sulphate and solvent was distilled out under reduced pressure to obtain the crude product and on recrystallization from methanol-chloroform, afforded crystalline material (0.78 gm; 74% yield), m.p. 216-218^{o}C.

Mol. Formula : $C_{18}H_{16}N_4O_2S_2$; Mol. Wt. 384

IR (KBr) cm^{-1} : 3235(γ_{NH}), 2946(γ_{C-H}), 1655(γ_{CONH}), 1060(γ_{S-O}), 747(γ_{C-S})

NMR (DMSO-d_6)δppm : 1.79-1.83 (4H, m, C*H*$_2$ at 6 & 7), 2.54-2.79 (4H, m, C*H*$_2$ at 5 & 8), 5.58 (2H, s, CH_2 at SC*H*$_2$), 7.39-7.16 (4H, m, Ar-*H*), 12.60 (1H, s, N*H*), 13.80 (1H, s, N*H*).

MS(m/e) : 368, 364, 335, 218.

2. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl) sulfinyl]methyl}-5,6,7,8-tetra-hydro[1]benzo-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*ii*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5,6,7,8-tetrahydro[1]benzothieno-[2,3-*d*]pyrimidin-4(3*H*)-one **(III*ii*,** 2.14 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was

added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 194-196^{o}C; Yield: 76%.

Mol. Formula : $C_{19}H_{18}N_4O_3S_2$; Mol. Wt. 414.5.

IR (KBr) cm^{-1} : 3054(γ_{NH}), 2934(γ_{C-H}), 1681(γ_{CONH}), 1045(γ_{S-O}), 716(γ_{C-S}).

3. Synthesis of methyl 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (IV*iii*)

Methyl 2-[(1*H*-benzimidazol-2-ylthio)methyl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]-pyrimidine 6-carboxylate **(III*iii*,** 2.08 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 210-214^{o}C; Yield: 10%.

Mol. formula : $C_{17}H_{14}N_4O_4S_2$; Mol. Wt. 402.4

IR (KBr) cm^{-1} : 3237(γ_{NH}), 3078(γ_{C-H}), 1726(γ_{COO}),1682(γ_{CONH}), 1097(γ_{S-O}), 743(γ_{C-S}).

4. Synthesis of methyl 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylate (IV*iv*)

Methyl-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate **(III*iv*,** 2.24 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 200-205^{o}C; Yield: 10%.

Mol. formula : $C_{18}H_{16}N_4O_5S_2$; Mol. Wt. 432.4.

IR (KBr) cm^{-1} : 3008(γ_{C-H}), 1722(γ_{COO-}), 1659(γ_{CONH}), 1026(γ_{S-O}), 761(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.67 (3H, s, C*H*$_3$ at 5), 3.88 (3H, s, OC*H*$_3$), 3.87 (3H, s, C*H*$_3$-O-CO-), 5.50 (2H, s, CH_2 at SC*H*$_2$), 7.03 (1H, dd, C*H* at imidazole, *J* = 2.30 & 6.62), 7.17 (1H, d, C*H* at imidazole, *J* = 2.2), 7.52 (1H, d, C*H* at imidazole, *J* = 8), 12.40 (1H, br s, N*H*), 13.20 (1H, s, N*H*).

5. Synthesis of ethyl 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-5-methyl-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidine-6-carboxylate (IV*v*)

Ethyl 2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-5-methyl-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidine 6-carboxylate **(III*v*,** 2.16 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 220-222^{o}C; Yield: 41%.

Mol. formula : $C_{18}H_{16}N_4O_4S_2$; Mol. Wt. 416.

IR(KBr) cm^{-1} : 3239(γ_{NH}), 2946(γ_{C-H}), 1717(γ_{COOEt}), 1670(γ_{CONH}), 1038(γ_{S-O}), 741(γ_{C-S}).

6. Synthesis of ethyl 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine 6-carboxylate (IV*vi*)

Ethyl 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-methyl-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidine 6-carboxylate **(III*vi*,** 2.30 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 200-207^{o}C; Yield: 43%.

Mol. formula : $C_{19}H_{18}N_4O_5S_2$; Mol. Wt. 446.5.

IR (KBr) cm^{-1} : 3175(γ_{NH}), 2978(γ_{C-H}), 1715(γ_{COO-}), 1659(γ_{CONH}), 1029(γ_{S-O}), 754(γ_{C-S}).

7. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*vii*)

2-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*vii*,** 1.84 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 160-162^oC; Yield: 65%.
Mol. formula : $C_{16}H_{14}N_4O_2S_2$; Mol. Wt. 358.
IR (KBr) cm^{-1} : 3379(γ_{NH}), 3057(γ_{C-H}), 1681(γ_{CONH}), 1055(γ_{S-O}), 740(γ_{C-S}).

8. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*viii*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5,6-dimethylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(III*viii*,** 2.0 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 200-202^oC; Yield: 60%.
Mol. formula : $C_{17}H_{16}N_4O_3S_2$; Mol. Wt. 388.4.
IR (KBr) cm^{-1} : 3174(γ_{NH}), 2893(γ_{C-H}), 1651(γ_{CONH}), 1050(γ_{S-O}), 801(γ_{C-S}).

9. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-phenylthieno[2,3-*d*]-pyrimidin-4(3*H*)-one (IV*ix*)

2-[(1*H*-Benzimidazol-2-ylthio)methyl]5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*ix*,** 2.10 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution,

methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 207-210^{o}C; Yield: 95%.
Mol. formula : $C_{20}H_{14}N_4O_2S_2$; Mol. Wt. 406.4
IR (KBr) cm^{-1} : 3193(γ_{NH}), 2971(γ_{C-H}), 1680(γ_{CONH}), 1046(γ_{S-O}), 740(γ_{C-S}).
MS(m/e) : 368, 364, 335, 218.

10. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-phenyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*x*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*x*,** 2. 26 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 195-198^{o}C; Yield: 91%.
Mol. formula : $C_{21}H_{16}N_4O_3S_2$; Mol. Wt. 436.5.
IR (KBr) cm^{-1} : 3120(γ_{NH}), 2883(γ_{C-H}), 1677(γ_{CONH}), 1046(γ_{S-O}), 697(γ_{C-S}).

11. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-(4-methoxy-phenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xi*).

2-[(1*H*-Benzimidazol-2-ylthio)methyl]-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*xi*,** 2.26 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 214-218^{o}C; Yield: 77%.

Mol. formula : $C_{21}H_{16}N_4O_3S_2$; Mol. Wt. 436.5

IR (KBr) cm^{-1} : 3335(γ_{NH}), 3058(γ_{C-H}), 1677(γ_{CONH}), 1046(γ_{S-O}), 745(γ_{C-S}).

12. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-(4-methoxyphenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xii*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-(4-methoxyphenyl)-thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(III*xii*,** 2.4 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 220-225^oC; Yield: 77%.

Mol. formula : $C_{22}H_{18}N_4O_4S_2$; Mol. Wt. 466.5.

IR (KBr) cm^{-1} : 3119(γ_{NH}), 2997(γ_{C-H}), 1675(γ_{CONH}), 1045(γ_{C-S}), 703(γ_{C-S}).

13. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-(4-methylphenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xiii*).

2-[(1*H*-Benzimidazol-2-ylthio)methyl]-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*xiii*,** 2.18 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 215-217^oC; Yield: 91%.

Mol. formula : $C_{21}H_{16}N_4O_2S_2$; Mol. Wt. 420.5

IR (KBr) cm^{-1} : 3195(γ_{NH}), 3055(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 739(γ_{C-S}).

NMR (DMSO-d_6)δppm : 2.43 (3H, s, C*H*$_3$), 5.50 (2H, s, C*H*$_2$ at SCH_2), 7.15-7-75 (9H, m, *H* at 6 and Ar-*H*), 12.56 (1H, s, N*H*), 13.50 (1H, s, N*H*).

14. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-(4-methyl-phenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xiv*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-5-(4-methylphenyl)-thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(III*xiv*,** 2.3 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 203-207^{o}C; Yield: 90%.
Mol. formula : $C_{22}H_{18}N_4O_3S_2$; Mol. Wt. 450.
IR (KBr) cm^{-1} : 3123(γ_{NH}), 2895(γ_{C-H}), 1678(γ_{CONH}), 1046(γ_{S-O}), 768(γ_{C-S}).

15. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-5-(4-bromophenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xv*)

2-[(1*H*-Benzimidazol-2-ylthio)methyl]-5-(4-bromophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*xv*,** 2.53 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0-2^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 214-217^{o}C; Yield: 72%.
Mol. formula : $C_{20}H_{13}BrN_4O_2S_2$; Mol. Wt. 485.3
IR (KBr) cm^{-1} : 3189(γ_{NH}), 3074(γ_{C-H}), 1678(γ_{CONH}), 1045(γ_{S-O}), 746(γ_{C-S}).

16. Synthesis of 5-(4-bromophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)-sulfinyl]-methyl}-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xvi*)

5-(4-Bromophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(III*xvi*,** 2.7 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To

this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 214-216^{o}C; Yield: 60%.
Mol. formula : $C_{21}H_{15}BrN_4O_3S_2$; Mol. Wt. 515.4.
IR (KBr) cm^{-1} : 3118(γ_{NH}), 2894(γ_{C-H}), 1677(γ_{CONH}), 1044(γ_{S-O}), 772(γ_{C-S}).
MS(m/e) : 496, 446, 332, 180.

17. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-5-(4-chloro-phenyl)-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xvii*)

2-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*xvii*,** 2.3 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 180-182^{o}C; Yield: 51%.
Mol. formula : $C_{20}H_{13}ClN_4O_2S_2$; Mol. Wt. 440.9.
IR (KBr) cm^{-1} : 3280(γ_{NH}), 1665(γ_{CONH}), 1056(γ_{S-O}), 743.18(γ_{C-S}).

18. Synthesis of 5-(4-chlorophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)-sulfinyl]-methyl}thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xviii*)

5-(4-Chlorophenyl)-2-{[(5-methoxy-1*H*-benzimidazol-2-yl)thio]methyl}thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(III*xviii*,** 2. 54 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 214-219^{o}C; Yield: 82%.

Mol. formula : $C_{21}H_{15}ClN_4O_3S_2$; Mol. Wt. 470.

IR (KBr) cm^{-1} : 3119(γ_{NH}), 2882(γ_{C-H}), 1677(γ_{CONH}), 1044(γ_{C-S}), 772(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.85 (3H, s, OC*H*$_3$), 5.52 (2H, s, CH_2 at SC*H*$_2$), 7.13 (1H, s, C*H* at 6), 6.81(1H, dd, C*H* at imidazole, *J* = 2.4 & 6.36), 7.05 (1H, d, C*H* at imidazole, *J* = 2.16), 7.45 (1H, d, C*H* at imidazole, *J* = 8.76), 7.30-7.55 (4H, m, Ar-*H*).

19. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-6-methyl-5-phenyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one (IV*xix*)

2-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)-6-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(III*xix***, 2.10 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 180-182^{o}C; Yield: 30%.

Mol. formula : $C_{21}H_{16}N_4O_2S_2$; Mol. Wt. 420.

IR (KBr) cm^{-1} : 3351(γ_{NH}), 3077(γ_{C-H}), 1681(γ_{CONH}), 1054(γ_{S-O}), 742(γ_{C-S}).

20. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-3,5,6,7-tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (IV*xx*)

2-[(1*H*-Benzimidazol-2-ylthio)methyl]-3,5,6,7-tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one **(III*xx*,** 1.90 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^{o}C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 221-225^{o}C; Yield: 60%.

Mol. formula : $C_{17}H_{14}N_4O_2S_2$; Mol. Wt. 370.4

IR (KBr) cm^{-1} : 3218(γ_{NH}), 2960(γ_{C-H}), 1661(γ_{CONH}), 1057(γ_{S-O}), 745(γ_{C-S}).

21. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-3,5,6,7-tetra-hydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (IV*xxi*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-3,5,6,7-tetrahydro-4*H*-cyclopenta-[4,5]thieno[2,3-*d*]pyrimidin-4-one **(III*xxi*,** 2.0 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 210-212^oC; Yield: 48%.

Mol. formula : $C_{18}H_{16}N_4O_3S_2$; Mol. Wt. 400.4.

IR (KBr) cm^{-1} : 3394(γ_{NH}), 2954(γ_{C-H}), 1659(γ_{CONH}), 1029(γ_{S-O}), 808(γ_{C-S}).

22. Synthesis of 2-[(1*H*-benzimidazol-2-ylsulfinyl)methyl]-3,5,6,7,8,9-hexa-hydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (IV*xxii*)

2-[(1*H*-Benzimidazol-2-ylthio)methyl]-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno-[2,3-*d*]pyrimidin-4-one **(III*xxii*,** 2.0 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 196-199^oC; Yield: 37%.

Mol. formula : $C_{19}H_{18}N_4O_2S_2$; Mol. Wt. 398.5

IR (KBr) cm^{-1} : 3250(γ_{NH}), 2916(γ_{C-H}), 1651(γ_{CONH}), 1057(γ_{S-O}), 744(γ_{C-S}).

NMR (DMSO-d_6)δppm : 1.68-1.71 (6H, m, C*H*$_2$ at 6, 7, & 8), 1.88 (2H, t, C*H*$_2$ at 5, *J* = 3.30), 3.30 (2H, t, C*H*$_2$ at 9, *J* = 5.32), 5.35 (2H, s, C*H*$_2$ at SCH_2), 7.20-7.69 (4H, m, *H* at imidazole), 12.30 (1H, s, N*H*), 13.20 (1H, s, N*H*).

23. Synthesis of 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (IV*xxiii*)

2-{[(5-Methoxy-1*H*-benzimidazol-2-yl)thio]methyl}-3,5,6,7,8,9-hexahydro-4*H*-cyclo-hepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (**III*xxiii***, 2.2 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml methylene dichloride by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 155-160^oC; Yield: 44%.
Mol. formula : $C_{20}H_{20}N_4O_3S_2$; Mol. Wt. 428.5.
IR (KBr) cm^{-1} : 3269(γ_{NH}), 2909(γ_{C-H}), 1672(γ_{CONH}), 1048(γ_{S-O}), 804(γ_{C-S}).
MS(m/e) : 408, 393, 380, 365, 352, 323, 309, 180

24. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)quinazolin-4(3*H*)-one (IV*xxiv*)

2-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)quinazolin-4(3*H*)-one **(III*xxiv*,** 1.66 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml dichloromethane by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 175-177^oC; Yield: 56%
Mol. Formula : $C_{16}H_{12}N_4O_2S$; Mol. Wt. 324.3
IR (KBr) cm^{-1} : 3059(γ_{NH}), 1676(γ_{CONH}), 1052(γ_{S-O}), 741(γ_{C-S}).

25. Synthesis of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)quinazolin-4(3*H*)-one (IV*xxv*)

2-((5-Methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)quinazolin-4(3*H*)-one **(III*xxv*,** 1.8 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml dichloromethane by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath

while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 110-112°C; Yield: 60%

Mol. Formula : $C_{17}H_{14}N_4O_3S$; Mol. Wt. 354.3

IR (KBr) cm^{-1} : 3351(γ_{NH}), 3076(γ_{C-H}), 1681(γ_{CONH}), 1029(γ_{S-O}), 776(γ_{C-S})

26. Synthesis of 2-(1*H*-benzoimidazol-2-sulfinylmethyl)-6,8-dimethyl-3*H*-9-thia-1,3,5-triazafluoren-4-one (IV*xxvi*)

6-(1*H*-benzoimidazol-2-ylsulfanylmethyl)-2,4-dimethyl-7*H*-9-thia-1,5,7-triazafluoren-8-one (**III*xxvi***, 2.1 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 140-143°C; Yield: 60%

Mol. Formula : $C_{19}H_{15}N_5O_2S_2$; Mol. Wt. 409.4

IR (KBr) cm^{-1} : 3345(γ_{NH}), 3030(γ_{C-H}), 1685(γ_{CONH}), 1040(γ_{S-O}), 768(γ_{C-S}).

27. Synthesis of 2-(5-methoxy-1*H*-benzoimidazol-2-sulfinylmethyl)-6,8-dimethyl-3*H*-9-thia-1,3,5-triazafluorene 4-one (IV*xxvii*)

6-(5-Methoxy-1*H*-benzoimidazol-2-ylsulfanylmethyl)-2,4-dimethyl-7*H*-9-thia-1,5,7-triazafluoren-8-one **(III*xxvii*,** 2.2 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 160-162°C; Yield: 64%

Mol. Formula : $C_{20}H_{17}N_5O_3S_2$; Mol. Wt. 439.5

IR (KBr) cm^{-1} : 3358(γ_{NH}), 2980(γ_{C-H}), 1682(γ_{CONH}), 1055(γ_{S-O}), 760(γ_{C-S}).

28. Syntheis of 2-(1*H*-benzoimidazole-2-sulfinylmethyl)-3*H*-1,3,9a,10-tetraaza-anthracene-4,9-dione (IV*xxviii*)

2-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)-3*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-dione (**III*xxviii***, 2.0 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 170-172^oC; Yield: 61%

Mol. Formula : $C_{18}H_{12}N_6O_3S$; Mol. Wt. 392.4

IR (KBr) cm^{-1} : 3059, 2909(γ_{C-H}), 1677(γ_{CONH}), 1053(γ_{S-O}), 741(γ_{C-S})

NMR (DMSO-d_6)δppm : 4.70 (2H, s, C*H*$_2$ at SCH_2), 7.02-8.06 (8H, m, Ar-*H*), 12.44 (1H, s, N*H*), 13.55 (1H, s, N*H*).

29. Synthesis of 2-(5-methoxy-1*H*-benzoimidazol-2-sulfinylmethyl)-3*H*-1,3,9a,10-tetraaza-anthracene 4,9-dione (IV*xxix*)

2-((5-Methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3*H*-[1,2,4]triazino[6,1-*b*]-quinazoline 4,10-dione (**IV*xxix***, 2.2 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 157-159^oC; Yield: 50%

Mol. Formula : $C_{19}H_{14}N_6O_4S$; Mol. Wt. 422.4

IR (KBr) cm^{-1} : 3184, 2922(γ_{C-H}), 1686(γ_{CONH}), 1061(γ_{S-O}), 779(γ_{C-S}).

NMR (DMSO-d_6)δppm : 3.81 (3H, s, OC*H*$_3$), 4.65 (2H, s, C*H*$_2$ at SCH_2), 7.02-8.06 (8H, m, Ar-*H*), 12.45 (1H, s, N*H*), 13.42 (1H, s, N*H*).

30. Synthesis of 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-6,7-dimethoxy-quinazolin-4(3*H*)-one (IV*xxx*)

2-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-6,7-dimethoxyquinazolin-4(3*H*)-one **(III*xxx*,** 1.98 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***).

M.P. : 122-124^oC; Yield: 56%
Mol. Formula : $C_{18}H_{16}N_4O_4S$; Mol. Wt. 384.4
IR (KBr) cm^{-1} : 2916(γ_{C-H}), 1655(γ_{CONH}), 1064(γ_{S-O}), 746(γ_{C-S})
MS(m/e) : 380, 366, 351, 203, 150.

31. Synthesis of 2-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-6,7-dimethoxyquinazolin-4(3*H*)-one (IV*xxxi*)

2-((5-Methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-6,7-dimethoxyquinazolin-4(3*H*)-one **(III*xxxi*,** 2.1 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 105-107^oC; Yield: 60%
Mol. Formula : $C_{19}H_{18}N_4O_5S$; Mol. Wt. 414.4
IR (KBr) cm^{-1} : 2916(γ_{C-H}), 1663(γ_{CONH}), 1026(γ_{S-O}).

32. Synthesis of 2-(1*H*-benzoimidazol-2-sulfinylmethyl)-8-methoxy-3*H*-benzo-[4,5]-thieno[2,3-*d*]pyrimidin-4-one (IV*xxxii*)

9-Methoxy-2-(1*H*-benzoimidazol-2-ylsulfanylmethyl)-3*H*-benzo[4,5]thieno[3,2-*d*]-pyrimidin-4-one (**III*xxxii***, 2.1 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was

chilled in an ice salt bath while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 195-197°C;Yield: 61%

Mol. Formula : $C_{19}H_{14}N_4O_3S_2$; Mol. Wt. 410.4

IR (KBr) cm^{-1} : 3068(γ_{C-H}), 1683(γ_{CONH}), 1022(γ_{S-O}), 739(γ_{C-S}).

33. Syntheis of 8-methoxy-2-(5-methoxy-1*H*-benzoimidazole-2-sulfinylmethyl)-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (IV*xxxiii*)

9-Methoxy-2-(5-methoxy-1*H*-benzoimidazol-2-ylsulfanylmethyl)-3*H*-benzo[4,5]-thieno-[3,2-*d*]pyrimidin-4-one **(III*xxxiii*,** 2.2 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml chloroform by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 125-127°C; Yield: 64%

Mol. Formula : $C_{20}H_{16}N_4O_4S_2$; Mol. Wt. 440.5

IR (KBr) cm^{-1} : 2917(γ_{C-H}), 1675(γ_{CONH}), 1028(γ_{S-O}), 784(γ_{C-S}).

34. Synthesis of 6-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (IV*xxxiv*)

6-((1*H*-Benzo[*d*]imidazol-2-ylthio)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one **(III*xxxiv*,** 2.2 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml dichloromethane by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0°C. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 132-134°C; Yield: 55%

Mol. Formula : $C_{20}H_{16}N_6O_2S_2$; Mol. Wt. 436.5

IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1678(γ_{CONH}), 1025(γ_{S-O}), 756(γ_{C-S}).

35. Synthesis of 6-((5-methoxy-1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (IV*xxxv*)

6-((5-Methoxy-1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(III*xxxv*,** 2.4 gm; 0.0054 mole) was dissolved in 125 ml methanol and 100 ml dichloromethane by stirring at room temprature. Thereafter, the reaction mixture was chilled in an ice salt bath while maintaining the temperature below 0^oC. To this clear solution, methanolic solution of *m*-CPBA (1.16 gm; 0.0065 mole) was added while stirring and the reation was continued for 30-45 mins. After completion of the reaction, worked up was done as for the compound **IV*i***.

M.P. : 172-175^oC; Yield: 40%

Mol. Formula : $C_{21}H_{18}N_6O_3S_2$; Mol. Wt. 466.5

IR (KBr) cm^{-1} : 2980(γ_{C-H}), 1670(γ_{CONH}), 1029(γ_{S-O}), 752(γ_{C-S}).

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PART-II

Novel Microwave Assisted Green Chemical Synthesis of Condensed 2-Substitutedpyrimidin-4(3*H*)-ones under Solvent Free Conditions, their MWI Assisted Facile and Rapid Chlorination and their Multidrug Reverting Activity

1. Synthesis of Pyrimidines and Condensed Pyrimidines through Reactions of Nitriles with ortho-Aminocarbonyl Substrates under Acidic Conditions

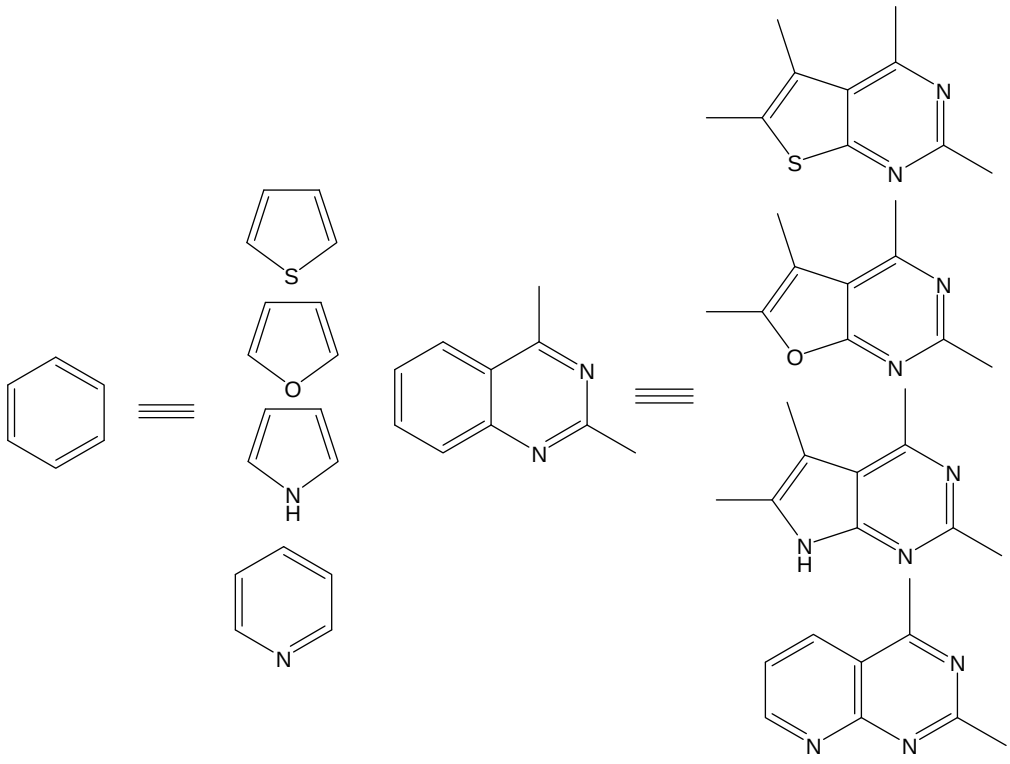
1. Synthesis of Pyrimidines and Condensed Pyrimidines through Reactions of Nitriles with *ortho*-Aminocarbonyl Substrates under Acidic Conditions: A Review

1.1 Introduction

Pyrimidines & condensed pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS. The pyrimidine nucleus has very wide biological and medicinal significance. For more details a comprehensive review[1] on this topic can be consulted.

A. Bioisosterism

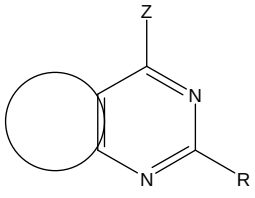
The bioisosterism[2] between benzene and various heterocycles, namely thiophene, furan, pyrrole, pyridine *etc*. is well known since long. Thus, various condensed pyrimidine systems like thienopyrimidines, furanopyrimidines, pyrrolopyrimidines, pyridopyrimidines *etc*., are the logical bioisosters of the bioactive quinazolines.



1.2 Synthesis of Condensed Pyrimidines: General Aspects

Logically, medicinal chemistry research worldwide routinely involves the synthesis and evaluation of bioisosteric molecules of existing drugs. As many of the drug molecules have quinazoline as the basic nucleus, the synthesis of condensed pyrimidines,

appropriately functionalised, especially at the 2- and 4-positions has attracted great attention of the medicinal chemists.

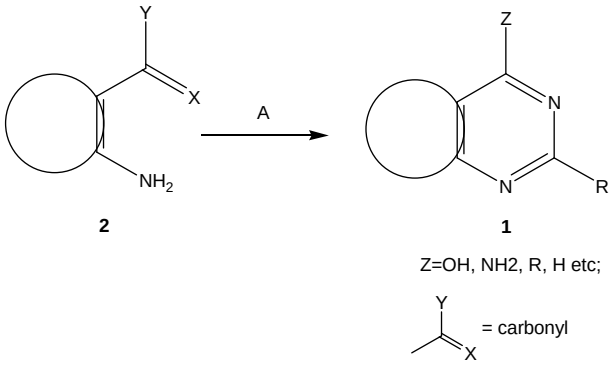


1

The synthesis of condensed pyrimidine systems is a very important process subject to improvement on various points and parameters. The regularly employed methods for synthesis of condensed pyrimidines involve mainly, two different approaches as mentioned below.

Approach A:

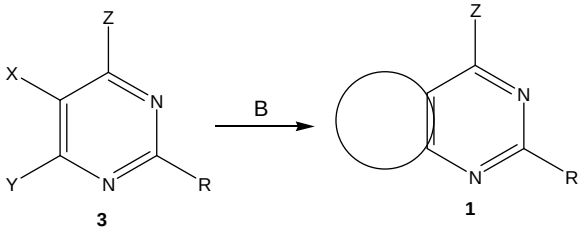
Annelation of pyrimidines on an appropriately substituted heterocycle[3].



2 **1**

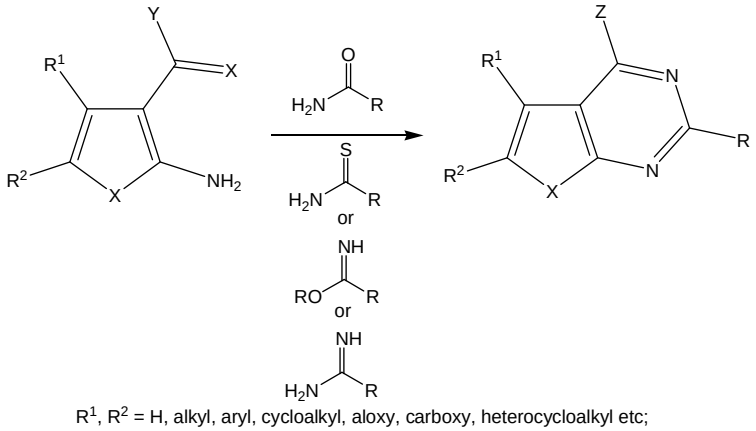
Approach B:

Annelation of a heterocycle on the appropriately substituted pyrimidine ring[3].



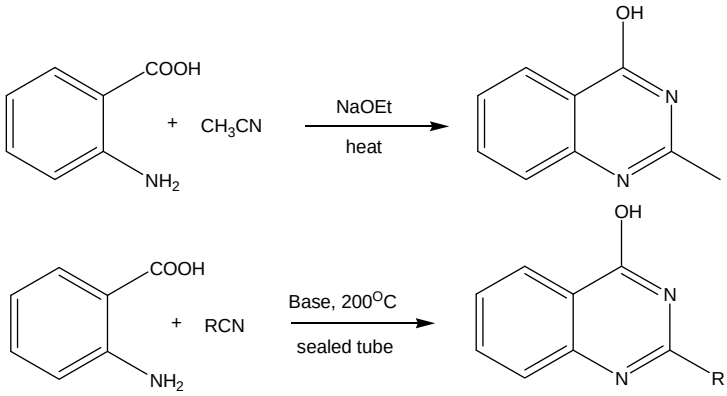
3 **1**

Approach A is the most widely used approach for the synthesis of condensed pyrimidines. Under this approach, a variety of *o*-aminocarbonyl substrates of various heterocycles have been cyclocondensed with a host of reagents namely amides, thioamides, imidates, amidines *etc.*, mostly under basic conditions, to afford various condensed pyrimidines, quinazolines, thienopyrimidines, pyrrolopyrimidines, triazolopyrimidines, pteridines, furanopyrimidines, pyridopyrimidines and many more.



R^1, R^2 = H, alkyl, aryl, cycloalkyl, aloxy, carboxy, heterocycloalkyl etc;
X = HC=CH, NH, S, O, CH=N, N=NH, etc;
Y = COOH, $COOR^3$, $CONH_2$, COR^3, CHO,CN, etc;
Z =OH, NH_2;
R = alkyl, aryl,aralkyl, etc.
R^3 = CH_3, C_2H_5, alkyl, aryl

However, the direct use of a nitrile (RCN) as a reagent to cyclocondense with *o*-amino carbonyl substrates to afford condensed pyrimidines has received rather scant attention. There are a few reports[3,4,5] available in the literature on such reactions under basic conditions. The major drawback of these reactions under basic conditions is poor product yields.

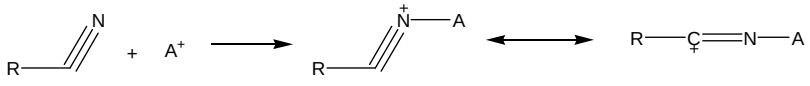


1.3 Reactions of Nitriles Under Acidic Conditions

Nitriles have played a major role in the synthesis of a variety of open chain and heterocyclic compounds[6]. The polar C≡N group of the nitrile is prone towards electrophilic attack at the nitrogen and nucleophilic attack at the carbon.

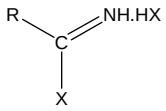
The enhanced electrophilicity of nitriles in the presence of halogen acids is known since long. The interaction of a nitrile **4,** with an acid or its complexation with a Lewis acid results in the formation of a species **5,** with enhanced electrophilicity and therefore, many of the reactions of nitriles with nucleophilic reagents are acid catalyzed. Halogen acids

have been found to be particularly effective in promoting the reaction of nitriles with a variety of nucleophiles.



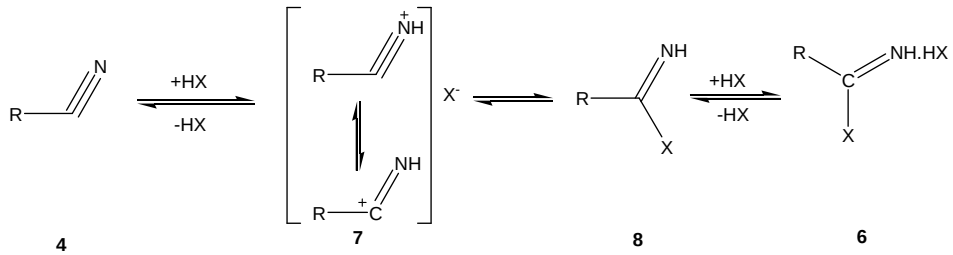
4 **5**

In the absence of other nucleophilic species, nitriles react with halogen acids, to yield unstable adducts of different compositions. The nature of these adducts, as well as, the possible involvement of such nitrile-halogen acid adducts in the hydrogen halide catalyzed reactions of nitriles with nucleophiles has been the subject of considerable discussion[6-16]. These adducts are of compositions, such as RCN.HX, 2 RCN.HX, 2 RCN.nHX *etc.*, depending upon the nature of the nitriles and the reaction conditions employed. The unstable, hygroscopic adducts resulting from the reaction of a variety of aliphatic and aromatic nitriles with halogen acids, at low temperatures, have been found to be of the general composition RCN.2HX. The structure **6** however, has been assigned to many of these adducts[7,17-21].



6

The sequence of reactions leading to the formation of imidoyl halide hydrohalide **6** from a nitrile **4** can be depicted as shown below. The protonation of the nitrile yields the nitrilium ion **7**, which combines with a halide ion to form imidoyl halide **8**. The imidoyl halide **8** thus formed, is sufficiently basic to react with another molecule of halogen acid to yield the imidoyl halide hydrohalide salt **6**. In this reversible reaction, the formation of imidoyl halide salt **6,** is frequently slow and is favoured by high concentrations of hydrogen halide (Scheme 1)[11,12].

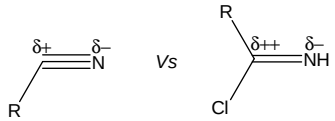


4 **7** **8** **6**

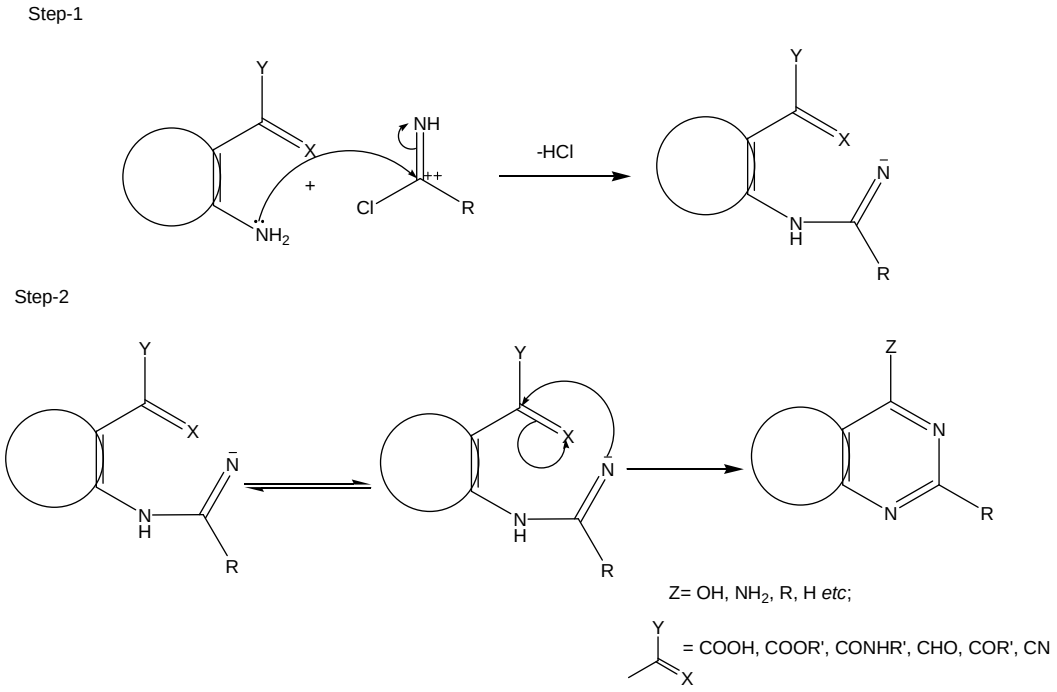
Scheme 1

This reactive intermediate, imidoyl halide, is formed *in situ* through the reaction of nitrile R-C≡N and halogen acid HX. The addition of HX is across the polar C≡N bond. The

electron withdrawing group X (Cl) further makes the nitrile C' more electrophilic or enhances its electrophilicity.

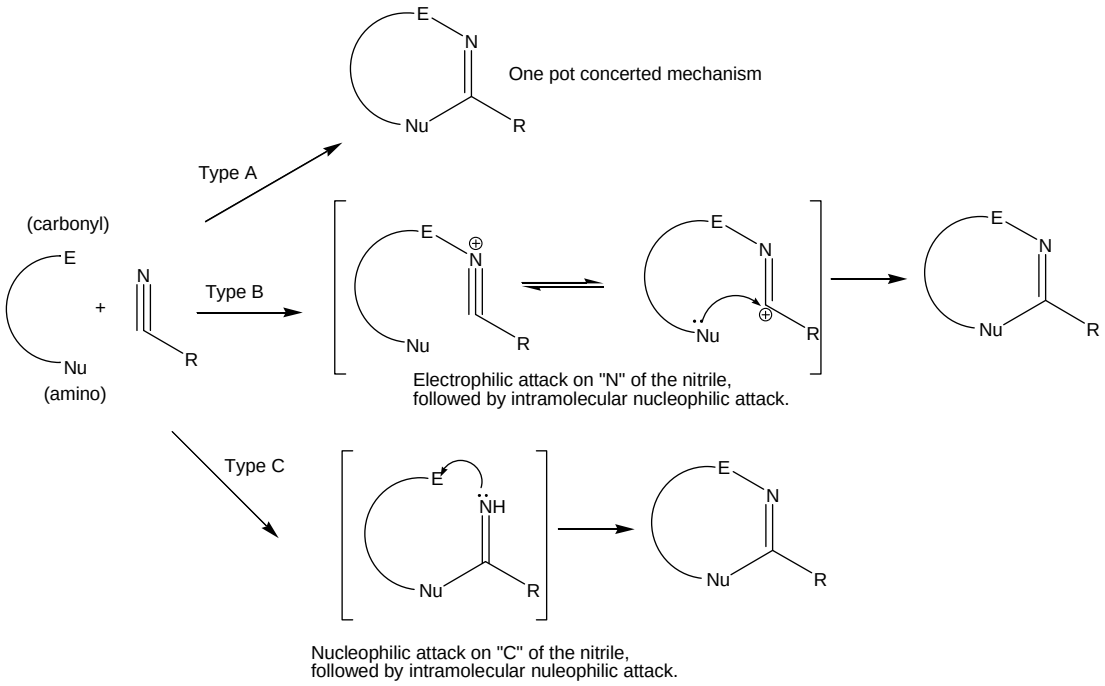


The imidoyl halide when is reacted with the *o*-aminocarbonyl substrate, attracts the electrons of the nucleophilic-NH_2 group of the substrate very readily as follows (Scheme 2).



Scheme 2

The reaction of a nitrile with an *o*-aminocarbonyl substrate possessing electrophilic and nucleophilic centers leads to the formation of an azaheterocycle through the incorporation of CN of the nitrile in the ring. The mechanism may be any one of the following three, a concerted cycloaddition process (Type A) or by discreet steps, involving either the initial electrophilic attack on the nitrile nitrogen (Type B) or by the initial electrophilic attack at the nitrile carbon (Type C), followed by ring closure[22] (Scheme 3).

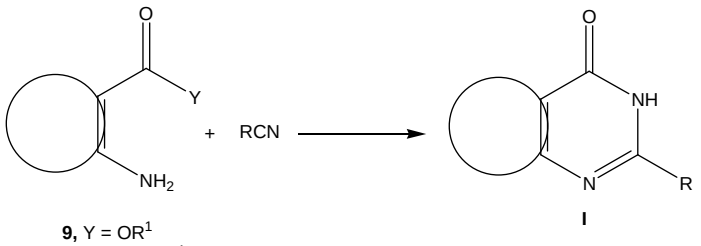


Scheme 3

Of the above three mechanisms the type C mechanism is the most favored mechanism and is mostly reported.

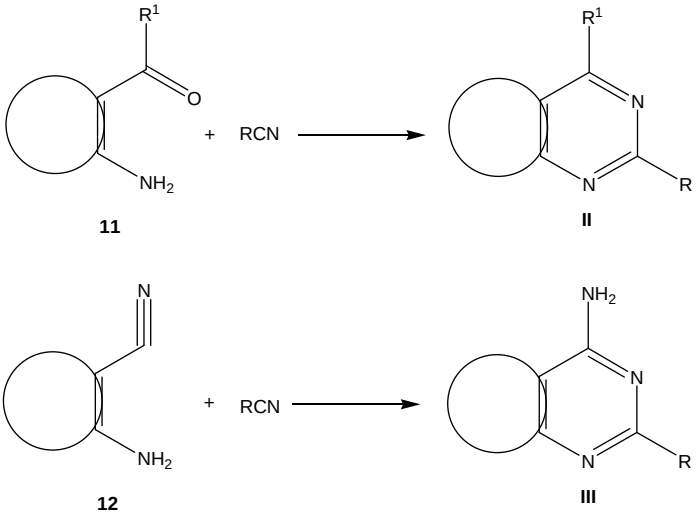
1.4 Synthesis of Various Condensed Pyrimidines under the Influence of Dry HCl Gas

This enhanced reactivity of nitriles in the presence of acids has been particularly exploited for the synthesis of condensed pyrimidines. A host of nitriles have been reacted with various *o*-aminocarbonyl compounds to obtain a variety of condensed pyrimidines. This approach has led to the development of a facile, one pot synthesis of condensed 2-substituted functionalised pyrimidines of wide applicability[23-30]. A variety of *o*-aminocarbonyl compounds, such as *o*-aminoesters **9**, *o*-aminoamides **10**, *o*-aminoketones **11** and *o*-aminonitriles **12** have been reacted with nitriles to obtain the corresponding condensed 4-oxo **I,** 4-aryl **II**, and 4-aminopyrimidines **III**.



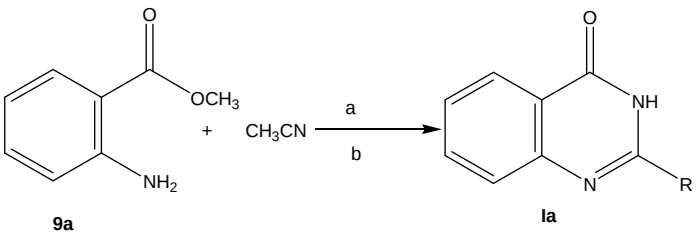
9, Y = OR^1
10a, Y = NHR^1
R^1 = H, alkyl, aryl, *etc.*

I



1.4.1 Synthesis of Condensed 4-Oxopyrimidines

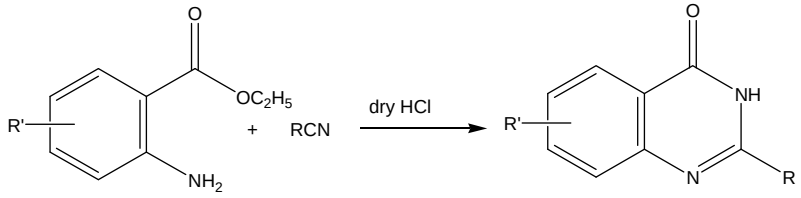
The reaction essentially consists of bubbling a stream of dry hydrogen chloride gas through a mixture of an *o*-aminoester **9** or *o*-aminoamide **10** substrate and the nitrile in a suitable solvent like dioxane at ambient temperature for a few hours. On basification the condensed 4-oxopyrimidines **I** are isolated in good yields (60-80%). This is exemplified by the reaction between methyl anthranilate **9a** and acetonitrile which when conducted in the presence of dry hydrogen chloride gas has been found to give 2-methylquinazolin-4-one **Ia** in 75% yield. This is higher in yields, than that obtained under basic conditions[4].



a = dry HCl gas, **b** = NaOEt/ EtOH

A series of 2-substitutedquinazolin-4-ones (Table 20) has been synthesized through the reaction of methyl anthranilates **9a**, with alkyl, aryl, aralkyl and heteroaryl nitriles under the influence of dry HCl gas. Further, the reaction has been found to be applicable to the condensation of a large variety of active methylene nitriles to obtain the corresponding condensed 2-substitutedmethylquinazolin-4-ones, which are otherwise inaccessible by the base catalyzed condensations (Table 20).

Table 20: 2-Substitutedquinazolin-4(3*H*)-ones

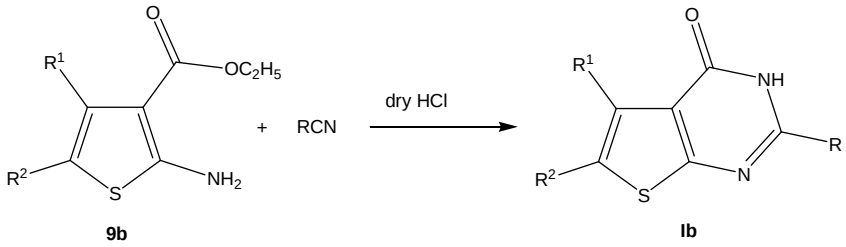


R'	R	Nitrile used	R. Solv	Yield (%)	Reference
H	CH_3-	CH_3CN	E	75	28
H	C_6H_5-	C_6H_5 CN	E- D	77	28
H	4-ClC_6H_4-	4-ClC_6H_4CN	E-D	70	28
H	$C_6H_5CH_2$-	C_6H_5 CH_2CN	E-D	64	28
H	3-C_5H_4N	3-C_5H_4N CN	E-D	58	28
H	$C_2H_5CO_2CH_2$-	$C_2H_5CO_2$ CH_2CN	E	69	23
H	$CH_3CO_2CH_2$-	$CH_3CO_2CH_2CN$	E	72	28
H	NH_2COCH_2-	NH_2COCH_2CN	E-C	68	28
H	$ClCH_2$-	$ClCH_2CN$	Di	72	28
H	Cl_2CH-	Cl_2CHCN	Di	73	31
H	4-$ClC_6H_4OCH_2$-	4-Cl $C_6H_4OCH_2CN$	E-D	65	28
H	4-$ClC_6H_4SCH_2$-	4-$ClC_6H_4SCH_2CN$	E-D	79	28
H	$C_6H_5SO_2CH_2$-	$C_6H_5SO_2CH_2CN$	E-D	67	28
H	$C_6H_5OCH_2CH_2$-	$C_6H_5OCH_2CH_2CN$	Di	75	32
H	4-$ClC_6H_4OCH_2CH_2$-	4-$ClC_6H_4OCH_2CH_2CN$	E	75	32
H	$C_6H_5NHCH_2CH_2$-	$C_6H_5NHCH_2CH_2CN$	Di	73	32
H	4-$CH_3C_6H_5NHCH_2CH_2$	4- $CH_3C_6H_5NHCH_2CH_2CN$	Di	72	32
H	$C_2H_5OCH_2CH_2$-	$C_2H_5OCH_2CH_2CN$	C	73	32
H	$C_{10}H_7OCH_2CH_2$-	$C_{10}H_7OCH_2CH_2CN$	E	51	32
I	$C_6H_5OCH_2CH_2$-	$C_6H_5OCH_2CH_2CN$	Di	77	32
I	4-$ClC_6H_4OCH_2CH_2$-	4-$ClC_6H_4OCH_2CH_2CN$	E-D	71	32
H	C_6H_5S-	C_6H_5SCN	E-D	55	33
H	CH_3S-	CH_3SCN	E-D	60	33

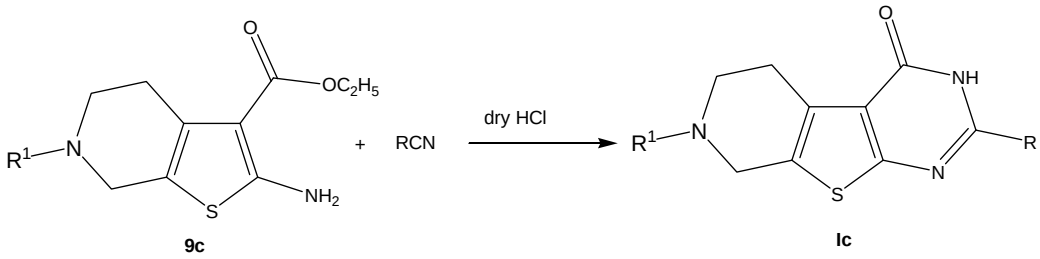
C= Chloroform, D=Dimethylformamide, Di= Dioxane, E= Ethanol

This method has been found to be equally applicable to the condensation of the *o*-amino esters and amides of a variety of substrates like thiophenes, pyridothiophenes, benzofurans, with a host of alkyl, aryl, aralky, heteroaryl nitriles as well as a range of α-substituted acetonitriles to give the corresponding condensed 2-substitutedpyrimidin-4(3*H*)-ones, namely, 2-substitutedthieno[2,3-*d*]pyrimidin-4(3*H*)-ones, 2-substituted

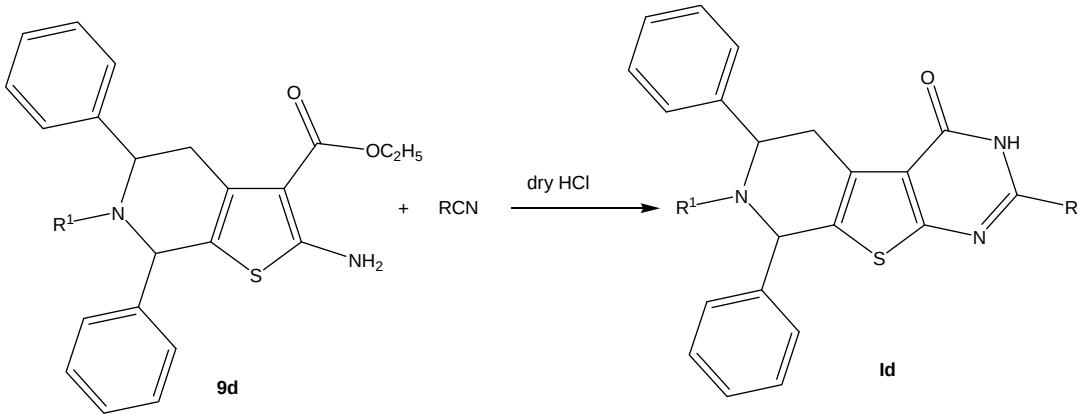
pyrido[4',3'-4,5]-thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (Table 21), 2-substitutedthieno[3,2-*d*]pyrimidin-4(3*H*)-ones, 2-substituted benzothieno[3,2-*d*]pyrimidin-4(3*H*)-ones, 2-substitutedpyrido-thieno-[3,2-*d*]pyrimidin-4(3*H*)-ones (Table 22), 2-substituted benzo furano[3,2-*d*]pyrimidin-4(3*H*)-ones (Table 23), 2-substituted-4*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-diones (Table 24) and 2-substitutedthieno [3,4-*d*]- and isothiazolo-[3,4-*d*]pyrimidin-4(3*H*)-ones (Table 25)**.**



2-Substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (Table 21)

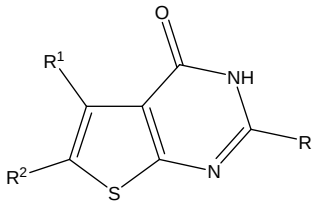


2-Substituted pyrido[4',3'-4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (Table 21)

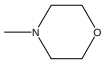


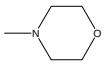
2-Substituted pyrido [4',3'-4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (Table 21)

Table 21: 2-Substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones



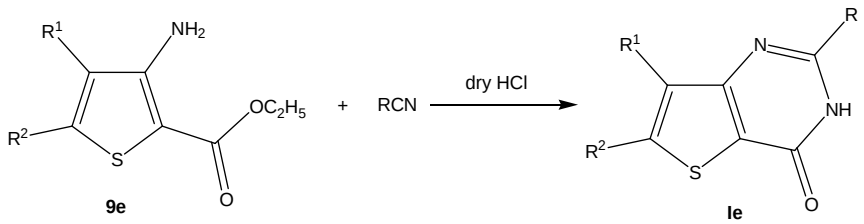
R^1	R^2	R	Yield (%)	R. Solv.	Reference
- $(CH_2)_4$-		-CH_3	85	E-D	24
- $(CH_2)_4$-		-$CH_2CO_2C_2H_5$	72	E	28
- $(CH_2)_4$-		-CH_2CONH_2	46	E-D	28
- $(CH_2)_4$-		-$CHClCH_2Cl$	40	Di	28
- $(CH_2)_4$-		-$CH_2C_6H_5$	83	E-D	34
- $(CH_2)_4$-		-CH_2Cl	81	E-C	25
- $(CH_2)_4$-		-$CH_2OC_6H_4Cl$-4	75	D	34
- $(CH_2)_4$-		-$CH_2SC_6H_4CH_3$-4	75	E-C	34
- $(CH_2)_4$-		-$CH_2SO_2C_6H_4CH_3$-4	58	E-C	34
- $(CH_2)_4$-		-$CH_2CO_2CH_3$	66	E-C	34
- $(CH_2)_4$-		-$CH_2NHSO_2C_6H_5NH_2$-4	50	E	34
- $(CH_2)_4$-		-$CH_2NHSO_2C_6H_4NH$-$COCH_3$-4	58	E-C	34
- $(CH_2)_4$-		-$CH_2SO_2C_6H_4Cl$-4	50	D	34
- $(CH_2)_4$-		-$CH_2SC_6H_4Cl$-4	70	E-C	34
- $(CH_2)_4$-		-$CH_2SC_6H_4NO_2$-4	65	M-C	36
- $(CH_2)_4$-		-$CH_2SO_2C_6H_4NO_2$-4	63	D	36
- $(CH_2)_4$-		-$CH_2NHSO_2C_6H_4$	63	E-C	36
- $(CH_2)_4$-		-$CH_2C_6H_4Cl$-4	75	E	31
- $(CH_2)_4$-		-$CH_2COC_6H_5$	62	Di	31
- $(CH_2)_4$-		-$CH_2NHC_6H_4$	56	C-P	31
- $(CH_2)_4$-		-CH_2CH_2Cl	50	E-C	31
-$(CH_2)_4$-		-C_6H_5	80	E-D	24
- $(CH_2)_4$-		-C_6H_4Cl-4	66	E-D	31
- $(CH_2)_4$-		3-C_5H_4N	60	E-D	28
- $(CH_2)_4$-		-$CO_2C_2H_5$	68	E-D	31
- $(CH_2)_4$-		-$SC_6H_4N(CH_3)_2$-4	56	E-C	37
- $(CH_2)_4$-		-$SC_{10}H_6OH$-4	50	M-C	37
- $(CH_2)_4$-		-$SC_6H_4CH_3$-4	59	E-C	37
- $(CH_2)_4$-		SC_2H_5	64	E-C	37
- $(CH_2)_4$-		-SC_3H_7	6.9	E-C	37

R^1	R^2	R	Yield (%)	R. Solv.	Reference
	$-(CH_2)_4-$	$-SCH_3$	82	E-C	37
	$-(CH_2)_4-$	$-SCH_2C_6H_5$	77	E-C	37
	$-(CH_2)_4-$	$-C_6H_3(OCH_3)_2$-3,4	60	E-C	31
	$-(CH_2)_4-$	$-NHC_6H_5$	57	E-D	26
	$-(CH_2)_4-$	$-NHC_6H_4CH_3$-4	45	E-D	26
	$-(CH_2)_4-$	$-NHC_6H_4OCH_3$-4	40	E-D	33
	$-(CH_2)_4-$	$-NHC_6H_4Cl$-4	69	E-C	26
	$-(CH_2)_4-$	$-NHC_6H_4Cl$-2	35	Di	26
	$-(CH_2)_4-$	$-NH_2$	68	*n*-P	26
	$-(CH_2)_4-$		62	E-C	26
	$-(CH_2)_4-$	$-CH=CHC_6H_5$	50	E-C	23
	$-(CH_2)_4-$	-SH	60	E-D	26, 38
	$-(CH_2)_3-$	$-CH_3$	73	E-D	28
	$-(CH_2)_3-$	$-CO_2C_2H_5$	65	E	28
	$-(CH_2)_3-$	$-CH_2CH_2Cl$	47	E-C	28
	$-(CH_2)_3-$	$-CH_2CO_2C_2H_5$	68	E	28
	$-(CH_2)_3-$	$-CH_2Cl$	70	Di	26
	$-(CH_2)_3-$	$-C_6H_5$	60	E-C	31
CH_3-	CH_3-	$-CH_3$	85	E-C	24
CH_3	CH_3-	3-C_5H_4N	66	Di	28
CH_3-	CH_3-	$-C_6H_5$	66	D-E	24
CH_3-	CH_3-	$-SC_6H_4N(CH_3)_2$-4	60	E-C	37
CH_3-	CH_3-	$-SC_{10}H_6OH$-4	51	M-C	37
CH_3-	CH_3-	$-SC_6H_4CH_3$-4	58	E-C	31
CH_3-	CH_3-	$-SC_6H_5$	64	E-C	37
CH_3-	CH_3-	$-SCH_3$	79	E-C	37
CH_3-	CH_3-	$-CH_2C_6H_5$	70	E-D	24
CH_3-	CH_3-	$-CH_2COC_6H_5$	57	Di	31
CH_3-	CH_3-	$-CH_2NHSO_2C_6H_5$	64	E-C	36
CH_3-	CH_3-	$-CH_2NHSO_2C_6H_4NH_2$-4	60	E	36
CH_3-	CH_3-	$-CH_2NHSO_2C_6H_4NH-COCH_3$-4	48	E-C	36
CH_3-	CH_3-	$-CH_2NHC_6H_5$	65	C-P	31
CH_3-	CH_3-	$-CH_2CO_2C_2H_5$	68	E	28
CH_3-	CH_3-	$-CH_2Cl$	78	E-C	26, 34
CH_3-	CH_3-	$-CH_2SC_6H_5$	74	E-C	36
CH_3-	CH_3-	$-CH_2SC_6H_4CH$-4	59	E-C	36

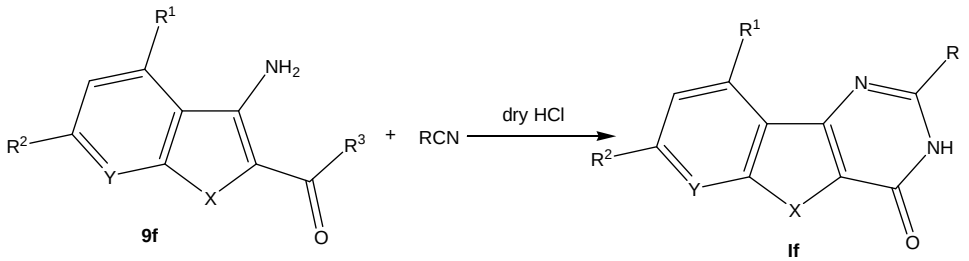
R^1	R^2	R	Yield (%)	R. Solv.	Reference
CH_3-	CH_3-	-$CH_2SC_6H_4NO_2$-4	71	E-C	36
CH_3-	CH_3-	-$CH_2SO_2C_6H_4CH_3$-4	50	E-C	36
CH_3-	CH_3-	-$C_6H_3(OCH_3)_2$-3,4	70	E-C	31
CH_3-	CH_3-	-$CO_2C_2H_5$	60	E	31
CH_3-	CH_3-	-$NHC_6H_4CH_3$-4	60	E-C	33
CH_3-	CH_3-	-$NHC_6H_4OCH_3$-4	53	E-C	33
CH_3-	CH_3-		57	Ch	26
CH_3-	CH_3-	-SH	47	E-D	38
CH_3-	CH_3-	-$CH_2SC_6H_4Cl$-4	59	E-C	26
C_6H_5-	H	-CH_3	74	E-C	24
C_6H_5-	H	-C_6H_5	56	Di	24
C_6H_5-	H	-$C_6H_3(OCH_3)_2$	60	E-C	31
C_6H_5-	H	-$NHC_6H_4CH_3$-4	62	E-C	33
C_6H_5-	H	-$CO_2C_2H_5$	70	C-P	31
C_6H_5-	H	-$CH_2C_6H_5$	50	E-C	24
C_6H_5-	H	-$CH_2CO_2C_2H_5$	70	E	28
C_6H_5-	H	-CH_2Cl	81	Ch	34
C_6H_5-	H	-$CH_2COC_6H_5$	58	E	31
C_6H_5-	H	-SH	54	D-E	38
4-$CH_3OC_6H_4$-	H	-CH_2Cl	98	Di	39
4-$CH_3OC_6H_4$-	H	-$CH_2CO_2C_2H_5$	88	E	39
4-$CH_3OC_6H_4$-	H	-$CH_2C_6H_5$	75	E-C	39
4-$CH_3OC_6H_4$-	H	-CH_3	90	D	39
4-ClC_6H_4-	H	CH_2Cl	65	M-C	26
4-$CH_3C_6H_4$-	H	-CH_2Cl	86	E-C	40
H	C_2H_5-	3-C_5H_4N	70	E-D	31
H	C_2H_5-	-CH_2Cl	98	Di	34
H	C_2H_5-	-$CH_2COC_6H_5$	67	E	31
H	C_2H_5-	-$C_6H_3(OCH_3)_2$-3,4	65	E-C	28
CH_3-	$COOC_2H_5$-	-$CH_2C_6H_5$	58	E-D	23, 31
CH_3-	$COOC_2H_5$-	-$CH_2COC_6H_5$	76	Di	31
CH_3-	$COOC_2H_5$-	-CH_2Cl	87	Di	34
CH_3-	$COOC_2H_5$-	-CH_3	80	D	23, 31
CH_3-	$COOC_2H_5$-	-$CO_2C_2H_5$	60	Ch	31
CH_3-	$COOC_2H_5$-	-$C_6H_3(OCH_3)_2$-3,4	50	E-C	28
C_6H_5-	CH_3-	-CH_2Cl	94	E-C	40
-$(CH_2)_2$-N-($CH_2C_6H_5$)CH_2-		-CH_3	77	D-P	28

R^1	R^2	R	Yield (%)	R. Solv.	Reference
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CH_2Cl$	54	D	28
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CH_2CO_2C_2H_5$	50	E-C	31
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CH_2OC_6H_4Cl$-4	61	Di	31
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CH_2C_6H_5$	60	E-C	28
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-C_6H_5$	55	E-C	28
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CH_2COC_6H_5$	76	Di	31
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-C_6H_3(OCH_3)_2$-3,4	50	E-C	28, 31
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-CO_2C_2H_5$	76	Ch	31
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-NHC_6H_4CH_3$-4	43	E-C	33
$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$-SC_6H_4N(CH_3)_2$-4	55	E-C	37
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-CH_2CO_2C_2H_5$	56	E	37
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-CH_2Cl$	50	E-C	41
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-CH_3$	64	E-D	41
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-CH_2C_6H_5$	59	E	41
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-CH_2OC_6H_4Cl$-4	51	Di	41
$-(CH_2)_2-N-(CH_3CO)CH_2-$		$-C_6H_5$	57	E-C	41
$-(CH_2)_2-N-(CH_3CO)CH_2-$		3-C_5H_4N	57	E-D	28, 31
$-CH_2CH(C_6H_5)N-(CH_3)CH(C_6H_5)-$		$-CH_3$	90	E-C	31
$-CH_2CH(C_6H_5)N-(CH_3)CH(C_6H_5)-$		$-CH_2CO_2C_2H_5$	75	E-C	31
$-CH_2CH(C_6H_5)NHCH(C_6H_5)-$		$-CH_2C_6H_5$	45	E-D	31

C = Chloroform, Ch = Cyclohexane, D = Dimethylformamide, Di = Dioxane, E = Ethanol, M = Methanol, P = Petroleum ether(60-80°C), n-P = n-Propanol



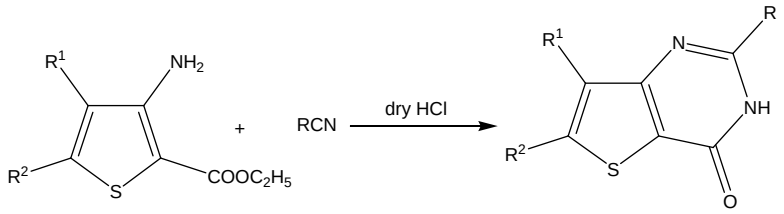
2-Substitutedthieno[3,2-*d*]pyrimidin-4(3*H*)-ones (Table 22)



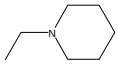
R^3 = alkoxy, NH_2
R^1,R^2 = H, CH_3
Y= CH, N; X = O, S

2-Substitutedbenzothieno[3,2-*d*]pyrimidin-4(3*H*)-ones, 2-Substitutedpyridothieno[3,2-*d*]-pyrimidin-4(3*H*)-ones (Table 3) and 2-Substitutedbenzofurano[3,2-*d*]pyrimidin-4(3*H*)-ones (Table 23)

Table 22: 2-Substitutedthieno[3,2-*d*]/benzo(b)thieno[3,2-*d*]/pyridothieno[3,2-*d*]-pyrimidin-4(3*H*)-ones.

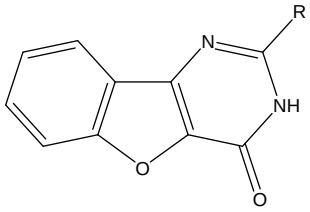


R_1	R_2	R	Yield (%)	R. Solv.	Reference
C_6H_5	H	$-CH_3$	80	C	42
C_6H_5	H	$-CH_2Cl$	82	E	42
C_6H_5	H	$-CH_2CH_2Cl$	76	C	42
C_6H_5	H	$-C_6H_5$	63	D	43
C_6H_5	H	-SH	54	D-E	43
H	C_6H_5	$-CH_3$	45	E-C	42
H	C_6H_5	$-CH_2Cl$	40	E-C	42
H	C_6H_5	$-CO_2C_2H_5$	25	E-C	42
H	C_6H_5	$-CH_2CO_2C_2H_5$	38	C-P	42
H	C_6H_5	$-CH_2CH_2Cl$	41	E-C	42
H	C_6H_5	$-C_6H_5$	35	E-C	42
H	C_6H_5	$-CH_2C_6H_5$	32	E-C	42
H	C_6H_5	$-CH_2C_6H_4Cl$-4	38	C-P	42
H	C_6H_5	$-CH_2C_6H_4NO_2$-4	52	C-P	42
H	C_6H_5	$-CH_2$=CH-C_6H_5	55	E-C	42
-CH=CH-CH=CH-		$-CH_3$	73	E-D	28, 35
-CH=CH-CH=CH-		$-CH_2C_6H_5$	69	E-D	28
-CH=CH-CH=CH-		$-CH_2Cl$	63	E-D	35
-CH=CH-CH=CH-		$-CH_2CO_2C_2H_5$	66	E-D	31
$-C(OCH_3)$=CH-CH=CH-		$-CH_2C_6H_5$	65	E-D	28
$-C(OCH_3)$=CH-CH=CH-		$-CH_2CO_2C_2H_5$	58	E-D	31
$-C(CH_3)$=CH-$C(CH_3)$=N-		$-CH_3$	62	E-C	35
$-C(CH_3)$=CH-$C(CH_3)$=N-		$-CH_2Cl$	68	E	35
$-C(CH_3)$=CH-$C(CH_3)$=N-		$-CH_2OCH_3$	62	D	44
$-C(CH_3)$=CH-$C(CH_3)$=N-		$-CH_2OC_2H_5$	64	E-C	44

R_1	R_2	R	Yield (%)	R. Solv.	Reference
-C(CH_3)=CH-C(CH_3)=N-		-CH_2OCOCH_3	60	A	44
-C(CH_3)=CH-C(CH_3)=N-		-$CH_2OCOC_6H_5$	56	E-C	44
-C(CH_3)=CH-C(CH_3)=N-		-$CHCl_2$	64	E-C	44
-C(CH_3)=CH-C(CH_3)=N-		-$CH_2N(C_2H_5)_2$	64	C	44
-C(CH_3)=CH-C(CH_3)=N-		-$CH_2NHCH(CH_3)_2$	61	M	44
-C(CH_3)=CH-C(CH_3)=N-			59	E-C	44

A = Gl.acetic acid, C = Chloroform, D = Dimethylformamide, E = Ethanol,M = Methanol, P= Petroleum ether(60-80^oC)

Table 23: 2-Substitutedbenzo(*b*)furo[3,2-*d*]pyrimidin-4(3*H*)-ones



R	Yield (%)	R. Solv.	Reference
-CH_3	66	B-M	45
-$CH_2CO_2C_2H_5$	60	B-M	45
-$CH_2C_6H_5$	65	B-M	45
-CH_2Cl	62	B-M	45
-CH_2Cl	17	M	46
-CH_2CH_2Cl	30	M	46
-$CO_2C_2H_5$	50	B-M	46
-$CH_2C_6H_4NO_2$	22	M	46
-$CH_2SC_6H_4Cl$-4	12	M	46

B=Benzene, M=Methanol

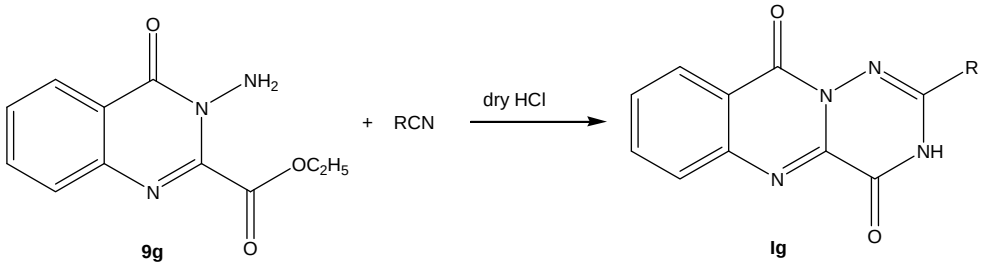


Table 24: 2-Substituted-4*H*-[1,2,4]triazino[6,1-*b*]qunazolin-4,10-diones

R	Yield (%)	R. Solv.	Reference
$-CH_2CH_2Cl$	43	A	47
$-CHCl_2$	43	A	47
$-CH_3$	53	E	32
$-CH_2CONH_2$	55	A	47
$-CH_2CONHC_6H_5$	43	A	47
$-C_6H_4(2-NH_2)$	45	A	47
$-CH_2CONHCH_3$	42	A	47
$-CH_2Cl$	53	E	32
$-COOC_2H_5$	46	A	47
$-CH_2C_6H_4Cl$-4	50	A	47
$-CH_2COOC_2H_5$	53	B	32
$-CH_2C_6H_5$	51	B-P	32
$-CH_2CH_2OC_6H_5$	52	E-D	32
$-C_6H_5$	35	A	47
$-CH_2SC_6H_5$	51	B	32
$-CH_2CH_2OC_6H_4Cl$-4	51	B-P	32

A=Gl.Acetic acid,B=Benzene,D=Dimethylformamide, E=Ethanol, P= Petroleum ether(60-80^oC)

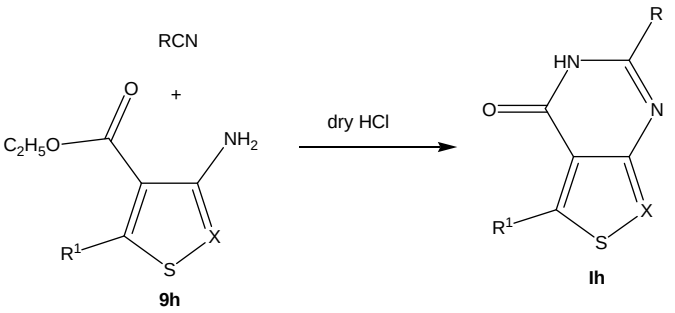


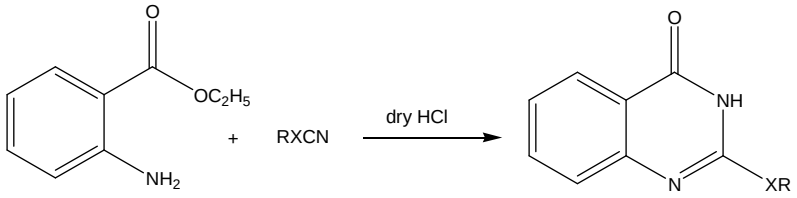
Table 25: 2-Substitutedthieno[3,4-*d*] and isothiazolo[3,4-*d*]pyrimidin-4(3*H*)-ones

R^1	X	R	Yield (%)	R. Solv.	Reference
SCH_3	$=C(COOC_2H_5)-$	SCH_3	70	E-D	31
NHC_6H_5	$=C(COOC_2H_5)-$	CH_3	52	E-D	31
SCH_3	=N-	CH_3	70	E-D	31

D = Dimethylformamide, E = Ethanol

Heteronitriles, such as thiocyanates have been found to react with methyl anthranilate **9a** and thiophene 2-amino-3-carboxylates **9b** in the presence of dry hydrogen chloride to

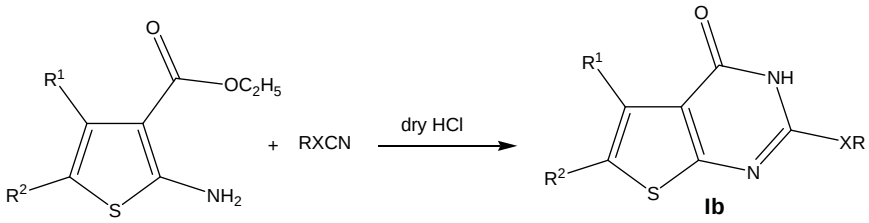
yield 2-alkyl and arylthioquinazolinones (Table 20), as well as**,** 2-alkyl or arylthiothieno[2,3-*d*]pyrimidin-4-ones (Table 21), 2-alkylthiothieno[3,2-*d*]pyrimidin-4-ones (Table 22) and 2-alkylthiothieno[3,4-*d*]pyrimidin-4-ones (Table 25), which are otherwise accessible only through two step syntheses.



9a **Ia**

X = S, O

2-Alkyl and arylthioquinazolinones (Table 20)

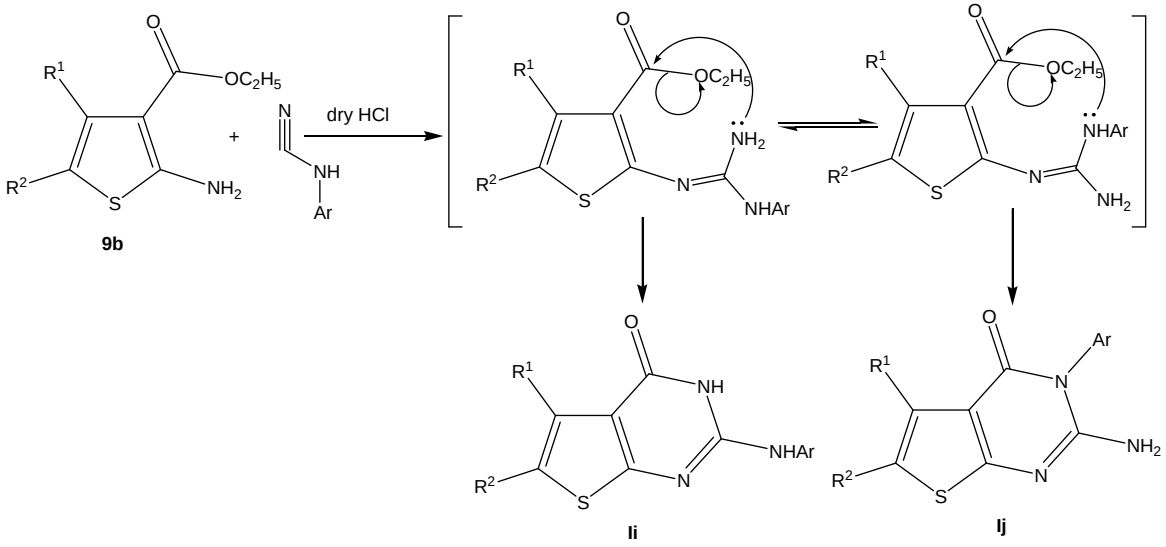


9b **Ib**

X = S, O

2-Alkyl and arylthiothieno[2,3-*d*]pyrimidin-4-ones (Table 2), 2-alkylthiothieno[3,2-*d*]pyrimidin-4-ones (Table 3) and 2-alkylthiothieno[3,4-*d*]pyrimidin-4-ones (Table 25).

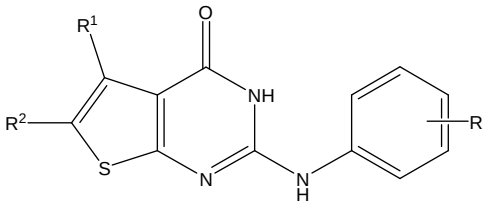
Similarly, simple cyanamide and dialkyl cyanamides yield 2-amino- and 2-dialkylaminothieno[2,3-*d*]pyrimidin-4(3*H*)-ones (Table 26) under these reaction conditions. However when *N*-monoaryl cyanamides were used two isomeric thienopyrimidin-4-ones **Ii** and **Ij** have been obtained as the condensation products of their dry HCl catalyzed reaction with thiophene *o*-aminoesters **9b.** The reaction proceeds *via* the transient guanidine intermediate, which cyclizes through two alternate pathways to afford the isomeric 2-aminothieno[2,3-*d*]pyrimidin-4-ones. (Scheme 4) (Tables 26 and 27).[26,33]



$R^1 = R_2 = -(CH_2)_4,-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$; $R_1 = R_2 = CH_3$, $R_1 = C_6H_5$, $R_2 = H$, Ar = C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, 4-ClC_6H_4, 2-ClC_6H_4.

Scheme 4

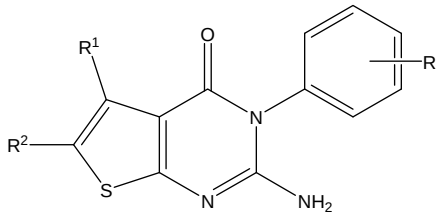
Table 26: 2-Arylaminothieno[2,3-*d*]pyrimidin-4(3*H*)-ones



R^1	R^2	R	Yield %	R. Solv	Reference
$(CH_2)_4$		H	57	E-D	26, 33
$(CH_2)_4$		4-CH_3	45	E-D	26, 33
$(CH_2)_4$		4-CH_3O	40	E-D	33
$(CH_2)_4$		4-Cl	69	E-C	33
$(CH_2)_4$		2-Cl	35	Di	26, 33
CH_3	CH_3	4-CH_3	60	E-C	33
CH_3	CH_3	4-CH_3O	53	E-C	33
C_6H_5	H	4-CH_3	62	E-C	33
-$(CH_2)_2$-N-$(CH_2C_6H_5)CH_2$-		4-CH_3	43	E-C	33

D=Dimethylformamide, C=Chloroform, E = Ethanol, Di=Dioxane

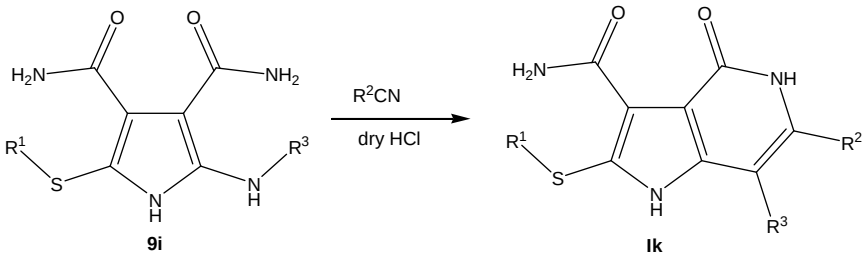
Table 27: 2-Amino-3-substitutedarylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones



R^1	R^2	R	Yield (%)	R. Solv.	Reference
-(-CH_2-)$_4$-		H	20	E-C	26, 33
-(-CH_2-)$_4$-		4-CH_3	25	E	26, 33
-(-CH_2-)$_4$-		4-CH_3O	18	B	33
-(-CH_2-)$_4$-		4-Cl	20	B	33
-(-CH_2-)$_4$-		2-Cl	30	E-C	26, 33
CH_3	CH_3	4-CH_3	19	E	33
CH_3	CH_3	4-CH_3O	23	E	33
C_6H_5	H	4-CH_3	27	E	33
-$(CH_2)_2$-N-($CH_2C_6H_5$)CH_2-		4-CH_3	22	E	33

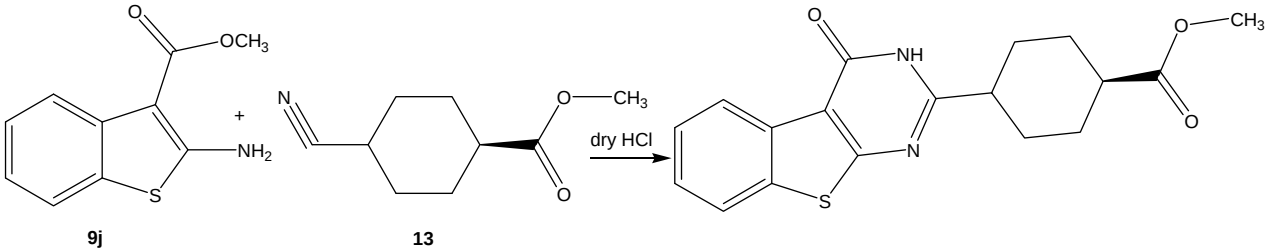
B=Benzene, C=Chloroform, E = Ethanol

Boehm *et al*.[48] have reported the synthesis of 2-substitutedpyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones **Ik** by reacting the pyrrole *o*-aminoester **9i** with various nitriles in the presence of dry hydrogen chloride.

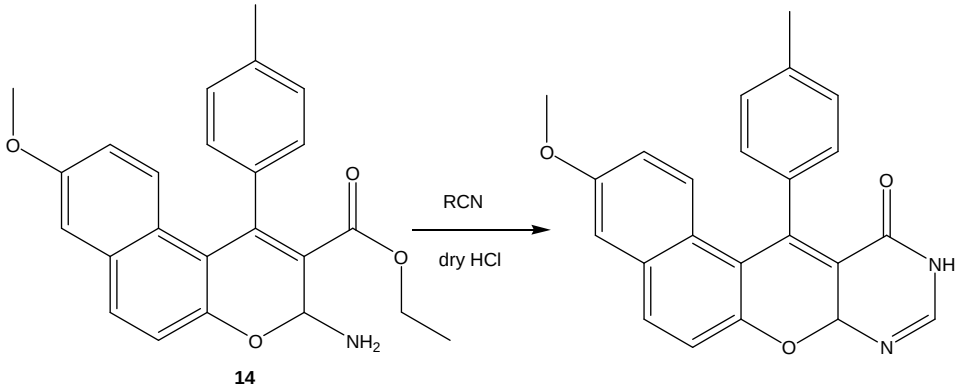


R^1 = alkyl, R^2 = alkyl, arylalkyl, carboxyalkyl, R^3 = H

Recently, Juraszyk and coworkers[49] have utilized the same approach to synthesize methyl *trans*-4-(4-oxo-3,4-dihydro[1]benzothieno[2,3-*d*]pyrimidin-2-yl)cyclo hexane carboxylate from 2-aminobenzothiophene-3-carboxylic ester **9j** and methyl *trans*-4-cynocyclohexane carboxylate **13** under the influence of dry HCl gas.

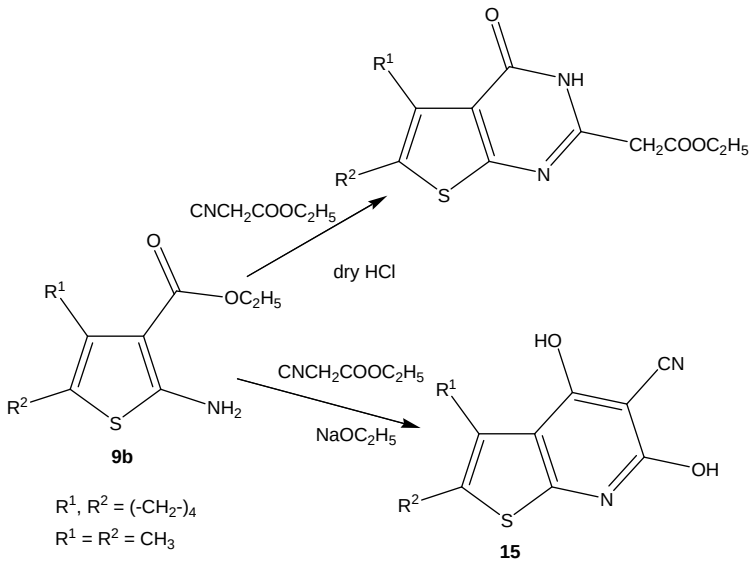


Similarly, Eid Fathy *et al.*,[50] have synthesized novel naphtha[2,1-*b*]pyrano[2,3-*d*]-pyrimidin-4-one derivatives from the corresponding *o*-amino ester **14** under the influence of dry HCl gas.

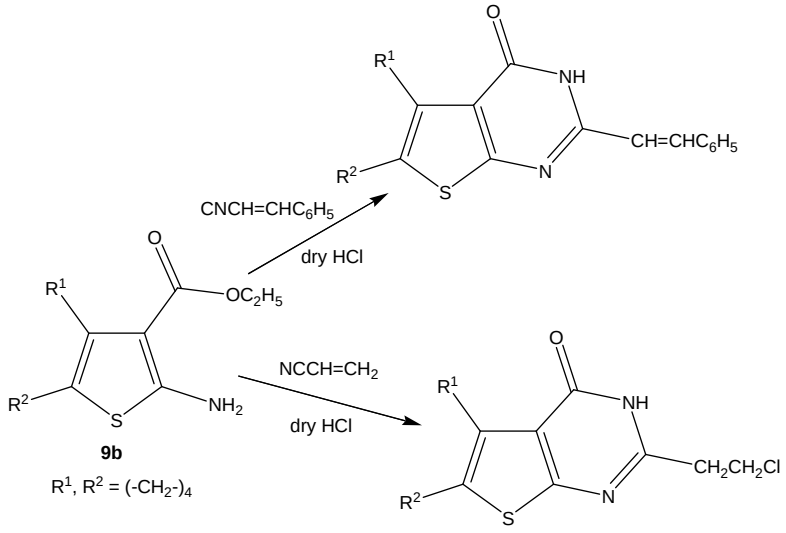


A.a Some Interesting Observations: Condensed 4-Oxopyrimidines

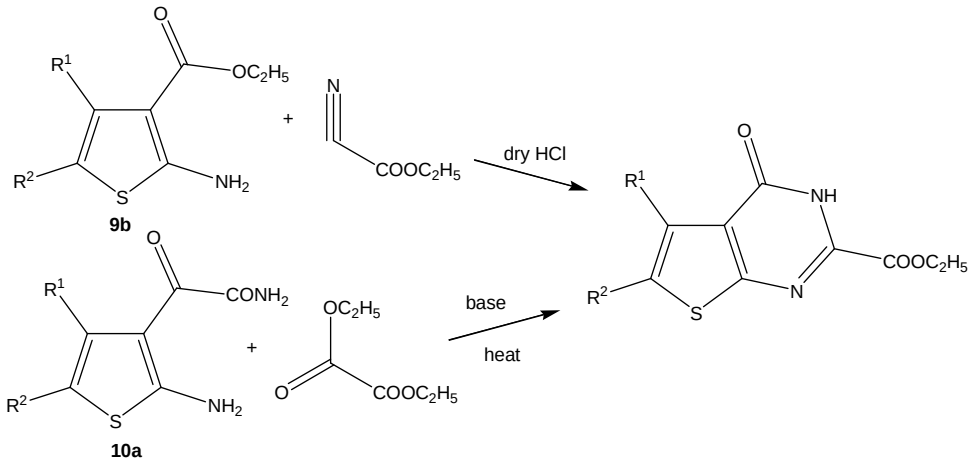
The condensation of thiophene *o*-aminoester **9b** with ethyl cyanoacetate, when effected in the presence of dry HCl gas yields the corresponding 2-carbethoxy-4-oxothienopyrimidine in 65% yields.[23,26] In contrast, the base catalyzed condensation, employing sodium ethoxide is reported to afford the 3-cyano-2,4-dihydroxy-thienopyridine[31]**15**.



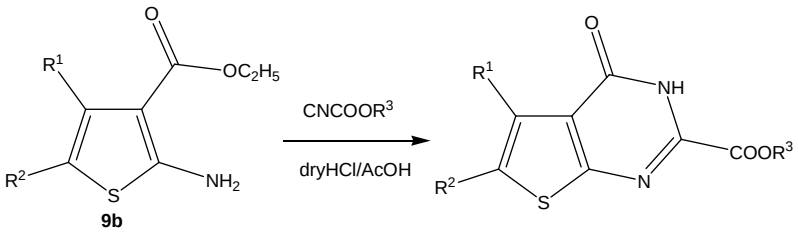
While, the use of cinnamonitrile in this condensation with the thiophene *o*-aminoester **9b** has yielded the expected 2-styrylthienopyrimidin-4-ones. On the other hand, acrylonitrile when condensed with 2-amino-3-carbethoxythiophene, yields 2-chloroethylthieno-pyrimidin-4-ones as the only product of the reaction.[23, 31] (Table 21).



The condensation of thiophene *o*-aminoesters with ethyl cyanoformate yielded the 2-carbethoxy-4-oxothienopyrimidin-4-one, which otherwise is, accessible only by the condensation of the *o*-aminoamide with diethyl oxalate at elevated temperature.[51]

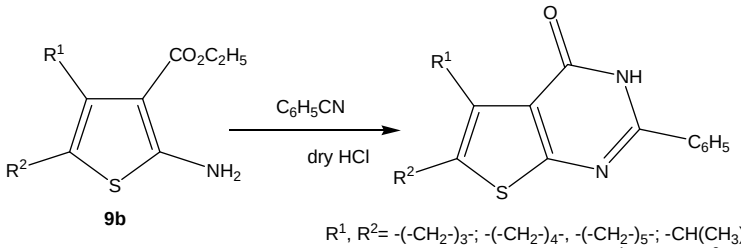


On this basis Madding & co-workers[52] have reported the synthesis of 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine-2-carboxylates *via* the HCl catalysed reactions of a mixture of the thiophene 3-carboxylates with activated nitriles. One of the derivatives, Tiprinast, 3,4-dihydro-5-methyl-6-(2-methylpropyl)-4-oxothieno[2,3-*d*]pyrimidine carboxylic acid is a proven orally active antiallergic and antiasthamatic drug.



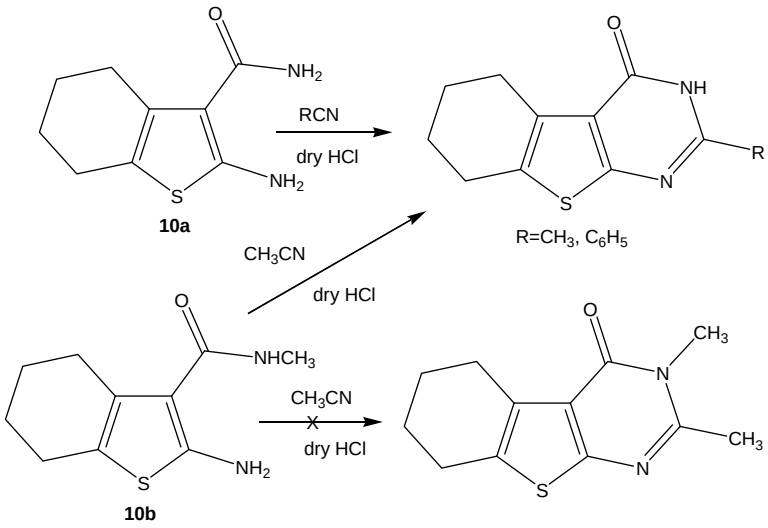
Tiprinast, $R^1 = CH_3$, $R_2 = (CH_3)_2CHCH_2^-$, $R^3 = H$.

Some European workers[53] have synthesized a series of 3-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones by the cyclization of 2-amino-3-carbethoxythiophenes with benzonitrile in the presence of dry HCl gas. These compounds have exhibited potent analgesic and anti-inflammatory activities.

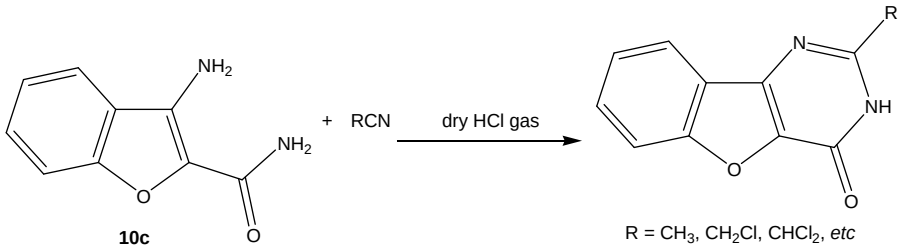


R^1, R^2= -(-CH_2-)$_3$-; -(-CH_2-)$_4$-, -(-CH_2-)$_5$-; -$CH(CH_3)$-(-CH_2-)$_3$-; -(-CH_2-)$_2$-$CH(CH_3)$-CH_2-CH_2-$CH(CH_3)$-(-CH_2-)$_2$-; $R^1 = C_6H_5$, R^2= H.

The dry HCl gas catalyzed reaction of 2-aminothiophene-3-carboxamide **10a** with acetonitrile and benzonitrile has yielded the corresponding 2-substituted-4-oxothienopyrimidines. Similar reaction of 2-amino-*N*-substituted thiophene-3-caboxamide **10b** with acetonitrile could be expected to yield the 3-*N*-substituted 2-substitutedthienopyrimidin-4-one. However, the reaction when actually conducted led to the formation of the 3-unsubstituted thienopyrimidin-4-one (R = CH_3), as the only product.[23,31,54]



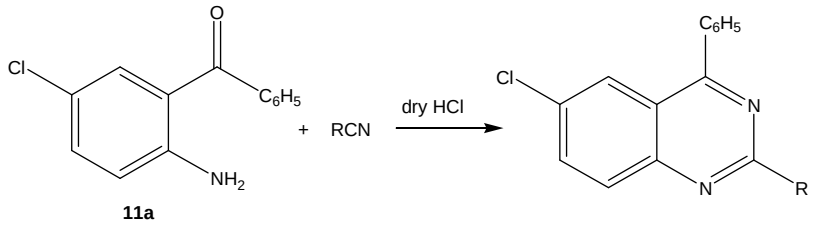
Similarly, benzofuro[3,2-*d*]-4-oxopyrimidines **10c** have been obtained by the reaction of the corresponding, *ortho*-aminocarboxamide derivative of the benzofuran, with nitriles (Table 23).[45,46]



The plausible explanation and proof for the reaction mechanism has been discussed in details in section **V** in the later part of this review.

1.4.2 Synthesis of Condensed 4-Arylpyrimidines

The hydrogen chloride catalyzed condensation has been found applicable to the synthesis of certain fully aromatic condensed pyrimidines by the reaction of *ortho*-aminoketones with nitriles. Thus, 2-amino-5-chlorobenzophenone **11a** has been reacted with aliphatic and aromatic nitriles to obtain the corresponding 2-substituted 4-phenyl-6-chloroquinazolines (Table 28).[23,31]



Similarly, 4-phenylthienopyrimidines have been obtained through the reaction of 2-amino 3-benzoylthiophenes **11b** with various nitriles.[23, 31]

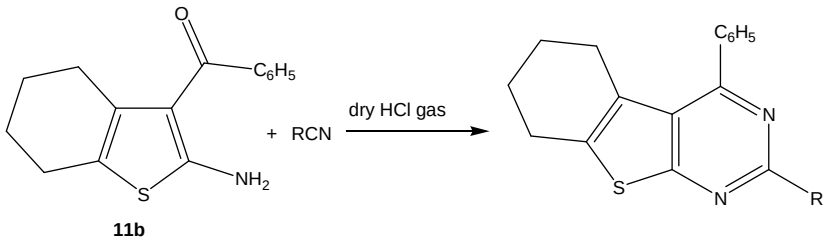
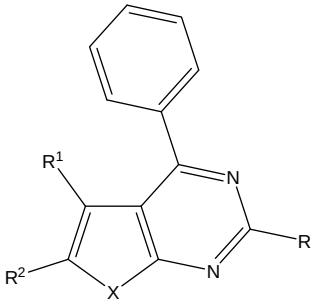


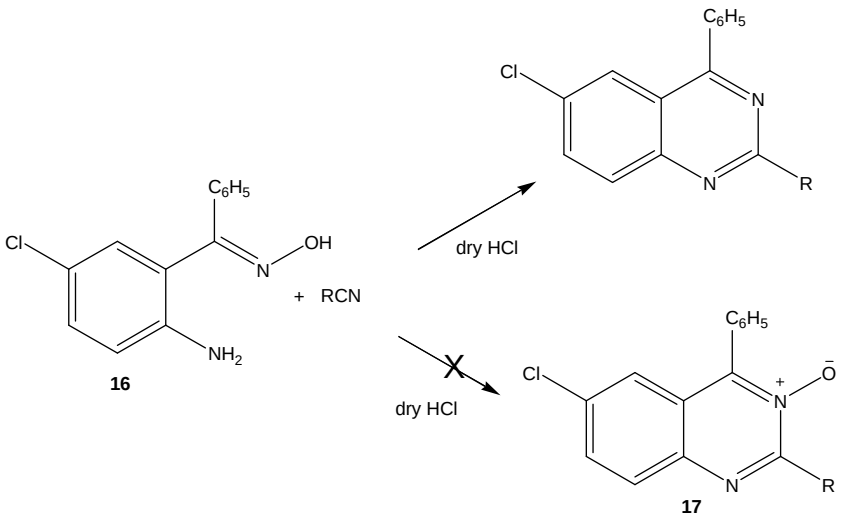
Table 28: Condensed 2-Substituted-4-phenylpyrimidines



R^1	R^2	R	X	Yield (%)	R. Solv.	Reference
H	Cl	$-CH_3$	-HC=CH-	70	B-H	31
H	Cl	$-CH_2Cl$	-HC=CH-	70	B-H	31
H	Cl	$-CH=C(C_6H_5)OH$	-HC=CH-	73	E	55
$-(-CH_2-)_4-$		$-CH_3$	S	54	E	31
$-(-CH_2-)_4-$		$-CH_2CO_2C_2H_5$	S	57	E	31
$-(-CH_2-)_4-$		$-CH_2Cl$	S	51	E	31

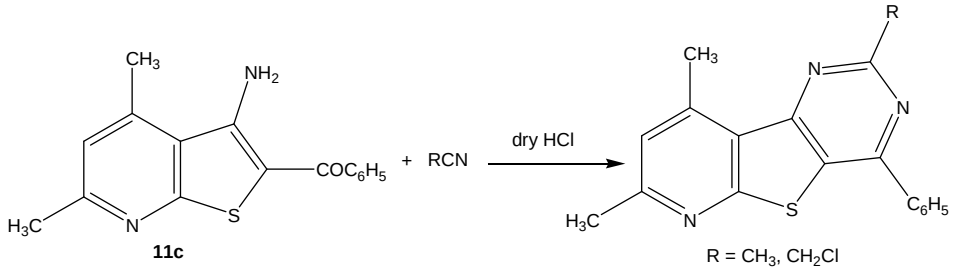
B = Benzene, C=Chloroform, H = Hexane, E = Ethanol

The condensation of *o*-aminoketoxime **16** with nitriles was found to yield the condensed 4-arylpyrimidines *via* the elimination of hydroxylamine, rather than the expected condensed 4-arylpyrimidin-*N*-oxides **17** (Table 28).[23]



Some novel 2-substituted-4-phenylpyrido[3,2-*d*]pyrimidines have also been obtained through the dry HCl gas catalysed condensation of the corresponding pyridothiophene *o*-aminoketone **11c** with nitriles like acetonitrile and chloroacetonitrile (Table 29).[56]

Table 29: 4-Phenylpyridothieno[3,2-*d*]pyrimidines

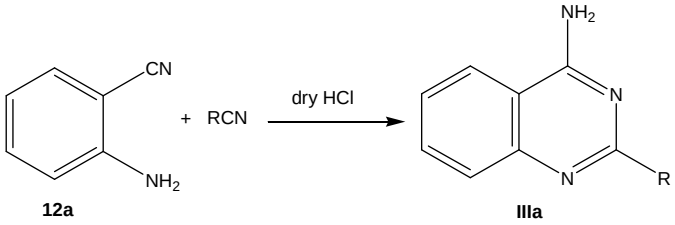


R	Yield (%)	R. Solv.	Reference
CH_3	66	E	55
CH_2Cl	55	E-C	55

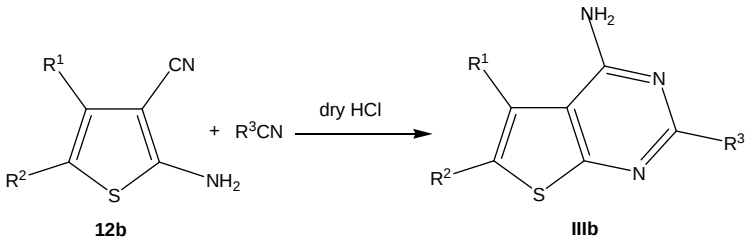
E = Ethanol, C = Chloroform

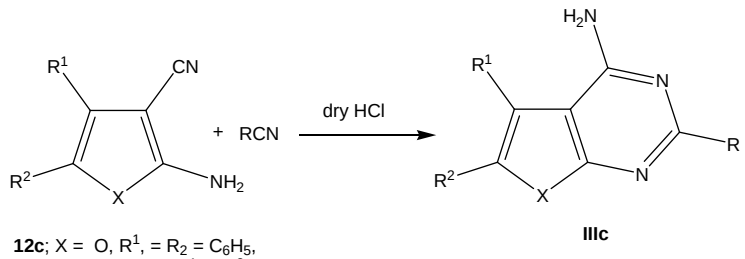
1.4.3 Synthesis of Condensed 4-Aminopyrimidines

This facile, dry HCl gas catalyzed one-pot synthesis of condensed pyrimidin-4(3*H*)-ones have been further extended to obtain condensed 4-aminopyrimidines through the reaction of nitriles with a variety of *o*-aminonitrile substrates. Thus, anthranilonitrile **12a** has been reacted with nitriles; acetonitrile, benzyl cyanide and benzoyl acetonitrile in presence of hydrogen chloride gas to give the corresponding 4-amino-2-substitutedquinazolines **III** in 40-65% yields (Table 30).[29,57]



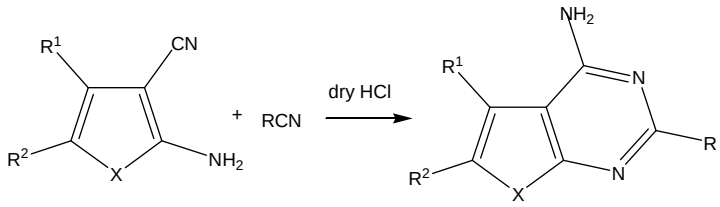
A host of nitriles has been found to react smoothly to give condensed 4-aminopyrimidines in good yields. Thus, the HCl (g) catalyzed reaction of nitrile with *o*-aminonitriles, for the synthesis of condensed pyrimidines can be said to be quite general in its scope. This hydrogen chloride catalysed condensation of nitriles, especially acetonitrile, benzonitrile, phenylacetonitrile, as well as heteronitriles like alkylthiocyanates, dialkyl and monoaryl cyanamides with thiophene *o*-aminonitriles, furan *o*-aminonitriles and pyrrole *o*-aminonitriles **12b-d** has been found to give the corresponding 2-substituted condensed 4-aminopyrimidines derivatives (Table 30) in good yields.[29,31,34,58]



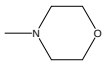


12c; X = O, R^1, = R_2 = C_6H_5,
12d; X= -N(C_6H_5)-, R^1, = R^2 = C_6H_5

Table 30: 4-Amino-2-substituted Condensed[2,3-*d*]pyrimidines



R^1	R^2	R	X	Yield (%)	R. Solv.	Reference
H	H	$-CH_3$	-HC=CH-	63	EA-Ch	29
H	H	$-CH_2C_6H_5$	-HC=CH-	40	EA-Ch	29
H	H	$-CH_2OC_6H_5$	-HC=CH-	40	E	29
$-(CH_2)_4-$		$-CH_3$	S	50	B	29
$-(CH_2)_4-$		$-CH_2C_6H_5$	S	43	B	29
$-(CH_2)_4-$		$-C_6H_5$	S	47	B	29
$-(CH_2)_4-$		$-SCH_3$	S	84	I	29
$-(CH_2)_4-$			S	40	C-H	29
$-(CH_2)_4-$		$2-C_5H_4N$	S	40	B-M	29
$-(CH_2)_4-$		$-NHC_6H_5$	S	33.8	E	34
$-(CH_2)_4-$		$-NHC_6H_4CH_3$-2	S	55.2	E-C	34
$-(CH_2)_4-$		$-NHC_6H_4CH_3$-4	S	41.8	E	34
$-(CH_2)_4-$		$-NHC_6H_4OCH_3$-2	S	61.3	E	34
$-(CH_2)_4-$		$-NHC_6H_4OCH_3$-4	S	61	E	34
$-(CH_2)_4-$		$-NHC_6H_40C_2H_5$-4	S	58.8	E-C	34
$-(CH_2)_4-$		$-NHC_6H_4Cl$-3	S	40	E-C	34
$-(CH_2)_4-$		$-NHC_6H_4Cl$-2	S	51.5	E-C	34

R^1	R^2	R	X	Yield (%)	R. Solv.	Reference
$-(CH_2)_4-$		$-NHC_6H_4Br$-4	S	56.1	E-C	34
$-(CH_2)_4-$		$-SC_2H_5$	S	68	B-h	57
$-(CH_2)_4-$		$-SC_3H_7$	S	79	B-h	57
$-(CH_2)_4-$		$-SC_4H_9$	S	84	B	57
$-(CH_2)_4-$		$-SCH_2C_6H_5$	S	70	B	57
$-CH_3$	$-CH_3$	$-SCH_3$	S	66	I	29
$-CH_3$	$-CH_3$		S	35	B-H	29
$-CH_3$	$-CH_3$	$-SC_3H_7$	S	83	I	57
$-CH_3$	$-CH_3$	$-SC_4H_9$	S	72	I	57
$-CH_3$	$-CH_3$	$-SCH_2C_6H_5$	S	71	E-C	57
$-CH_2CH_2N(CH_2C_6H_5)CH_2-$		$3-C_6H_4N$	S	43	D-E	31
$-(CH_2)_5-$		$-SCH_2C_6H_5$	S	56	E-C	57
$-C_6H_5$	$-C_6H_5$	$-CH_3$	O	68	B	29
$-C_6H_5$	$-C_6H_5$	$-C_6H_5$	O	47	B-M	29
$-C_6H_5$	$-C_6H_5$	$-CHCl_2$	O	55	B-H	29
$-C_6H_5$	$-C_6H_5$	$-CO_2C_2H_5$	O	35	B-H	29
$-C_6H_5$	$-C_6H_5$	$-CH_3$	$-N(C_6H_5)-$	45	E-D	39

B=Benzene, H = Hexane, I = Isopropanol , C = Chloroform, E =Ethanol, M = Methanol, D = Dimethylformamide, Ch =Cyclohexane, EA =Ethylacetate

The hydrogen chloride catalysis has also been extended to the synthesis of some novel 2-substituted 4-aminobenzo(*b*)furo[3,2-*d*]pyrimidines (Table 31) from the corresponding 3-amino-carbethoxybenzo(*b*)furan **12e.** However, the yields of the products are below 50%.

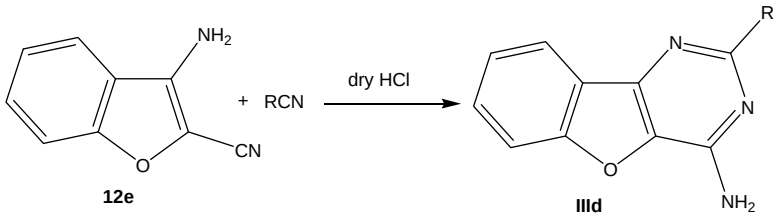
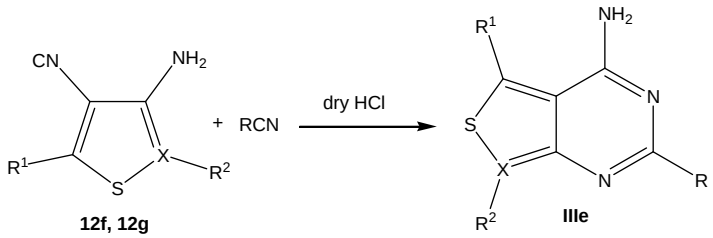


Table 31: 4-Amino-2-substituted benzo(*b*)furo[3,2-*d*]pyrimidines

R	Yield (%)	R. Solv.	Reference
$-CH_3$	35	B	45
$-C_6H_5$	48	B	45
SC_6H_5	17	B-M	46
$CO_2C_2H_5$	24	B	46
$CHCl_2$	17	B-M	46
$CH_2C_6H_5$	21	B-M	46
CH_2CH_2Cl	18	B-M	46
$CH_2C_6H_4NO_2$	19	B-M	46
$CH_2SC_6H_4Cl$	22	B-P	46

B=Benzene, M=Methanol, P=Petroleum ether

Angular condensed 4-aminopyrimidines, namely the 4-aminothieno[3,4-*d*]pyrimidines and 4-aminoisothiazolo[3,4-*d*]pyrimidine (Table 32) have also been synthesized through the reaction of 3-amino-4-cyanothiophene **12f** and 3-amino-4-cyanoisothiazole **12g** with nitriles in the presence of dry hydrogen chloride.[31,58]



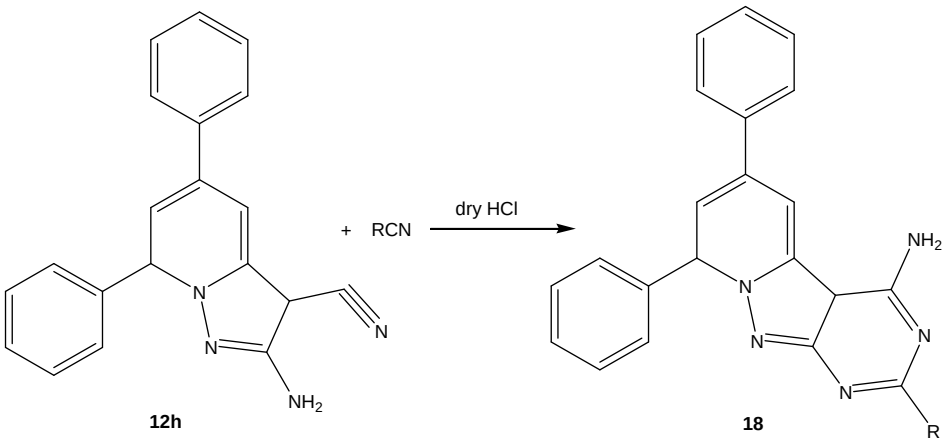
12f, 12g **IIIe**

Table 32: 4-Amino-2-substitutedthieno[3,4-*d*]pyrimidines and 4-aminoisothiazolo-[3,4-*d*]pyrimidines

R^1	R^2	X	R	Yield (%)	R. Solv.	Reference
$-SCH_3$	$COOC_2H_5$	C	CH_3	64	D-E	31
$-SCH_3$	$COOC_2H_5$	C	C_6H_5	51	E	31
$-SCH_3$	$COOC_2H_5$	C	$CO_2C_2H_5$	60	P-C	58
	$COOC_2H_5$	C	$CO_2C_2H_5$	50	P-C	58
$-SCH_3$	---	N	CH_3	45	E-D	31

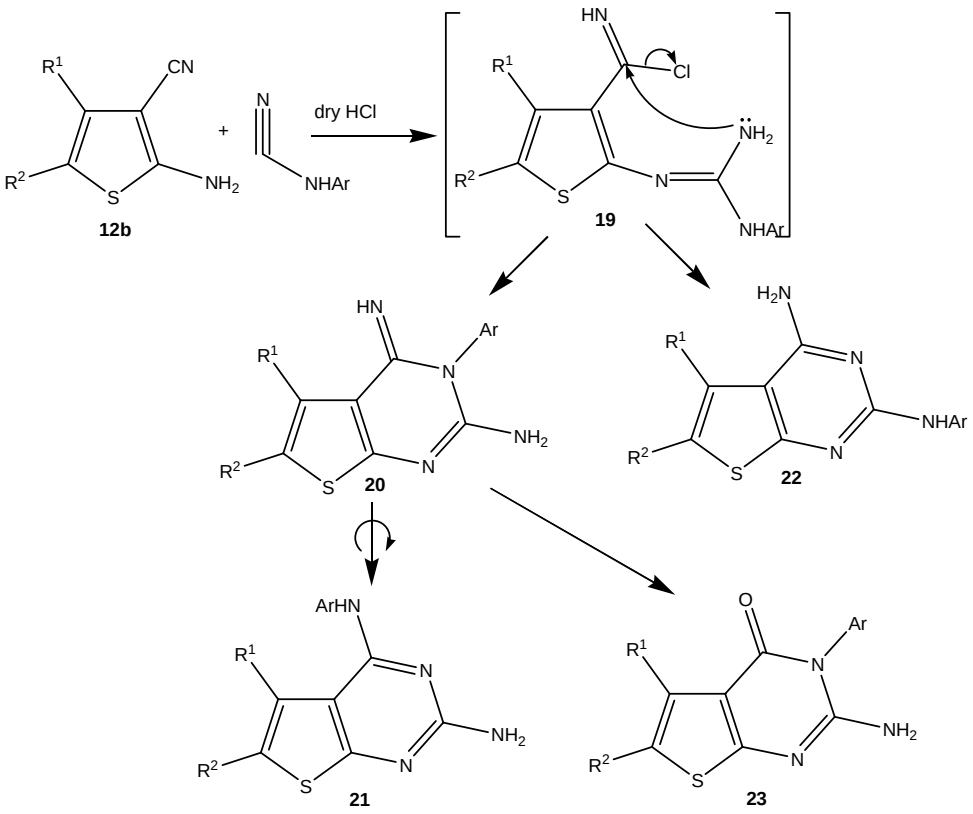
D = Diethylformamide, E = Ethanol, P = Petroleum ether C = Chloroform

Molina and co-worker[59] have reacted condensed pyrazole *o*-aminonitrile **12h** with aliphatic nitriles to yield the corresponding 4-amino-2-substitutedpyrazolopyrimidines **18.**



Interestingly, when mono arylcyanamides were reacted with thiophene *o*-aminonitriles under the influence of dry HCl gas, a mixture of two products was obtained. The major product was 2-amino-3-aryl-4-iminothieno[2,3-*d*]pyrimidine **20**, while the minor product was 2-amino-3-arylthieno[2,3-*d*]pyrimidin-4-one **23** (Scheme 5).

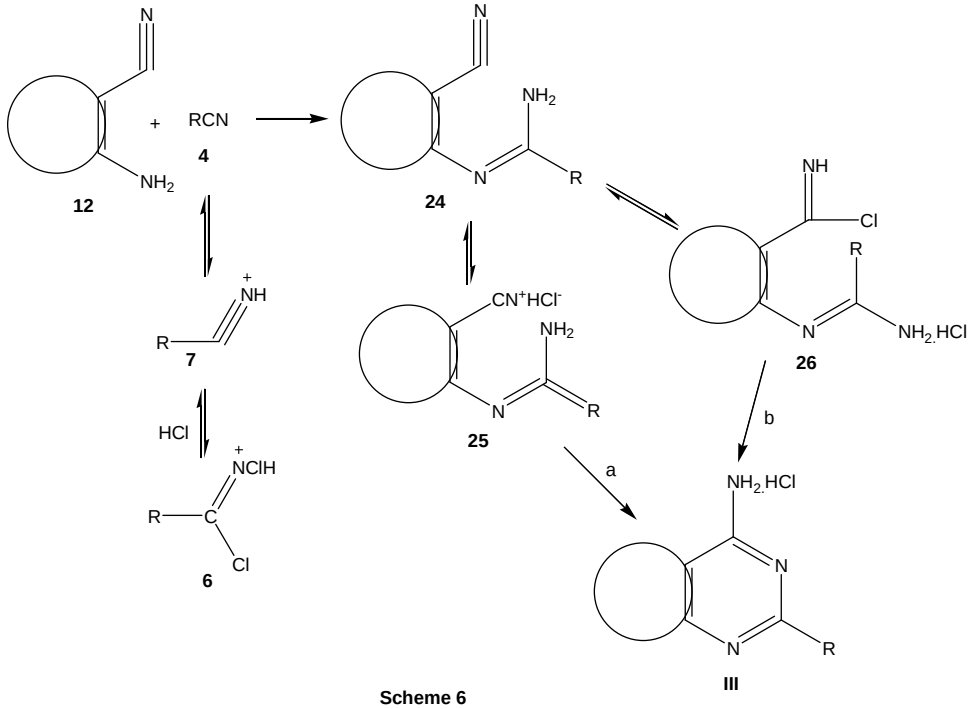
The proposed reaction mechanism envisages the possibility of the formation of three isomeric 2,4-diamoaminothieno[2,3-*d*]pyrimidines **20**-**22** through the alternate modes of cyclization of the guanidine intermediate **19** and Dimroth rearrangement of one of the isomers to the third isomer. However, the structural proof to the actual products was given through the unequivocal synthesis of three isomeric 2,4-diamoaminothieno[2,3-*d*]pyrimidines (Table 30) as well as, the 2-amino-3-arylthieno[2,3-*d*]pyrimidin-4-one (Table 27 and 29).[60,61] Compound **23** is infact the antifact of the reaction, arising through the hydrolysis of **20** during the workup.



Scheme 5

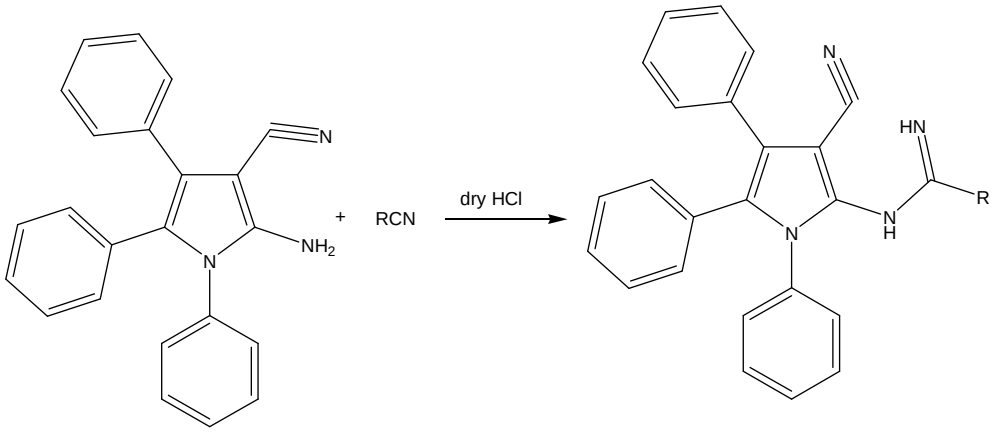
A Plausible explanation for the reaction mechanism involved in the condensation of an *o*-aminonitrile substrate **12** and a nitrile under the influence of dry HCl gas yield a 2-substituted condensed 4-aminopyrimidine **III** is discussed below.

These reactions, possibly, proceed through *o*-cyanoamidine intermediate **24** formed by the nucleophilic attack of the amino nitrogen on the *N*-protonated nitrilium species **7**, as the imidoyl halide, hydrohalide **6**. The *o*-cyanoamidine intermediate undergoes intramolecular cyclization through the nuceophilic attack by the amidine nitrogen on the carbon to yield the condensed 4-aminopyridine **III** as the observed product (path 'a'). The intramolecular cyclisation of *o*-cyanoamidine **24** is facilitated by the protonation of the cyano function **25** under the reaction conditions employed. Such *ortho* functionalised amidines have been presumed to be the intermediates in a variety of condensed pyrimidine synthesis through the reaction of *o*-aminocarbonyl derivatives with imidoyl derivatives. In view of the known tendency of the nitriles to form imidoyl halides in the presence of halogen acids, an alternate pathway involving the formation of *o*-amidinoimidoylchloride **26** and its intramolecular cyclisation to condensed 4-aminopyrimidines **III** also seems plausible (path 'b') (Scheme 6).



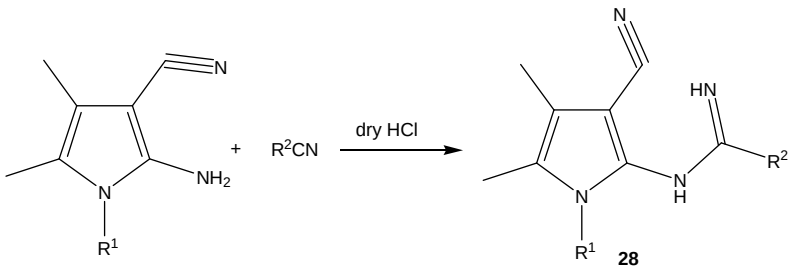
Scheme 6

An open chain intermediate **27** has been isolated in the reaction of the *o*-aminonitrile of triphenylpyrrole **12i** with nitriles; benzonitrile, phenylacetonitrile and chloroacetonitrile in presence of dry hydrogen chloride.[60]



R = C_6H_5 ,$CH_2C_6H_5$,CH_2Cl

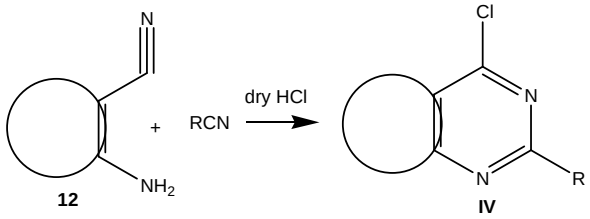
Eger *et. al.*,[62] have also isolated such amidine intermediate **28** in reaction of trimethylpyrrole *o*-aminonitrile **12j** with cyanamide and acetonitrile.



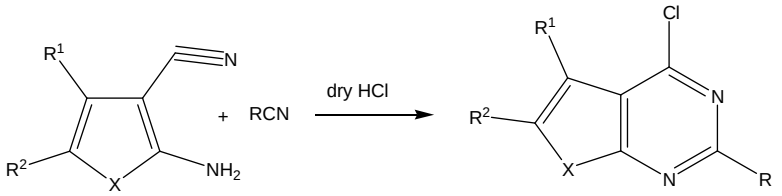
$R^1 = C_6H_5$, $R_2 = NH_2$
$R^1 = CH(CH_3)C_6H_5$, $R_2 = NH_2$
$R^1 = CH(CH_3)C_6H_5$, $R_2 = CH_3$

1.4.4 Synthesis of Condensed 4-Chloropyrimidines

In some of the reactions of *o*-aminonitriles with nitriles in presence of dry hydrogen chloride, interestingly 4-chloropyrimidine **IV** has been found to be the sole product formed.

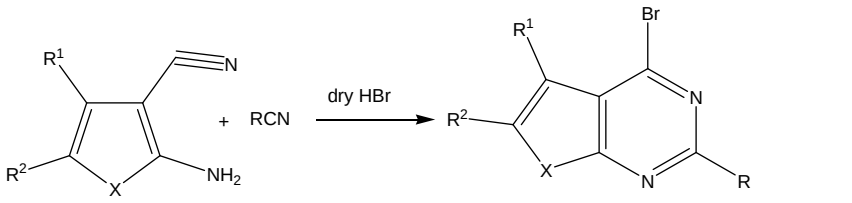


For example, the reaction of chloroacetonitrile and dichloroacetonitrile with anthranilonitrile **12a** in presence of excess of dry hydrogen chloride gas has been found to yield 4-chloro-2-chloromethylquinazolines in 85% yield. Surprisingly the expected 4-amino-2-chloromethylquinazoline was found to be totally absent.[29,61] Similarly, this hydrogen chloride catalysed condensation of active nitriles, especially, chloroacetonitrile and dichloroacetonitrile with thiophene *o*-aminonitriles **12b** and furan *o*-aminonitriles **12c** has been found to give the corresponding 2-substituted condensed 4-chloropyrimidines derivatives in good yields.[29]



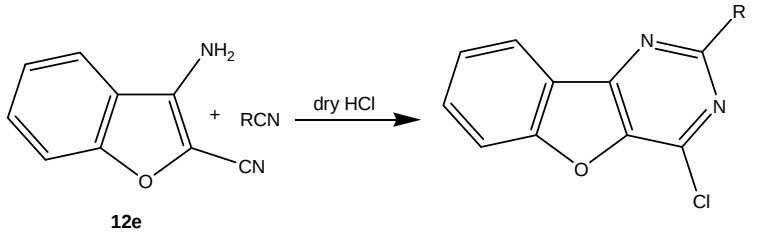
$R^1 = R^2 = H$, X = -CH=CH-; $R^1 = R^2 = CH_3$, X = S; R = CH_2Cl, $CHCl_2$
$R^1, R^2 = -(CH_2)_4-$, X = S; R = CH_2Cl, $CHCl_2$;
$R^1 = R^2 = C_6H_5$, X = O; R = CH_2Cl

Interestingly, when chloroacetonitrile and dichloroacetinitrile are reacted with anthranilonitrile **12a**, thiophene *o*-aminonitriles **12b**, furan *o*-amino nitrile **12c**, as well as, 3-amino-2-cyanopyridothiophene **12k**, in presence of dry HBr gas. The corresponding condensed 4-bromo-2-substitutedpyrimidine is the sole product.[63]

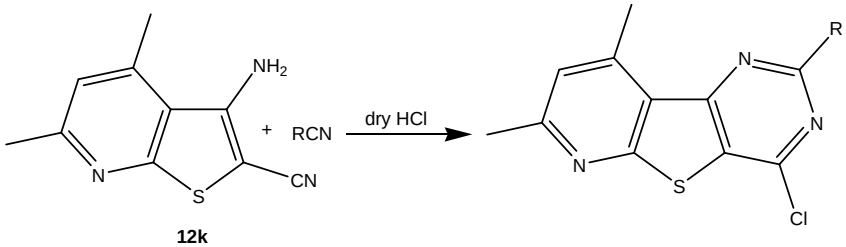


R^1 = R^2 = H, CH_3, C_6H_5, R^1, R^2 = -$(CH_2)_4$-,
X = -CH=CH-, S, O; R = CH_2Cl, $CHCl_2$

The hydrogen chloride catalysed reaction has been further utilized for the synthesis of some novel 2-substituted-4-chlorobenzo(*b*)furo[3,2-*d*]pyrimidines (Table 33)[54] and 4-chloropyridothieno[3,2-*d*]pyrimidines[55] from the corresponding 3-amino-carbethoxy-benzo(*b*)furan **12e** and 3-amino-2-cyanopyridothiophene **12k.**

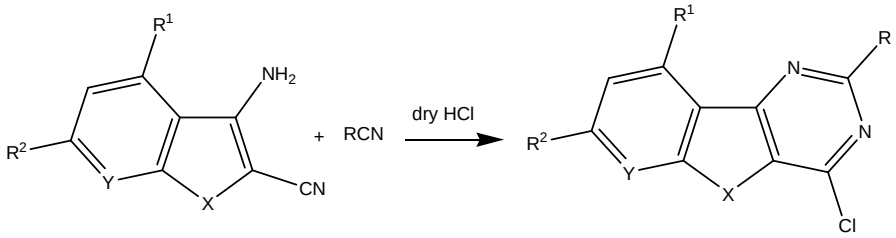


12e



12k

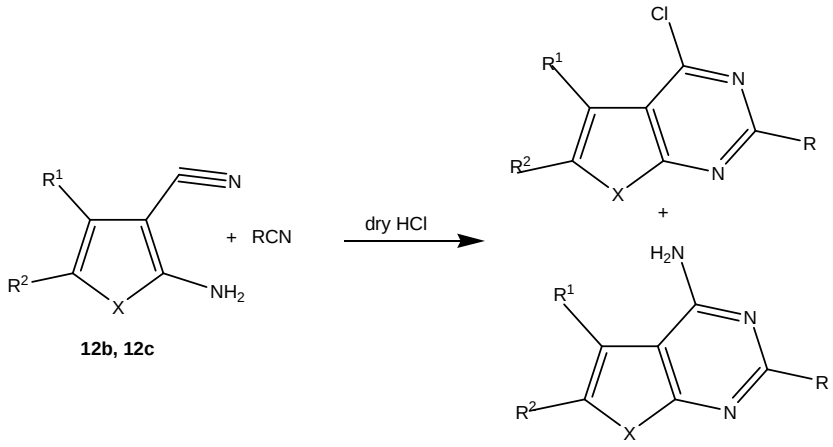
Table 33: 4-Chloro-2-substitutedbenzofuro[3,2-*d*]pyrimidines and 4-chloro-2-substituted pyridothieno[3,2-*d*]pyrimidine



R^1	R^2	X	Y	R	Yield (%)	R. Solv.	Reference
H	H	O	=CH-	CH_2Cl	68	H	54
H	H	O	=CH-	$CHCl_2$	21	P	54
CH_3	CH_3	S	=N-	CH_2Cl	60	B-H	54

B = Benzene, H = *Hexane, P = Petroleum Ether (60-80^oC)*

In contrast to the exclusive formation of condensed 2-substituted 4-chloropyrimidines as seen particularly in the reactions of mono & dichloroacetonitriles with various *o*-aminonitrile substrates, the condensation of the nitriles bearing moderately electron withdrawing groups, such as ethyl cyanoacetate, ethyl cyanoformate, phenylthioacetonitrile, phenoxyacetonitrile, phenylsulphonylacetonitrile, 4-nitrobenzo-nitrile, 4-nitrobenzyl cyanide, 3-cyanopyridine & 4-cyanopyridine, with thiophene *o*-aminonitriles **12b** and furan *o*-aminonitrile **12c** has been found to give a mixture of the corresponding condensed 2-substituted 4-amino- and 4-chloropyrimidines.[29]



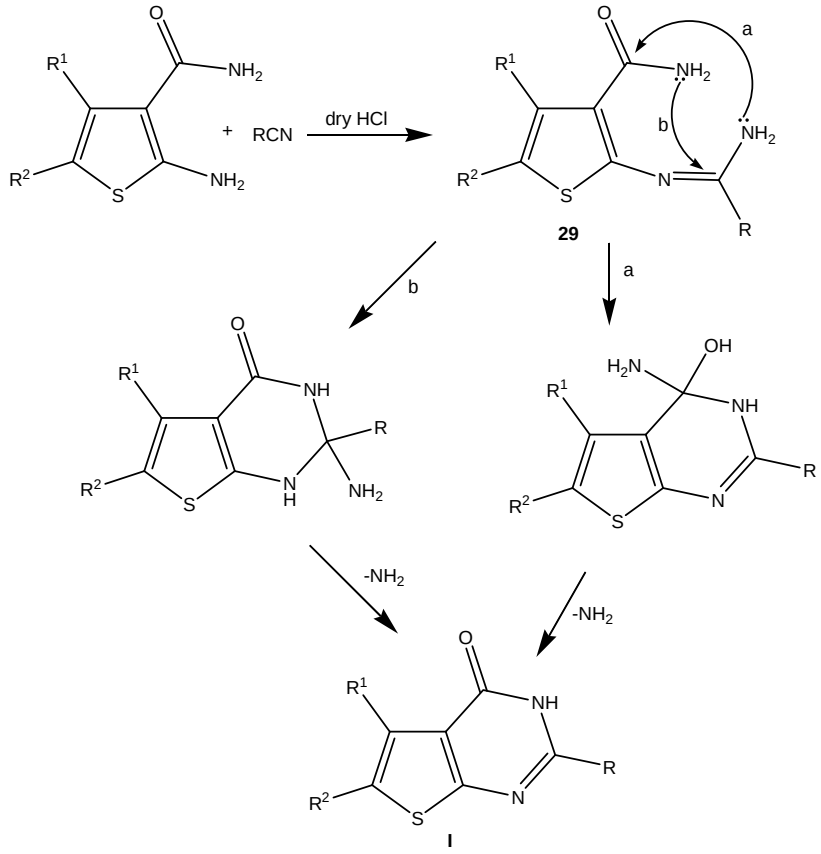
R^1, R^2= -(-CH_2-)$_4$-, C_6H_5
X= S, O
R= $C_2H_5COOCH_2$, C_2H_5COO, 4-$NO_2C_6H_4CH_2$,
4-$NO_2C_6H_4$,$C_6H_5OCH_2$, $C_6H_5SCH_2$,$C_6H_5SO_2CH_2$,
4-$ClC_6H_5SO_2CH_2$, 3-C_5H_4N, 4-C_5H_4N

1.5 Investigations in the Reaction Mechanisms for Product Formation, Disproportionations, as well as, Isolation and Cyclizations of Intermediates Involved.

1.5.1 Isolation of intermediate amidines in the synthesis of condensed 4-oxopyrimidines and their dry HCl gas catalyzed cyclization to condensed 2,3-disubstitutedthieno[2,3-*d*]pyrimidin-4-ones and condensed 3-unsubstituted-thieno[2,3-*d*]pyrimidin-4-ones

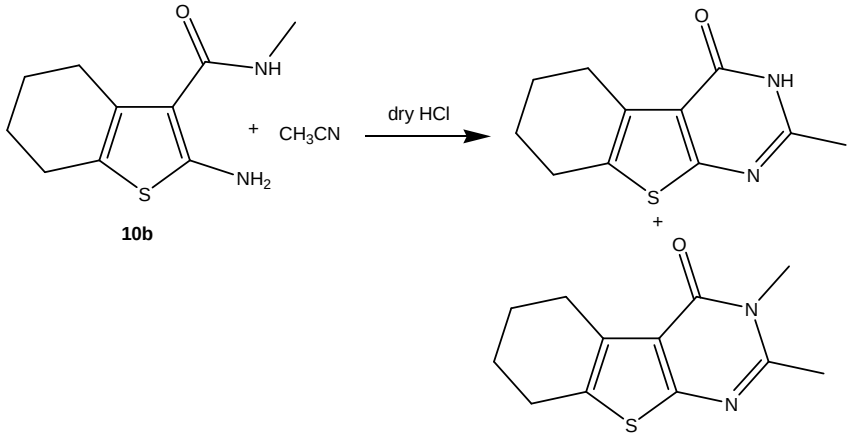
The reaction of 2-aminothiophene-3-carboxamide **10b** with acetonitrile and benzonitrile in presence of dry HCl has been found to yield the corresponding 2-substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **I.** These pyrimidines could conceivably arise by the loss of ammonia from the amidine intermediate **29** by either of the pathways involving

the nucleophilic attack by the amidine nitrogen on the amide carbonyl group (path 'a') or through the nucleophilic attack of the amide nitrogen on the amidine carbon (path 'b') (Scheme 7).



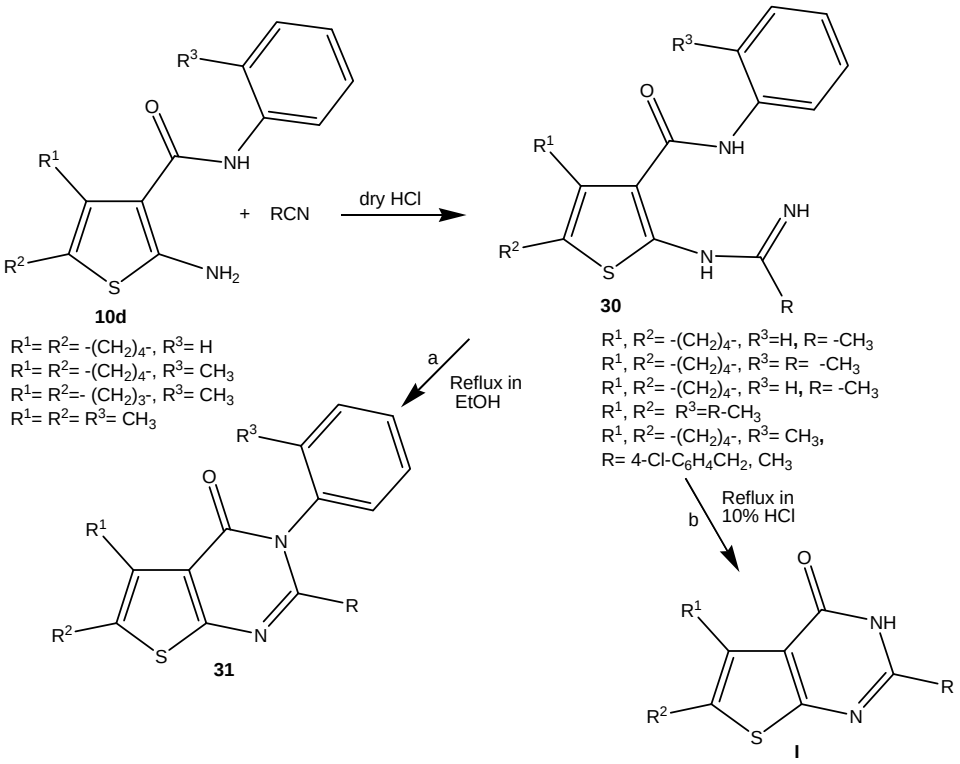
Scheme 7

The fact that the reaction of *o*-amino-*N*-methylcarboxamide **10b** with acetonitrile leads to the exclusive formation of 3-*N*-unsubstitutedthienopyrimidin-4(3*H*)-one and not to 3-*N*-methyltheinopyrimidin-4-one indicates that the reaction with the amides, presumably proceeds by the pathway 'a' involving the loss of NH_3 from the amide function.[28]



The condensation reaction of *o*-aminocarbonyl substrate with nitriles, presumably, proceeds by the nucleophilic addition of the amino group of the substrate to the nitrile or to a reaction species derived from the nitriles to yield the *o*-functionalized amidine intermediate, which undergoes intramolecular cyclisation to yield the pyrimidine.

Generally, such intermediates are not isolated due to their unstable nature. However, isolation of such amidine intermediates **30** has been reported in the reaction of thiophene *o*-aminoanilides **10d** with nitriles under acidic conditions.[28]



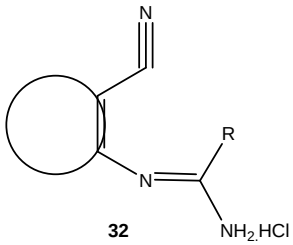
These intermediate amidines **30** when heated in absolute ethanol (path 'a') have been found to give the 2,3-disubstitutedthieno[2,3-*d*]pyrimidin-4-ones **31**, while on heating in acidic media they have been found to yield the 3-unsubstituted thieno[2,3-*d*]pyrimidin-4-ones **I.**[28]

1.5.2 Isolation of intermediate amidines and their dry HCl gas catalyzed cyclization to mononuclear 4-chloropyrimidines

A perusal of the comparative yields of the condensed 4-chlorothienopyrimidines **III** and 4-aminothienopyrimidines **IV** obtained in the reaction of acetonitrile and substituted

acetonitrile with thiophene *o*-aminonitrile indicates that 4-chlorothienopyrimidine formation can be observed in the reactions of thiophene-*o*-aminonitrile with nitriles possessing strong electron withdrawing substituent. Moreover, it appears that the yield of 4-chlorothienopyrimidine increases progressively with an increase in the –I effect of the substituent, reaching maximum with dichloroacetonitrile.[60]

Thus, the product formation in these reactions appears to depend upon the reactivity of amidine carbon of the *o*-cyanoamidine intermediate **32** towards nucleophilic attack which in turn can be expected to depend upon the nature of the nitrile component employed in the condensed pyrimidine synthesis. The formation of condensed 4-chloropyrimidines **IV** in the reaction of nitriles, possessing electron withdrawing substituent with *o*-aminonitriles **12** can be attributed to a low electron density at the amidine carbon of the *o*-cyanoamidine **32** intermediate because of the electron withdrawing effect of the substituent, which makes amidine carbon prone to nucleophilic attack by the incipient imidoyl nitrogen.



32

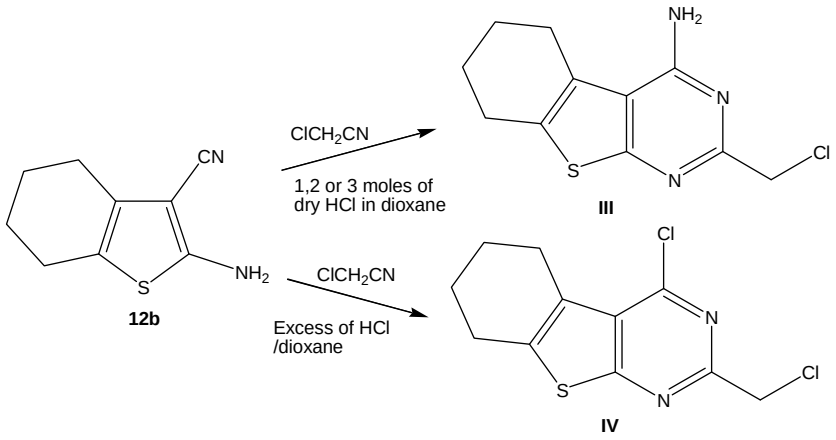
On the other hand, the nitriles, which do not possess an electron withdrawing substituent, lead to an amidine intermediate with a higher electron density at the amidine carbon, therefore an alternative pathway involving the nucleophilic attack of the amidine nitrogen on the cyano or imidoyl carbon predominates to yield the condensed 4-aminopyrimidine, **III.**

It has been found that changes in reaction temperature and rate of flow of hydrogen chloride, do not affect either the nature of the product or its yield. Nor the solvent of the reaction has any influence on the product nature or disproportionation.

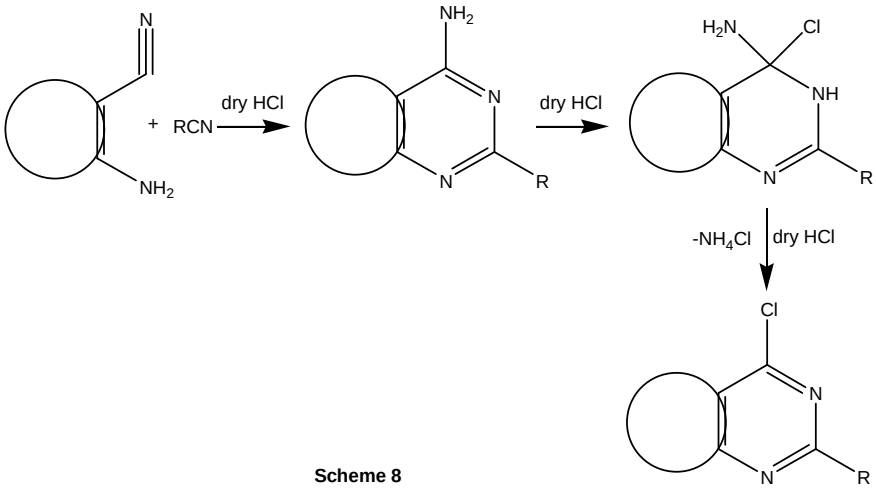
However, it has been observed that the amount of hydrogen chloride does play some role in influencing the nature of product formed. Thus, in a set of experiment the condensation

of equimolar quantities of thiophene *o*-aminonitrile and chloroacetonitrile was affected by employing dry HCl in different molar quantities.

With 1, 2 and 3 molar equivalents of a solution of dry HCl in dioxane, the product of the reaction was not the 4-chlorothienopyrimidine, but instead, the 4-aminothienopyrimidine. Addition of further excess of dry HCl, however, led to the formation the 4-chloropyrimidine.[60]



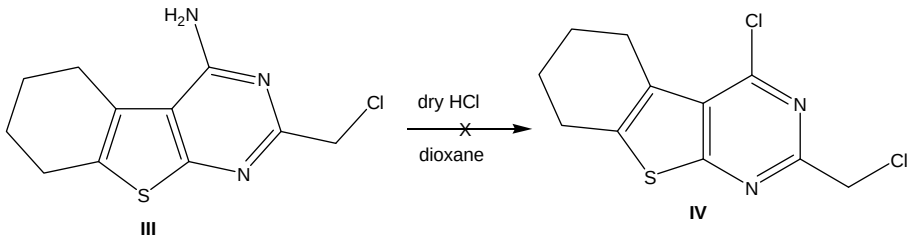
The above observations indicated the possibility of the formation of 4-chloropyrimidine through the 4-aminopyrimidine in the presence of excess of dry HCl gas, by the addition-elimination of HCl and NH_4Cl (Scheme 8).



Scheme 8

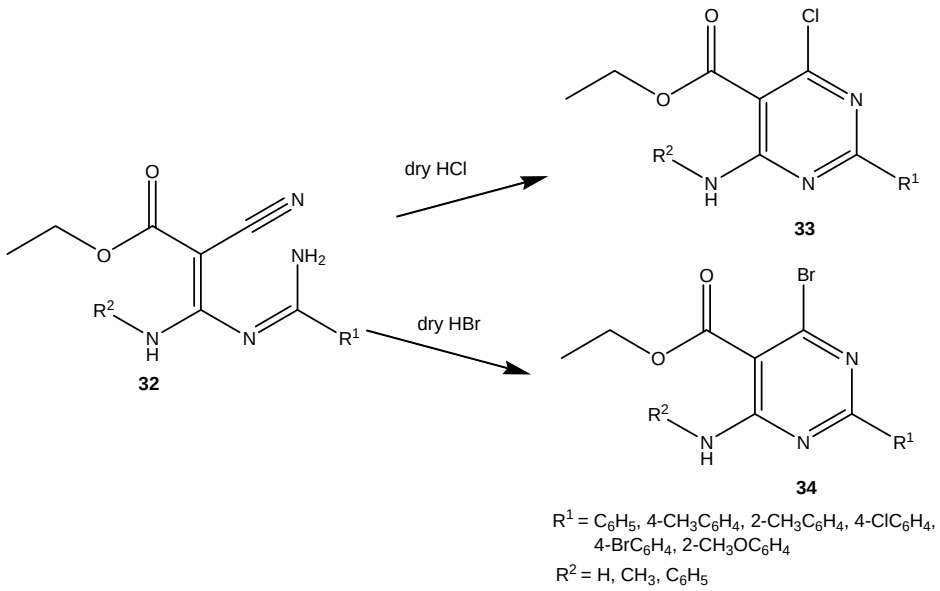
However, the possibility of the 4-aminopyrimidine as an intermediate in the formation of 4-chloropyrimidine has been excluded experimentally by bubbling excess of dry HCl gas through the solution of the preformed 4-aminothienopyrimidine in dioxane, under same standard reaction conditions. The workup of the reaction mixture didn't yield the

expected 4-chlorothienopyrimidine, instead the unchanged 4-aminopyrimidine was recovered.[60]



III **IV**

This one-pot formation of 4-chloropyrimidines is indeed novel, especially, in view of the fact that 4-chloropyrimidines are normally prepared through multistep synthesis, involving the preparation of the corresponding 4-oxopyrimidine, followed by its chlorination with $POCl_3$. The formation of 4-chloropyrimidines presumably proceeds through the transient *o*-cyanoamidine intermediates, **32** especially in view of the demonstrated isolability and also the cyclization of acyclic analogs of *o*-cyanoamidines, namely the *N*-(cyanovinyl)amidines **33** to 4-chloropyrimidines **34** in the presence of hydrogen chloride under essentially the same condition.[63,64]



32 **33** **34**

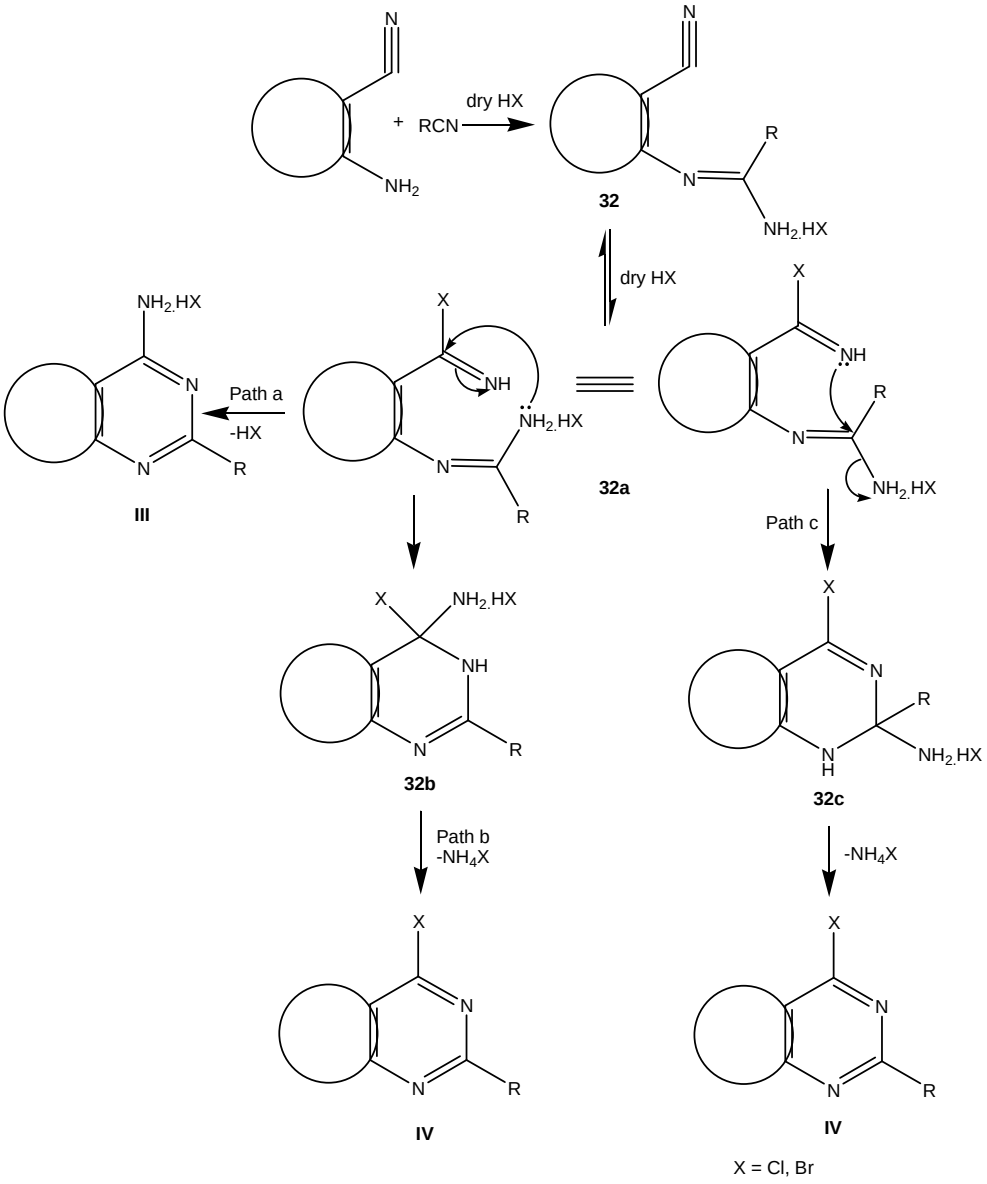
R^1 = C_6H_5, 4-$CH_3C_6H_4$, 2-$CH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 2-$CH_3OC_6H_4$

R^2 = H, CH_3, C_6H_5

On these lines, a plausible mechanism has been proposed[29,63,64] for the formation of condensed functionalised 4-halopyrimidines in these reactions under the influence of dry HCl or HBr gas.

It appears reasonable to assume that under the reaction conditions employed, the CN groups of both, the substrate, *o*-aminonitrile and the nitrile are activated by protonation or by the formation of hydrogen halide adducts. The initial condensation between the two components or their activated forms can be expected to result in the formation of the

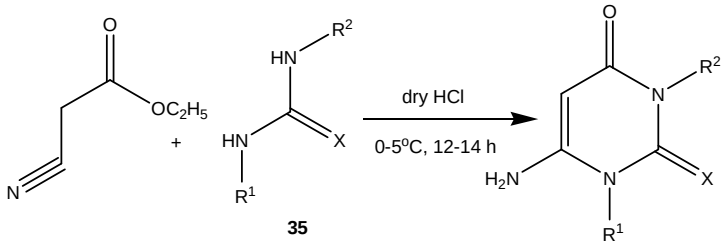
amidine hydrohalide or its hydrohalide adduct. Assuming that the imidoyl halide derivatives is the common intermediate, the formation of 4-aminopyrimidines, **III** can take place by 'path a' from the cyclic adduct and that of the 4-halopyrimidines **IV** by the 'path b' or 'path c' (Scheme 9)**.**



Scheme 9

1.6 Synthesis of Various Mononuclear Pyrimidines under Influence of Dry HCl Gas

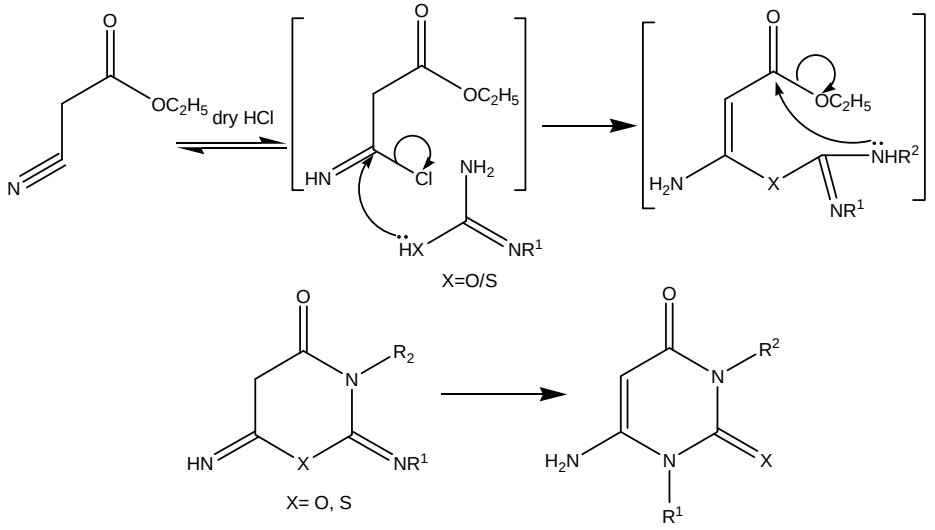
This novel reaction has been extended to the synthesis of monocyclic pyrimidines. Thus, ethyl cyanoacetate has been condensed with monoaryl and diaryl thioureas **35** to yield 6-amino-1-aryl and 6-amino-1, 3-diaryl thiouracils.[65,66]



R^1= H, 3-$CH_3C_6H_4$, 3-ClC_6H_4, C_6H_5, 4-$CH_3C_6H_4$, 2-$CH_3C_6H_4$, 4-ClC_6H_4, 2-ClC_6H_4, 4-$CH_3OC_6H_4$, 2-$CH_3OC_6H_4$
R^2= H, 3-$CH_3C_6H_4$, 3-ClC_6H_4
X= S, O

Novel series of 6-amino-1,3-diaryl-2-thiouracils, 6-aminouracil, 6-amino-2-thiouracil, 6-amino-1-aryluracils and 6-amino-1-aryl-2-thiouracils were synthesized[65] through the dry HCl catalyzed cycolcondensation of ethyl cyanoacetate with *symm*-diarylthioureas, urea, thiourea, monoarylureas and monoarylthioureas, respectively. The reaction involves the condensation of ethyl cyanoacetate with an appropriate urea or thiourea in the presence of dry HCl gas in dioxane at 0-5^{o}C for 12-14 hours. However, ethyl cyanoacetate failed to react with 1,3-diarylureas may be due to weaker nucleophilicity of the latter.

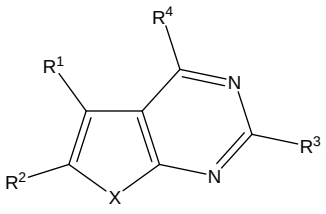
As an extension, on similar condensation benzoylacetonitrile with simple thiourea yielded 6-amino-4-phenyl-2-thoxopyrimidine.[65] Ethyl cyanoacetate reacts with ureas and thioureas presumably through the initial nucleophilic attack on the sulphur or oxygen atom of urea or thiourea on the protonated nitrile or imidoyl halide to yield imino oxide or sulphide intermediate, followed by its intramolecular cyclization through the corresponding oxazine or thiazine. The oxazine or thiazine intermediate may then under go a Dimroth rearrangement under the reaction condition to yield then corresponding 6-aminouracils or 6-amino-2-thiouracils (Scheme 10).[66]



Scheme 10

1.7 Synthesis of Condensed 4-Oxopyrimidines by Novel Acid Catalyzed Microwave Assisted Reaction of Nitriles with *o*-Aminoesters under Solvent Free Conditions.

Encouraging results in the MWI based syntheses of thiophene *o*-aminoesters involving Gewald reaction[67], as well as, thienopyrimidine bioisosteres of gefitinib[68] under microwave irradiation conditions, prompt for the use of MWI to be extended to the one-pot cyclocondensation of the nitriles with various *o*-aminoester substrates under solvent free conditions for generating compound libraries of condensed pyrimidines **36**.



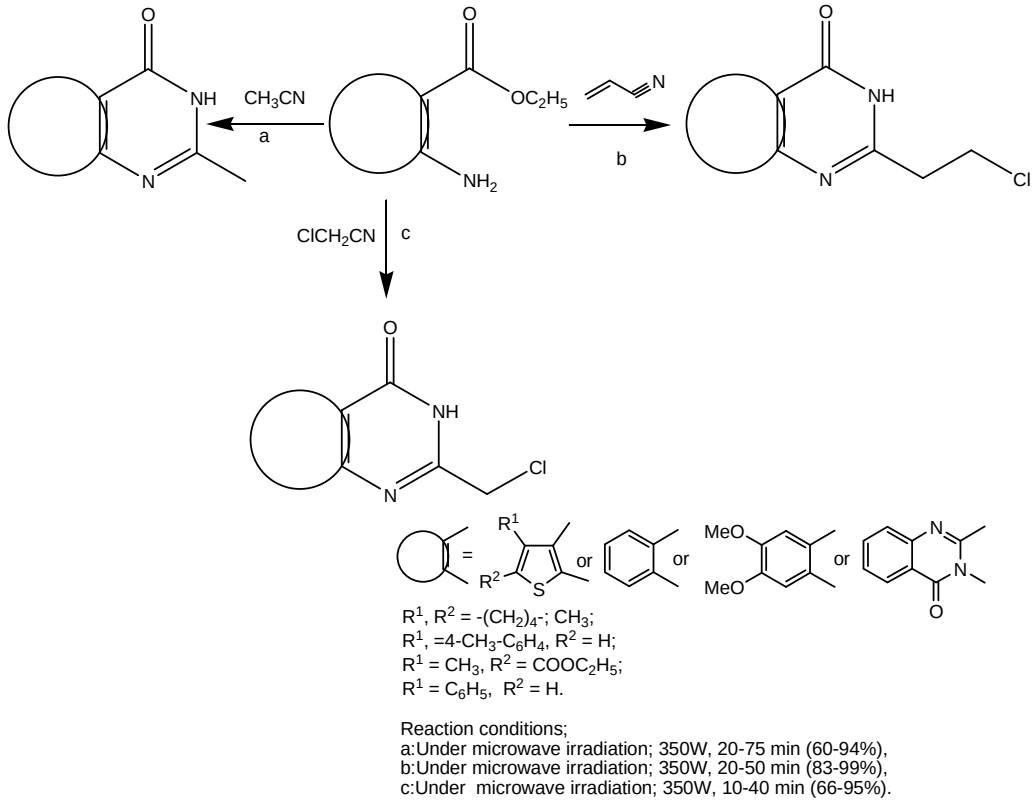
36

X = S, -CH=CH-
R^1, R^2 = H, alkyl, aryl, cycloalkyl, carboalkoxy, carbocyclic, heterocyclic, *etc*

A novel microwave assisted green synthesis of the bioactive condensed 2-substitutedpyrimidin-4(3*H*)-ones **I** under solvent free conditions has been reported for the first time.[69] The unusually rapid synthetic methodology involves the cyclocondensation of a variety of nitriles with *o*-aminoesters of benzene **9a**, thiophene **9b**, quinazolinone **9g** and dimethoxybenzene **35** in the presence of catalytic amount of conc. HCl alone or with the Lewis acid, $AlCl_3$. This novel synthesis involving nitriles as the building blocks, under microwave irradiation for these condensed 2-substitutedpyrimidin-4(3*H*)-ones requires only 10-75 min as compared to the conventional reaction protocols requiring 6-12 h, thereby showing a significant acceleration in reaction rates (Table 34) (Scheme 11).

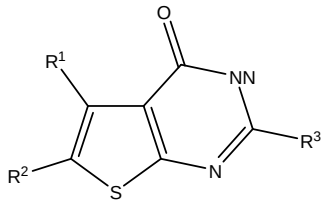
The reaction proceeds through the same activated electrophilic nitrile derivatives, the imidoyl halide intermediate & affords the products in yields superior to that by the conventional protocols. Coupled with simple workup procedures and superior yields the methodology is eminently suitable for the generation of diverse libraries of condensed 2-substitutedpyrimidin-4(3*H*)-ones employing parallel synthesis procedures.

It is therefore really interesting, that this acid catalysed cyclocondensation reaction has been made adaptable to high throughput synthesis, for the generation of diverse libraries of condensed pyrimidines **36** with four diversity points for further functionalization, if necessary.



Scheme 11

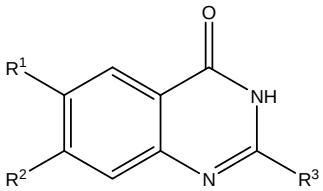
Table 34: **Physical data of 2-substitutedthieno[2,3-*d*]pyrimidin-4(3*H*)-ones**

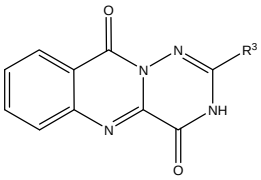
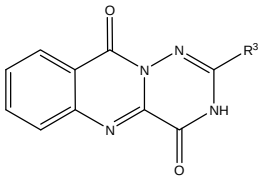


			Conventional Method			Microwave-Assisted Method		
R^1	**R^2**	**R^3**	**Yield (%)**	**Mp (^oC)**	**Time (h)**	**Yield (%)**	**Mp (^oC)**	**Time (min.)**
-(CH_2)$_4$-		CH_3	66	300	8-10	68	298	75
CH_3	CH_3	CH_3	76	256	10-12	89	256	60
4-$CH_3C_6H_4$	H	CH_3	93	245	8-10	94	248	55
CH_3	OOC_2H_5	CH_3	71	262	8-10	85	262	40
C_6H_5	H	CH_3	69	264	8-10	75	264	65
-(CH_2)$_4$-		CH_2CH_2Cl	84	220	8-10	96*	218	45
CH_3	CH_3	CH_2CH_2Cl	85	200	20-24	99	201	50
4-$CH_3C_6H_4$	H	CH_2CH_2Cl	91	168	10-12	92*	169	35
CH_3	$COOC_2H_5$	CH_2CH_2Cl	85	167	8-10	88	167	20
C_6H_5	H	CH_2CH_2Cl	94	268	8-10	96	267	45
-(CH_2)$_4$-		CH_2Cl	77	259	6-8	90	258	30
CH_3	CH_3	CH_2Cl	83	257	8-10	91	256	25
4-$CH_3C_6H_4$	H	CH_2Cl	88	248	6-8	93	249	40
CH_3	$COOC_2H_5$	CH_2Cl	86	225	6-8	95	225	10
C_6H_5	H	CH_2Cl	90	215	6-8	91	214	35

** Catalytic amount of anhydrous. $AlCl_3$ was added to the reaction mixture*

Table 35: **Physical data of other condensed 2-substitutedpyrimidin-4(3*H*)-ones**



R^1	R^2	R^3	Conventional Method			Microwave-Assisted Method		
			Yield (%)	M.P (^oC)	Time (h)	Yield (%)	M.P (^oC)	Time (min.)
H	H	CH_3	70	240	8-10	80	240	45
OCH_3	OCH_3	CH_3	62	239	8-10	70	240	45
		CH_3	52	243	8-10	71	243	20
H	H	CH_2CH_2Cl	80	200	8-10	83	202	30
H	H	CH_2Cl	90	242	6-8	94	241	30
OCH_3	OCH_3	CH_2Cl	65	242	6-8	70	242	25
		CH_2Cl	53	240	8-10	66	241	20

1.8 Scope and Limitations

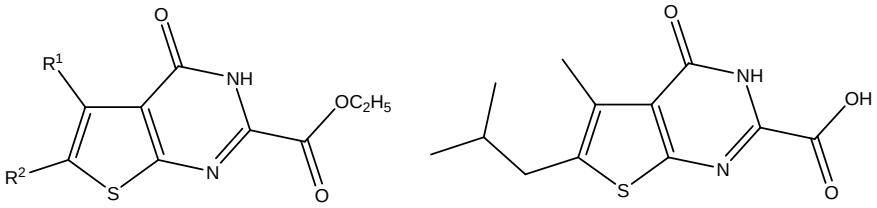
The synthesis of condensed 2-substitutedpyrimidines is in general carried out by initially introducing the appropriate *o*-aminocarbonyl substrate and a nitrile into a suitable solvent like dry dioxane and then passing a stream of dry hydrogen chloride gas under ambient temperature through the reaction mixture. However, it is possible to significantly increase reaction yield and purity of reaction product and further to shorten the reaction time if initially an excess of acid is dissolved in the solvent, preferably the solvent is saturated with the acid.

An excess of acid is an amount of acid so large that after quantitative reaction of compounds subsequent precipitation as salt unbound acid still remains in this solution. This amount of acid is to be already present in the reaction mixture at the start of reaction.

It has proved to be appropriate for the solvent to be selected from the group consisting of ethers, esters, alcohols, water, formamides, amines, carboxylic acid, but particularly important & suitable solvent is dioxane

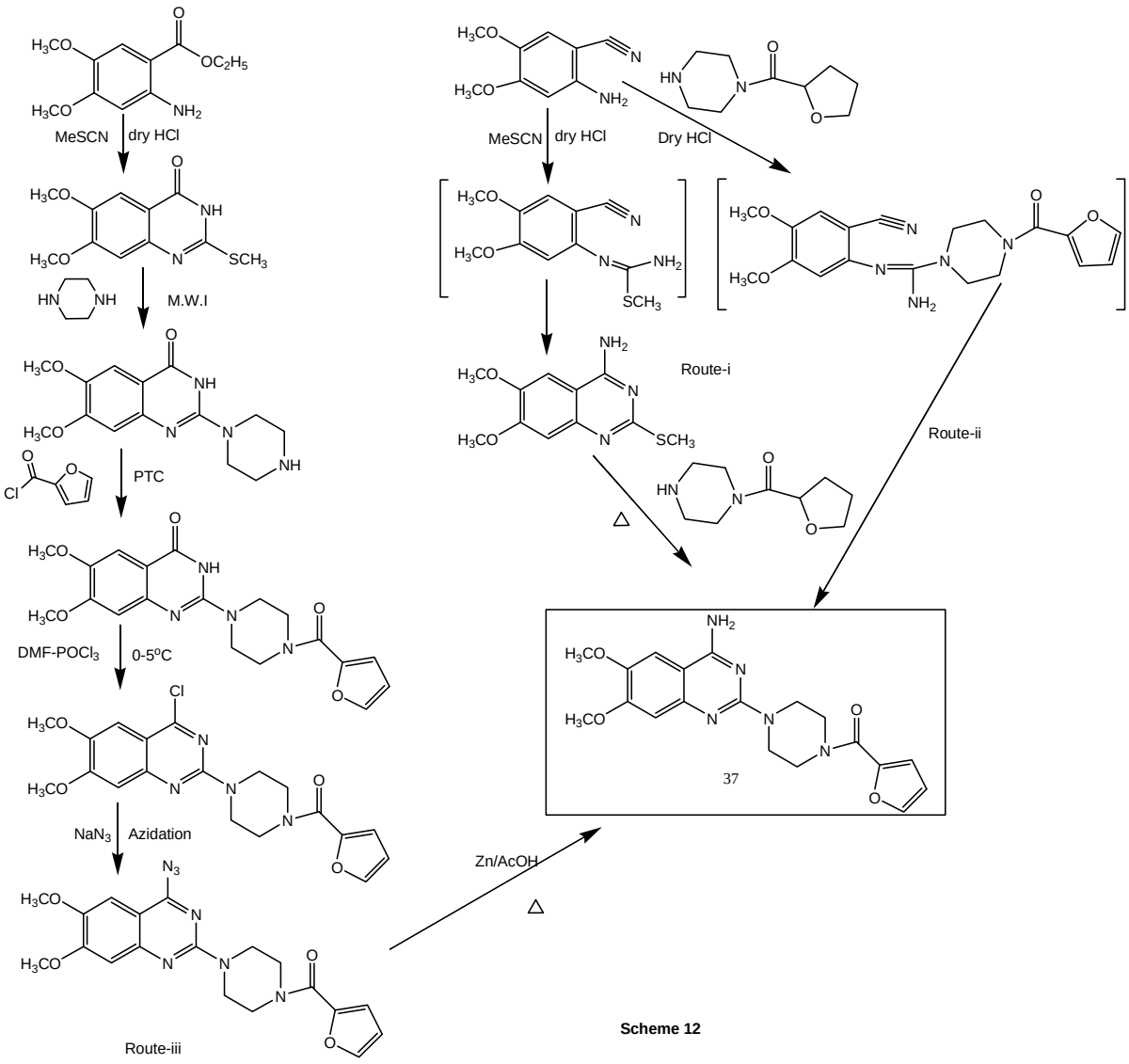
The acids are suitably selected from the group consisting of Bronsted acid & Lewis acids in particular hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, particularly suitable are gaseous acid *e.g.*, hydrogen chloride.
The reaction is suitably carried out at temperature of from -10^{o}C to 100^{o}C, preferably 0^{o}C to 60^{o}C, in particular 10^{o}C to 50^{o}C. The addition of acid to the mixture is continued during the reaction. Thus, it is possible to achieve nearly quantitative precipitation of compound as salt of acid.

Madding & co-workers[52] have reported the synthesis of 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine 2-carboxylates *via* the HCl catalysed reactions of thiophene 3-carboxylates with activated nitriles. One of the derivatives, Tiprinast, 3,4-dihydro-5-methyl-6-(2-methylpropyl)-4-oxothieno[2,3-*d*]pyrimidinecarboxylic acid is a proven orally active antiallergic and antiasthamatic drug.



Tiprinast

There are three reported routes for the synthesis and manufacture of Prazosin **38**, a selective α_1-adrenoreceptor antagonist antihypertensive drug. However, these presently used routes are having disadvantages (Scheme 12)[70,71] of very low overall yields 8-10, or use of thiophosgene, use of drastic reaction conditions as well as prolonged reaction times and lastly longer and multistep syntheses, which increases the overall cost of the product.
Many of the key steps in this synthesis have been modified and replaced with simpler reactants and drastic reaction conditions have been replaced by this novel nitrile reaction under acidic conditions.[72] Thus, many more uses of this novel reaction condition can be explored for the syntheses of API and drug intermediates as well as specialty fine chemicals.

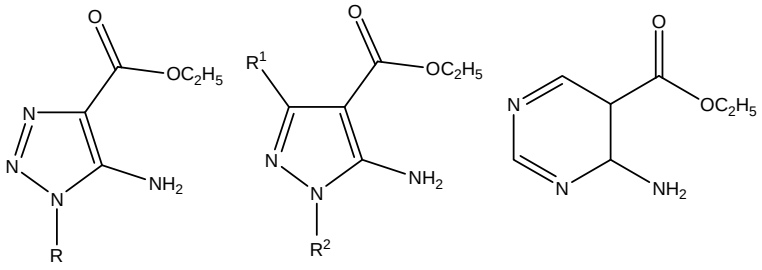


Scheme 12

The potential of this reaction for parallel synthesis by judiciously modifying the reaction conditions to generate novel libraries of NCE's of pyrimidine and condensed pyrimidines is also quite good. With the successful application MWI in speeding up this reaction, the potential of this reaction for the parallel synthesis of NCE's is much more.

A few limitations to this reaction, especially its inability to proceed to completion with a few typical *o*-aminocarbonyl substrates are noted below.

This one-pot, hydrogen chloride catalyzed reaction has found to fail with the *o*-amino carbonyl substrates of 1, 2, 3-triazole **38,** pyrazole **39**, and pyrimidine **40**.



1.9 Conclusions

Interestingly, this novel and interesting reaction can be explored to prepare a variety of drugs and drug intermediates, through almost one pot condensations and to afford products in good yields as well as purity.

Secondly, the reaction can also be modified suitably in its reaction conditions to be exploit and use for high throughput synthesis of compound libraries for New Drug Discovery Research (NDDR).

1.10 References

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2. Impact of Microwave Assisted Heating on the Combinatorial and Parallel Syntheses of Compound Libraries for New Drug Discovery Research

2. Impact of Microwave Assisted Heating on the Combinatorial and Parallel Syntheses of Compound Libraries for New Drug Discovery Research: A Review

2.1 Introduction

Medicinal chemistry has benefited tremendously from the technological advances in the field of combinatorial chemistry and high-throughput parallel synthesis. Developments of methods and technologies have helped accelerate the design, synthesis, purification, and analysis of compound libraries. These new tools have had a significant impact on both lead identification and lead optimization in New Drug Discovery Research (NDDR). Large compound libraries can now be designed and synthesized in very short time to provide valuable leads for new therapeutic targets.[1] Once a chemist has developed a suitable high-speed synthesis of a lead, it is now possible to synthesize and purify hundreds of molecules in parallel to discover new leads and/or to derive structure–activity relationships (SAR) in unprecedented timeframes.

Microwave-assisted heating under controlled conditions has been shown to be an invaluable technology for medicinal chemistry and drug discovery applications since it often dramatically reduces reaction times, typically from days or hours to minutes or even seconds. Compound libraries can then be rapidly synthesized in either a parallel or sequential (automated) format using this new, enabling technology.

Microwave synthesis has the potential to influence medicinal chemistry efforts in at least three major phases of NDDR

1. Generation of a discovery library;
2. Hit-to-lead efforts and
3. Lead optimization.

A common theme throughout this drug discovery and development process is speed. To the pharmaceutical industry and the medicinal chemist, time truly does equal money, and microwave chemistry has become a central tool in this fast-paced, time-sensitive field.

The short reaction times required by microwave synthesis make it ideal for rapid reaction scouting and optimization, allowing rapid synthesis of large number of NCE's.[1,2]

Microwave heating can readily be adapted to a parallel or automatic sequential processing format. In particular, the latter technique allows for the rapid testing of new ideas and high-speed optimization of reaction conditions. The fact that a "yes or no answer" for a particular chemical transformation can often be obtained within few a minutes (as opposed to several hours in a conventional protocol), has contributed significantly to the acceptance of microwave chemistry both in industry and academia. The recently reported incorporation of real time, in situ monitoring of microwave-assisted reactions by Raman spectroscopy allows a further increase in efficiency and speed in microwave chemistry.[3]

2.2 Theory of Microwave Assisted Heating

Microwave radiation is an electromagnetic radiation in the frequency range of 0.3 to 300 GHz, corresponding to wavelengths of 1 cm to 1 m. The microwave region of the electromagnetic spectrum (Figure-1) therefore lies between infrared and radio frequencies. All domestic "kitchen" microwave ovens and all dedicated microwave reactors for chemical syntheses commercially available today operate at a frequency of 2.45GHz (corresponding to a wavelength of 12.25 cm) in order to avoid interference with telecommunication and cellular phone frequencies.

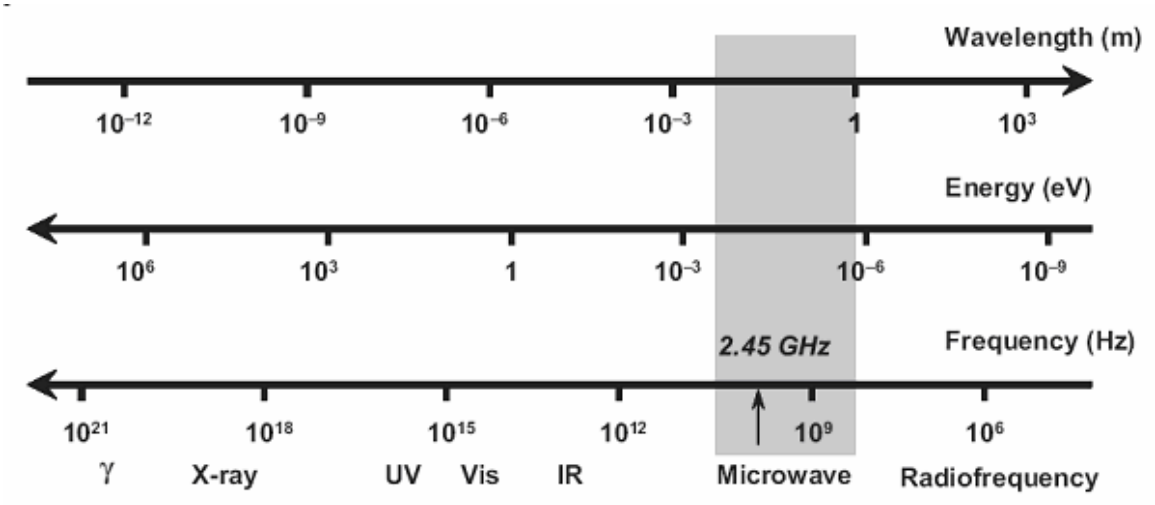


Figure-1 The electromagnetic spectrum

Microwave heating is either by any of the following three mechanisms[4-6]:

1. Dielectric heating involving dipolar polarization
2. Heating by ionic conductance and
3. Heating by interfacial polarization.

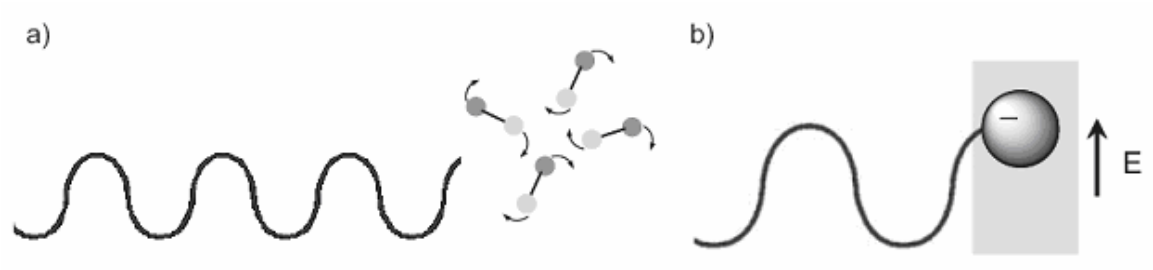


Figure-2 (a) Dipolar polarization mechanism (b) Dipolar molecular try to align with an oscillating electric field. Ionic conduction mechanism: Ions in solution will move in the electric field.

2.2.1 Dielectric Properties

The heating characteristics of a particular material (for example, a solvent) under microwave irradiation conditions are dependent on the dielectric properties of the material. The ability of a specific substance to convert electromagnetic energy into heat at a given frequency and temperature is determined by the so-called loss tangent, (tan δ). The loss factor is expressed as the quotient tan $\delta = \varepsilon''/\varepsilon'$, where ε'' is the dielectric loss, indicative of the efficiency with which electromagnetic radiation is converted into heat, and ε' is the dielectric constant describing the polarizability of the molecules in the electric field. A reaction medium with a high tan δ is required for efficient absorption and, consequently, for rapid heating. Materials with a high dielectric constant such as water (ε' at 25^oC = 80.4) may not necessarily also have a high tan δ value. In fact, ethanol has a significantly lower dielectric constant (ε' at 25^oC = 24.3), but heats much more rapidly than water in a microwave field due to its higher loss tangent (tan δ: ethanol = 0.941, water = 0.123). In general, solvents can be classified as high (tan $\delta > 0.5$), medium (tan δ 0.1-0.5), or low microwave-absorbing (tan $\delta < 0.1$).

2.2.2 Microwave versus Conventional Thermal Heating

Microwave irradiation produces efficient internal heating (in core volumetric heating) by direct coupling of microwave energy with the molecules (solvents, reagents, catalysts) that are present in the reaction mixture. Since the reaction vessels employed are typically made out of (nearly) microwave-transparent materials such as borosilicate glass, quartz or teflon, the radiation passes through the walls of the vessel and an inverted temperature gradient as compared to conventional thermal heating results[7] (Figure-3).

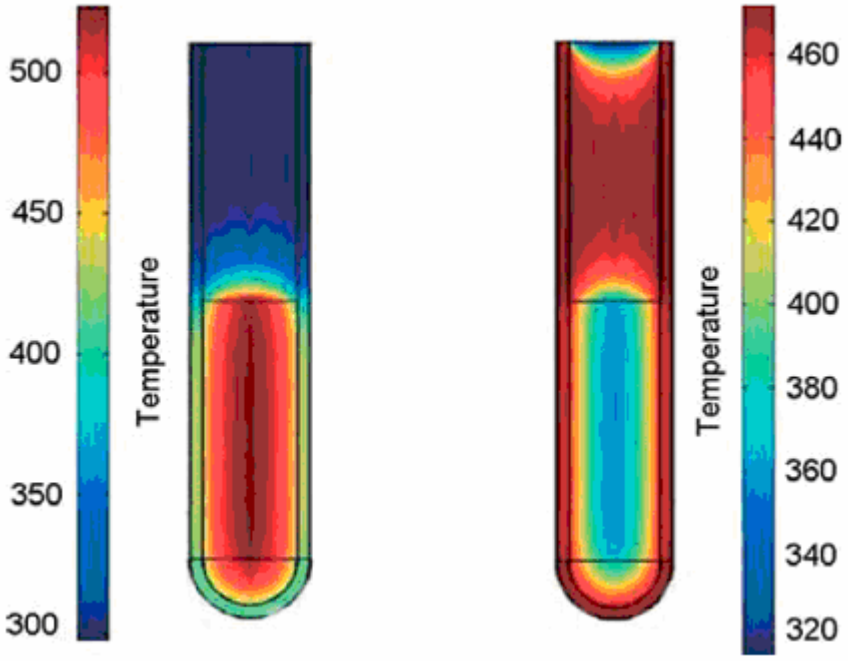


Figure-3. Microwave irradiation (left) compared to heating in an oil bath (right). Microwave irradiation raises the temperature of the whole volume simultaneously (bulk heating), whereas in the oil heated tube the reaction mixture in contact with the vessel wall is heated first. (Temperature in ^{o}K)

2.3. Applications of MWI in Combinatorial Parallel Syntheses of Compounds for New Drug Discovery Research

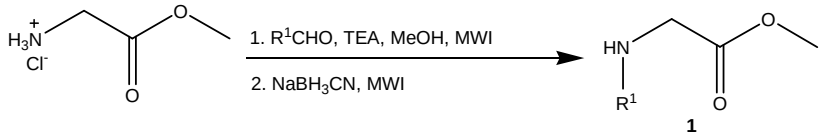
The current trend in the pharmaceutical industry is to generate comparatively small, focused libraries containing ~30-300 compounds for a typical drug discovery project. In this report, we are highlighting some reports of past few years on the generation of diverse libraries of compounds through combinatorial or parallel synthesis under MWI.

2.3.1 Library Synthesis of Acyclic and Heterocyclic NCE's.

Herein, an exhaustive account on the microwave assisted syntheses of diverse library of acyclic to heterocyclic NCE's is discussed. Around 40 examples documented in literature are covered.

An easy and convenient microwave-assisted synthesis of *N*-alkylated glycine methyl esters **1** (10 compounds) has been described, involving reductive alkylations of several

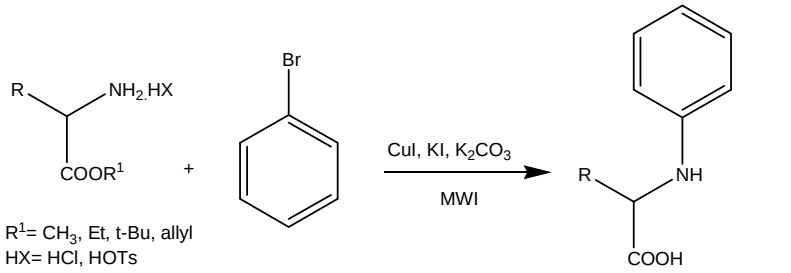
glycine methyl esters in the presence of sodium cynoborohydride ($NaBH_3CN$). Good yields and short reaction times are the main aspects of these procedures[8].



1

R^1 = 2,6-Cl Ph, 2,6-methyl Ph, 3,5 dimethyl-4-hydroxy Ph, 4-Cl Ph, 3-NO_2 Ph *etc.*

Rottger *et al.,* [9] have successfully developed a general method for the microwave-induced *N*-arylation of amino acids in water providing moderate to high yields and less than 6% racemization. A diverse set of amino acids **2** (20 derivatives) and differently substituted aryl bromides were fully reacted after 40 min of microwave radiation. In addition, various amino acid esters could be *N*-arylated with simultaneous deprotection, generating the free acid as product.

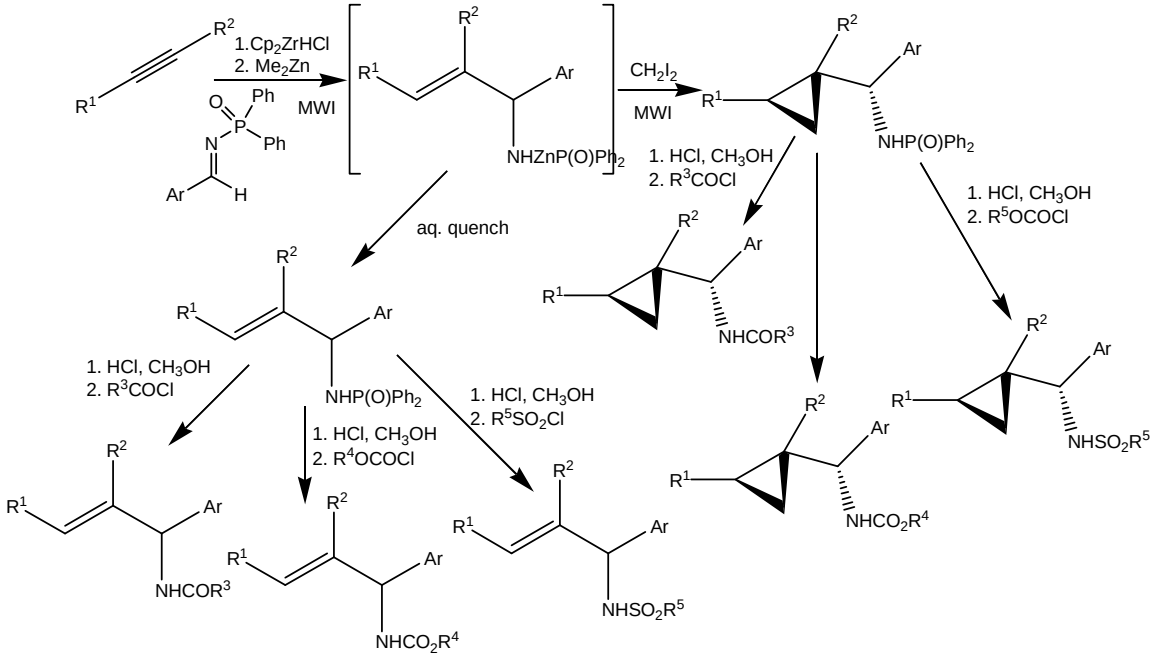


R^1= CH_3, Et, t-Bu, allyl
HX= HCl, HOTs

2

Amino acid ester= L-Leu, L-Phe, L-Lcu *etc*
R= H, CH_2Ph

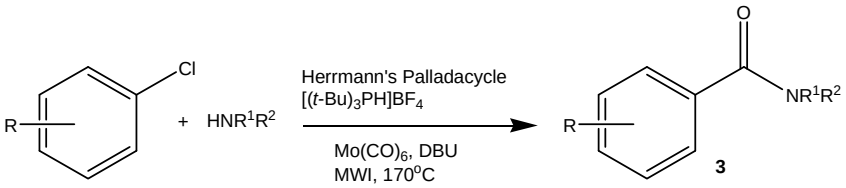
Wipf *et al.,*[10] have developed an expeditious divergent multi-component reaction method, combining the advantages of microwave reaction acceleration and combinatorial technologies with a libraries-from-libraries concept to prepare 20 allylic amides and C-cyclopropylalkylamides and create an expanded 100-member library as in scheme-1. The library building blocks consisted of 3 alkynes, 7 phosphinoylimines, 10 acid chlorides, 6 carbamoyl chlorides, and 9 sulfonyl chlorides (Scheme-1).



R^1= H, *n*-C_3H_7, *o*-C_6H_{11}
R^2 = *n*-C_4H_9, *n*-C_3H_7, CH_3
Ar = Ph, 4-ClC_6H_5, 3-ClC_6H_5 *etc.*
R^3 = Ph, CH_2Ph, CH_2CH_2Ph, *t*-Bu, 4-PhC_6H_5 *etc.*
R^4 = CH_2Ph, $CH_2CH(CH_2)_2$, *i*-Bu, 2-$ClC_6H_4CH_2$ *etc.*
R^5 = Ph, 8-quinoline, 4-ClC_6H_5, CH_3, 4-PhC_6H_4 *etc.*

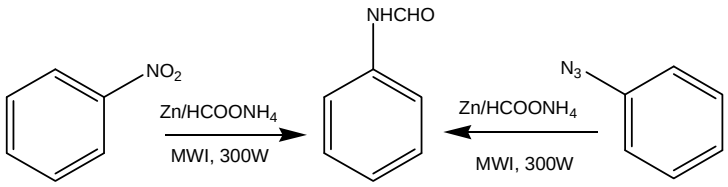
Scheme-1

A robust and straightforward palladium-catalyzed aminocarbonylation protocol that rapidly transforms aryl chlorides into a variety of benzamides **3** has been developed by Lagerlund *et al.*[11] This microwave method includes the use of commercially available molybdenum hexacarbonyl [$Mo(CO)_6$] as a solid carbon monoxide source. This procedure affords a convenient and versatile alternative for small-scale carbonylative applications relative to existing methods starting from aryl bromides or aryl iodides. A library of 18 compounds has been reported.



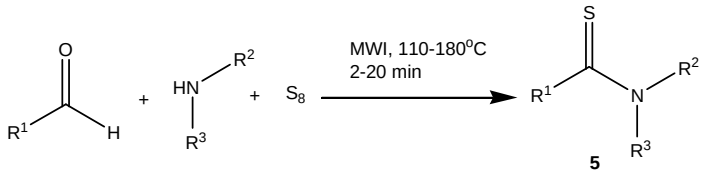
R = 4-OCH_3, 2,6-Me, 4-COOMe, 4-CF_3 etc.
HNR^1R^2= BuNH, n-BuNH, t-BuNH, piperidine, aniline

Microwave mediated reduction of nitro and azido arenes to *N*-arylformamides **4** (31 compounds) using Zn-$HCOONH_4$ has been described.[12] Interestingly, the reaction conditions are identical for both protocols.



4

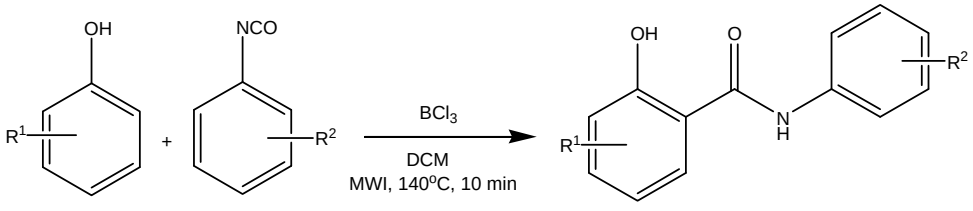
A microwave-enhanced variation of the Kindler thioamide synthesis has been introduced by taking advantage of the sealed vessel capabilities of a dedicated single-mode microwave reactor. A diverse selection of 13 aldehyde and 12 amine precursors was utilized in the construction of a representative 34-member library of substituted thioamides **5**. The three-component condensations were carried out by employing microwave flash heating at 110-180°C for 2-20 min. A simple workup protocol allows the isolation of synthetically valuable primary, secondary and tertiary thioamide building blocks in 83% average yield and >90% purity.[13]



5

R^1 = Ph, 4-NO_2Ph, CH_2Ph, 3-NO_2Ph *etc*
R^2=R^3= H, H, -$(CH_2)_5$-, -$(CH_2)_4$-, H, $PhCH_2$, H, Propyl *etc*

Zhang *et al.*,[14] have demonstrated the utility of microwave- assisted, boron trichloride (BCl_3)-mediated coupling of phenols with aryl isocyanates to make salicylamide-based exploratory library **6** of a total 16 compounds. The effect of diverse substitution groups, especially neutral and electron-withdrawing groups on the coupling reactions, has been analyzed.

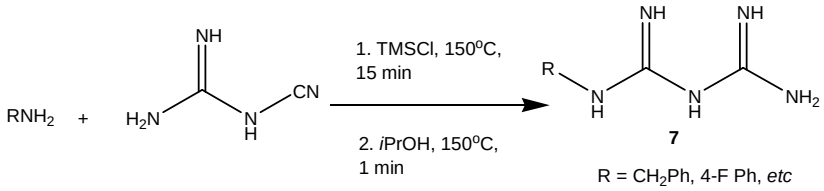


6

R^1 = H, 2-allyl, 3-MeO, 4-Cl, 4-CO_2Me;
R^2 = 4-Me, 4-MeO, 4-NO_2,

Mayer *et al.*,[15] have produced an array of alkyl- and aryl based biguanide compounds **7** using microwave irradiation and using trimethylsilyl chloride (TMSCl) for the first time

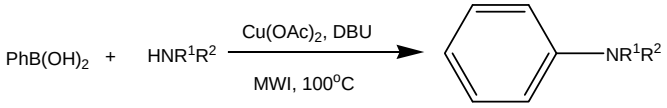
as an excellent and practical catalyst for the formation of alkyl and aryl biguanides. Using these methods, a 60-compound collection was prepared.



7

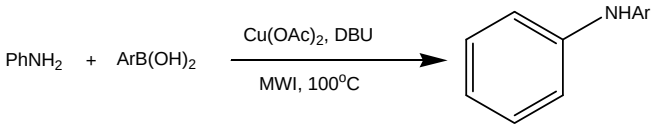
R = CH_2Ph, 4-F Ph, *etc*

An effective microwave assisted method for the amination of phenols and arylboronic acids with various amines and anilines under the catalysis of cupper(II)acetate ($Cu(OAc)_2$) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) has been reported[16] (22 compounds).



8

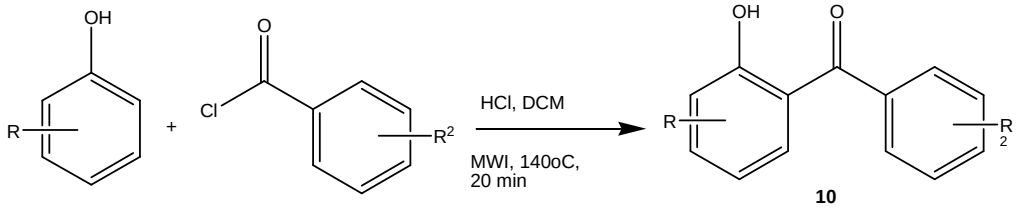
NR^1R^2= Isopropyl amine, morpholine, aniline, anisidine *etc.*



9

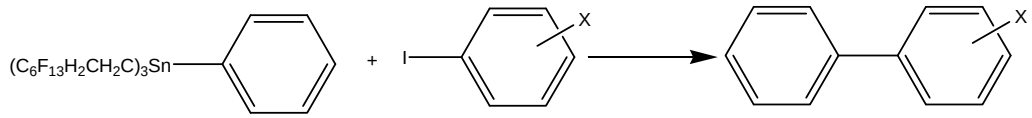
Ar = Ph, 4-CH_3OPh, 3-CH_3OPh, 4-NO_2Ph, 4-ClPh, 4-CH_3Ph

A novel and efficient microwave-assisted, boron trichloride (BCl_3) mediated coupling reaction to synthesize *o*-(hydroxyaryl)-(aryl)methanone structures **10** from phenols and acyl chlorides has been described for the generation of a library of 40 compounds.[17]



10

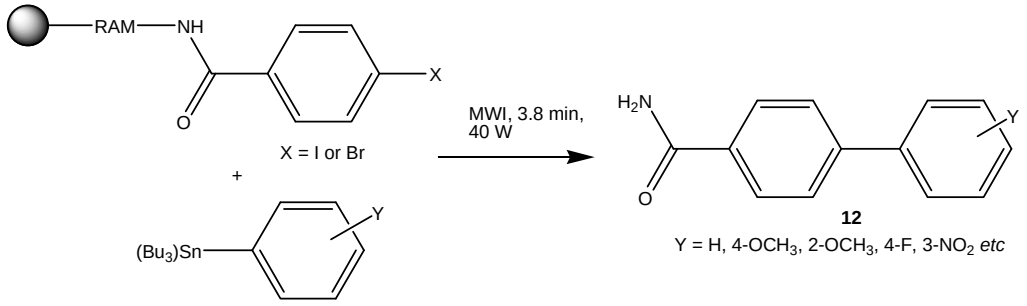
A palladium-catalyzed fluorous Stille cross-coupling reaction with organic halides or triflates requiring only 90-120 seconds for completion under microwave irradiation has been studied. Conventional thermal reactions require about 1 day. Fourteen different coupling products **11** were synthesized and isolated in good yields after three-phase extraction.[18]



11

X = 4-OCH_3, 2-CF_3, 4-Ph, 4-CH_3, 4-$COCH_3$, 3-CHO, *etc*

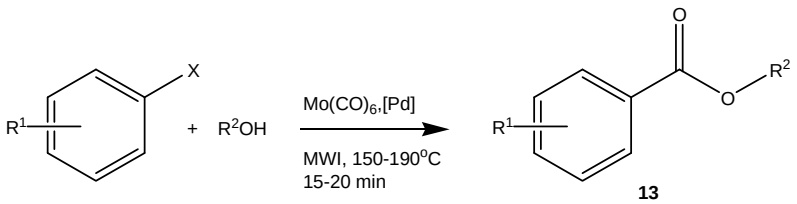
Microwave-assisted palladium-catalyzed coupling of aryl and heteroaryl boronic acids with iodo- and bromo-substituted benzoic acids, anchored to tentagel S RAM, provided high isolated yields of coupled products **12** after a reaction time of 3.8 min. In all 16 compounds have been synthesized.[19]



12

Y = H, 4-OCH_3, 2-OCH_3, 4-F, 3-NO_2 *etc*

Georgsson *et al.*,[20] has described a noninert palladium catalyzed method for the synthesis of ester-protected carboxylic acids **13** from aryl iodides and bromides, employing molybdenum hexacarbonyl [$Mo(CO)_6$] as a convenient solid carbon monoxide source. Thus, butyl-, benzyl- and trimethylsilylethyl esters were smoothly prepared after only 15-20 minutes of microwave heating. This in situ carbonylation route couples a facile experimental procedure to handle "carbon monoxide gas" in a highthroughput manner, with the rapid reaction speed associated with single-mode microwave irradiation. The methodology is quite applicable to today's modern synthetic techniques, both in solution and in solid-phase organic chemistry. In all 16 compounds have been reported.



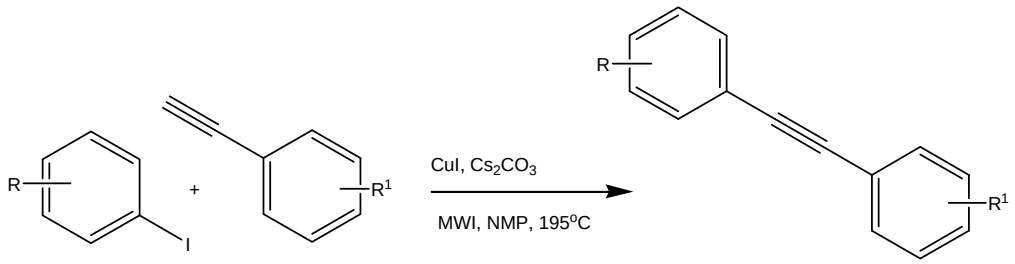
13

X = I, Br

R^1 = 1-naphthyl, 4-OCH_3, 2-CH_3, 4-CF_3, 3-Br
R^2 = *n*-Bu, $CH_2CH_2Si(CH_3)_3$, *t*-Bu

An efficient copper-catalyzed cross-coupling of aryl iodides with aryl acetylenes to give compounds of structure **14** under microwave irradiation has been described by Huan *et*

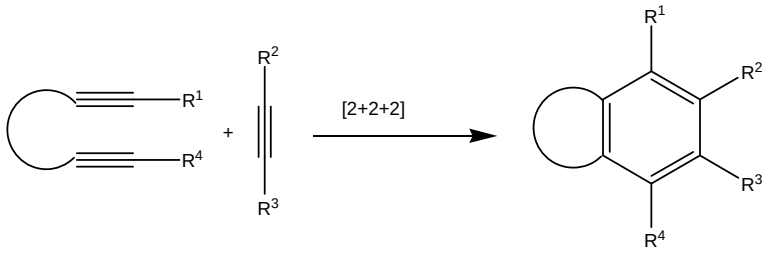
al.[21] The reaction proceeds under microwave heating with 10 mol % CuI and 2 equiv cesium carbonate (Cs_2CO_3) with 43-87% yields.



14

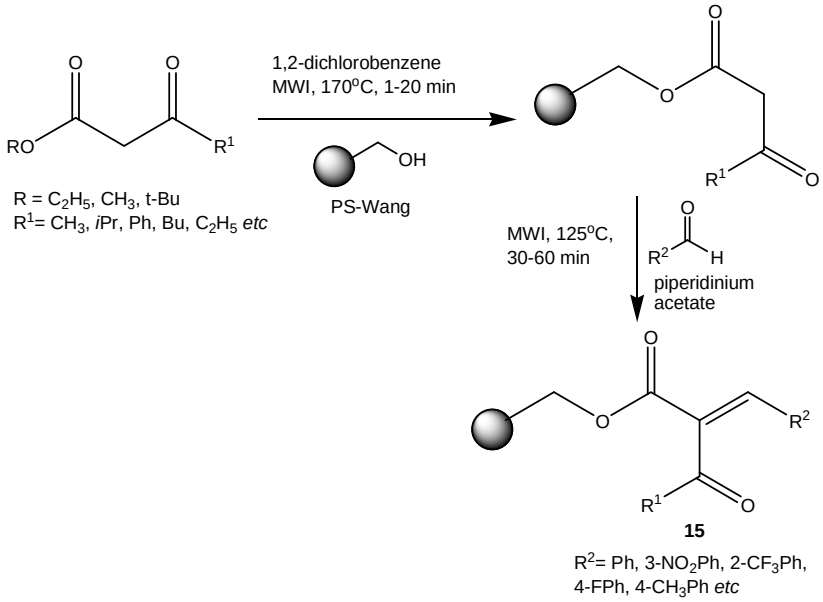
R = 4-*t*-Bu, 4-CN, 3-OCH_3, 4-CHO *etc*
R^1 = 4-CH_3, 4-OCH_3, 4-F, 2-F, 2-Cl, 2-Br *etc*.

Young *et al.*,[22] have demonstrated the reactivity enhancing effects of microwave irradiation combined with the effects of spatial diyne separation on a polymeric support on the ruthenium-catalyzed [2+2+2] cyclotrimerization reaction. The conducted transformations were highly efficient and a high level of chemoselectivity was observed. Microwave-irradiation did not affect the regioselectivity of the cyclotrimerization reaction when differentially substituted diyne precursors were used (Scheme-2). In all, 34 compounds have been reported.

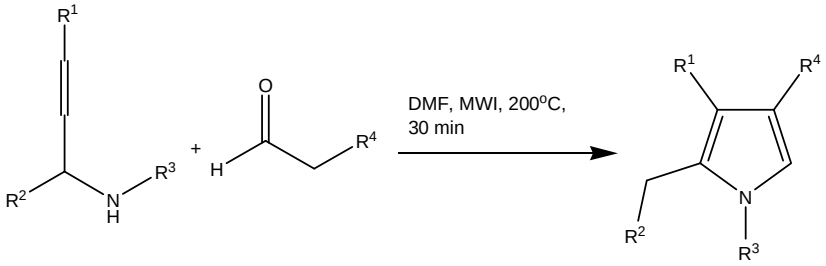


Scheme-2

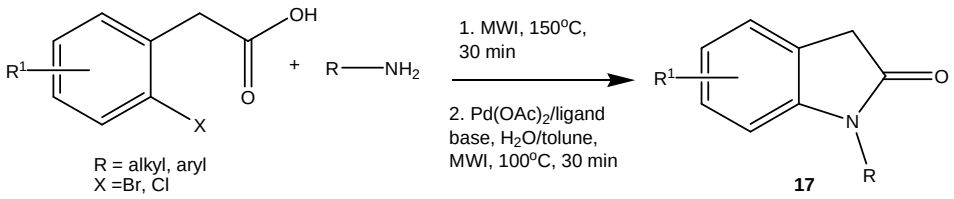
A microwave-assisted parallel solid-phase synthesis of a collection of 21 polymer-bound enones **15** has been developed. The two-step protocol involves initial high-speed acetoacetylation of polystyrene Wang resin with a selection of seven common β-ketoesters. When microwave flash heating at 170°C was employed, complete conversions were achieved within 1-10 minutes, against several hours for completion in the conventional heating protocol. Significant rate enhancements were also observed for the subsequent microwave-heated Knoevenagel condensations with a second set of 13 different aldehydes. Reaction times were reduced to 30-60 min at 125°C in the microwave protocol compared to 1-2 days using conventional thermal conditions.[23]



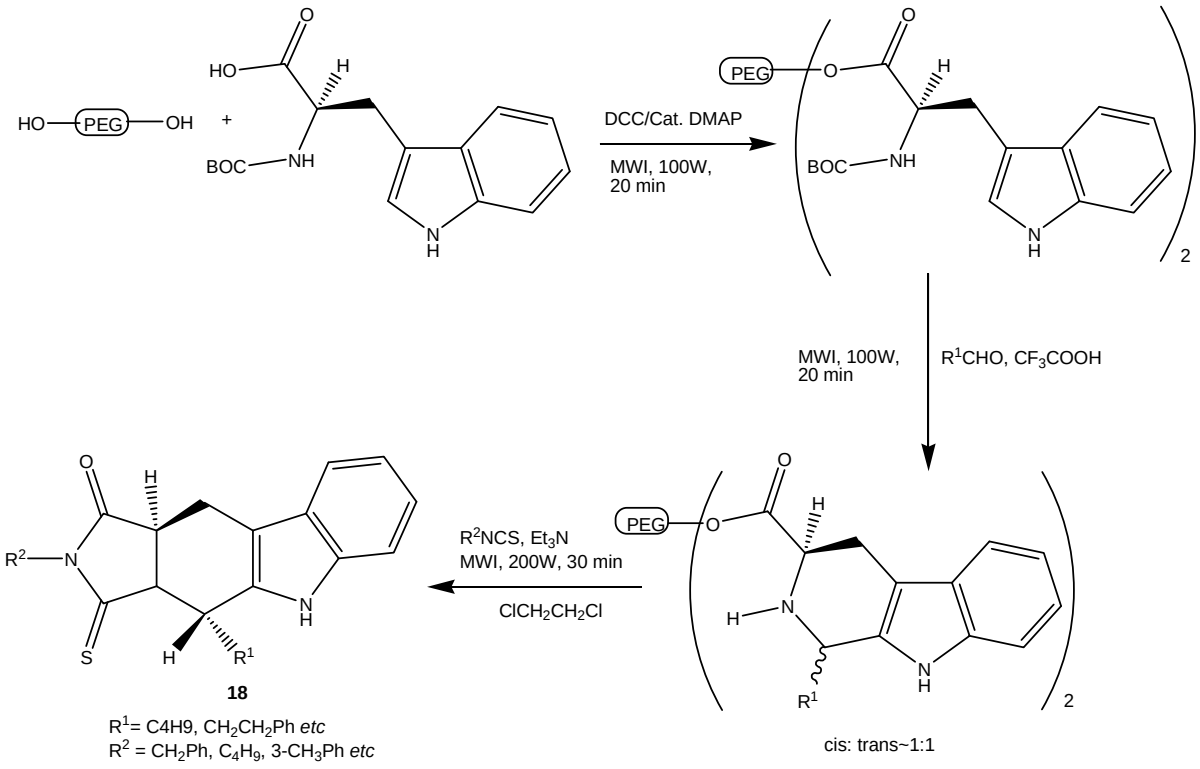
A diverse collection of pyrroles **16** (20 compounds) has been prepared using a one-pot, domino aldehyde/amine condensation, [3,3]-aza-Claisen rearrangement followed by imine-allene cyclization strategy. This protocol was accelerated by microwave irradiation and provided very good levels of conversion after reacting for only 30 min.[24]



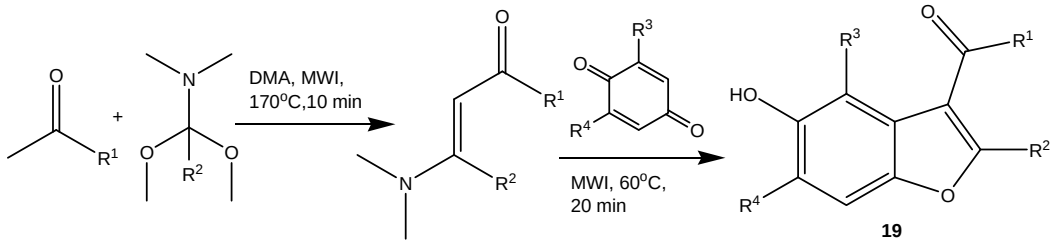
A general method has been developed for the synthesis of *N*-substituted oxindoles **17**. The two-step process involves initial microwave-assisted amide bond formation between 2-haloarylacetic acids and various alkylamines and anilines, followed by a palladium-catalyzed intramolecular amidation under aqueous conditions. In case of alkylamines, the procedure can be carried out as a one-pot process without isolation of the intermediate amide.[25]



Chang *et al.*[26] have demonstrated a microwave-assisted traceless, liquid-phase methodology to assemble substituted indole alkaloids with exceedingly high stereoselectivity. All the steps in this synthetic sequence have been accomplished under focused microwave irradiation, resulting in significantly reduced reaction times from hours to minutes in enhanced yields. This rapid synthesis of tetracyclic tetrahydro-β-carboline pharmacophore **18** with two points of diversity has the potential for the creation of a diverse array of polycyclic fused heterocyclic systems, closely resembling biologically active natural products. In all 15 compounds have been reported by them.



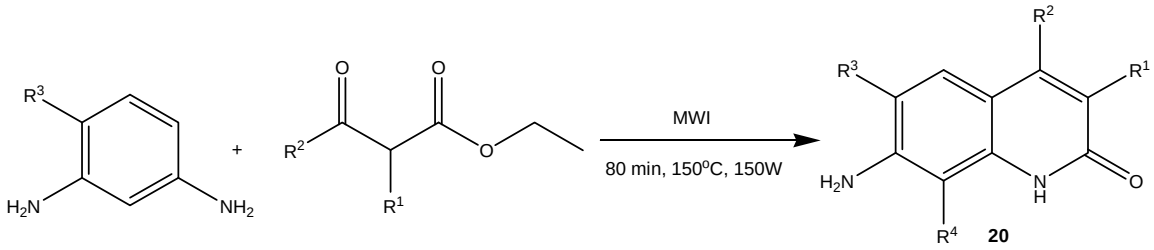
Some Chinese workers[27] have developed an efficient synthetic method to generate structurally diverse and medicinally interesting 3-acyl-5-hydroxybenzofurans **19** *via* a one-pot two-step reaction sequence under microwave irradiation. The method was employed to rapidly construct twenty-six different 3-acyl-5-hydroxybenzofurans.



19

R^1= Ph, 4-CH_3Ph, CH_3, 2-pyridine-2-yl *etc.*
R^2= H, CH_3
R^3, R^4= H, Br, Cl, $COOCH_3$

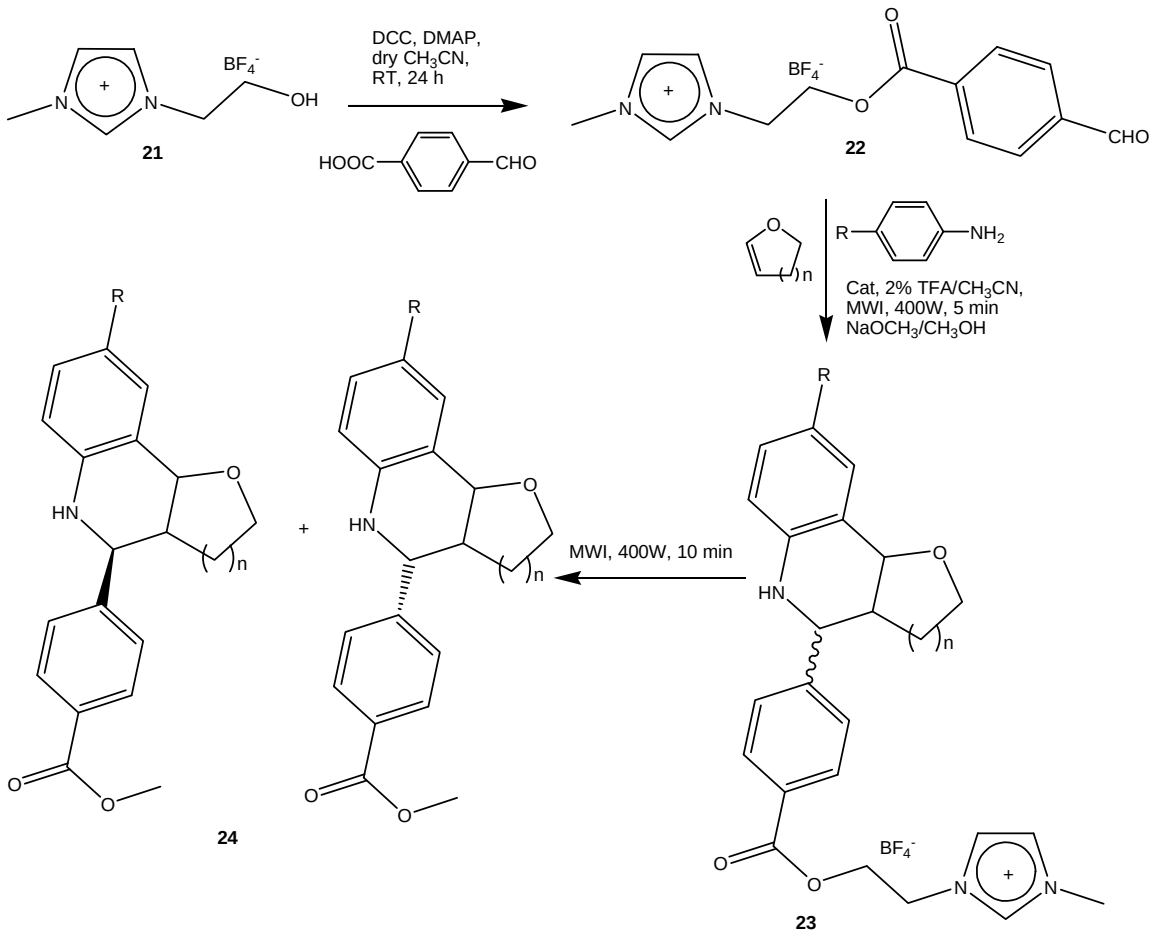
A highly efficient microwave-assisted method was successfully developed for the synthesis of a library of carbostyril analogues **20** (15 compounds). The reaction time for synthesis of carbostyril analogues was drastically reduced from a reported 18-58 hrs to only 80 min. Compounds obtained directly from each synthesis were more than 90% pure and did not require any further purification.[28]



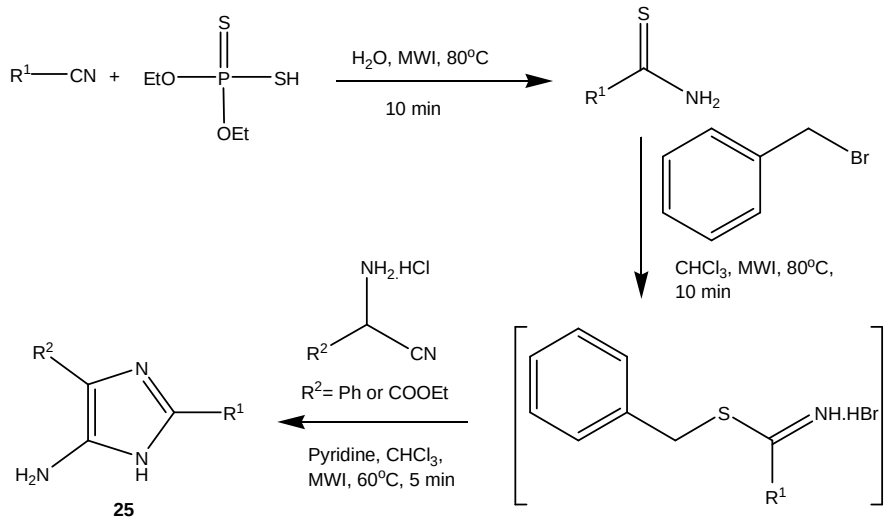
20

R^1=H, CH_3
R^2=CH_3, C_2H_5, C_3H_7, CF_3
R^3=H, CH_3
R^4=H, CH_3

Korean workers[29] have reported the application of functionalized ionic liquids as the soluble support for the synthesis of tetrahydropyrano- and tetrahydrofuranoquinolines **24** (10 compounds) under microwave irradiation. The efficient preparation of the functionalized 1-[2-(4-benzoyloxy)ethyl]-3-methylimidazolium tetrafluoroborate-bound aldehyde **22** was realized by the reaction of an ionic liquid (IL), 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ([2-hydemim] [BF_4]) **21**, and 4-formylbenzoic acid in dry acetonitrile with dicyclo-hexylcarbodiimide (DCC) and 5% dimethylamino pyridine (DMAP) as catalysts to afford the functionalized IL-bound benzaldehyde **22** in high yields. The cyclization of **22** to **23** and removel of the IL-binding to afford isomeric mixture **23**, were under MWI.

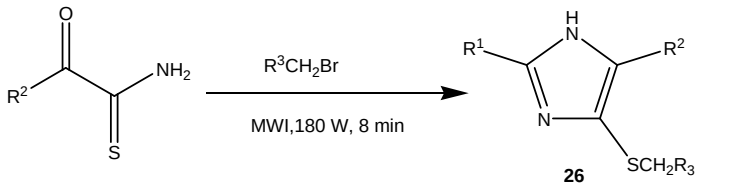


A microwave-assisted parallel synthesis of 2,4-disubstituted 5-aminoimidazoles **25** (15 compounds) has been developed. Significant rate enhancement was observed for all steps in the three-step protocol. The overall reaction time was shortened to 25 min, as compared to 53 hrs for the conventional procedures.[30]



A methodology for the microwave parallel synthesis of library has been described which involves the use of an array of expandable reaction vessels that can accommodate

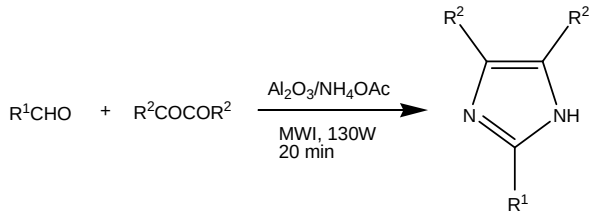
pressure buildup within the vessel due to heating without loss of volatile solvents or reagents. A demonstration 24-membered library of substituted 4(5)-sulfanyl-1*H*-imidazoles **26** was generated by microwave procedures, achieving a reduction from 12 hrs to 16 min in library generation time for the microwave approach.[31]



26

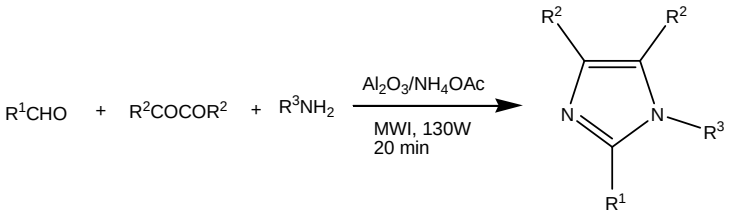
R^1= 4-FPh, C4H9, 3-NO3Ph, *etc*
R^2 =Ph
R^3= $COOH$, $CONH_2$
CH_2OH, CH_3

The solvent-free microwave-assisted synthesis of 2,4,5-substituted imidazoles **27** and 1,2,4,5-substituted imidazoles **28** (8 compounds) has been reported. Imidazoles were obtained as a result of the condensation of a 1,2-dicarbonyl compound with an aldehyde and an amine using acidic alumina impregnated with ammonium acetate as the solid support.[32]



27

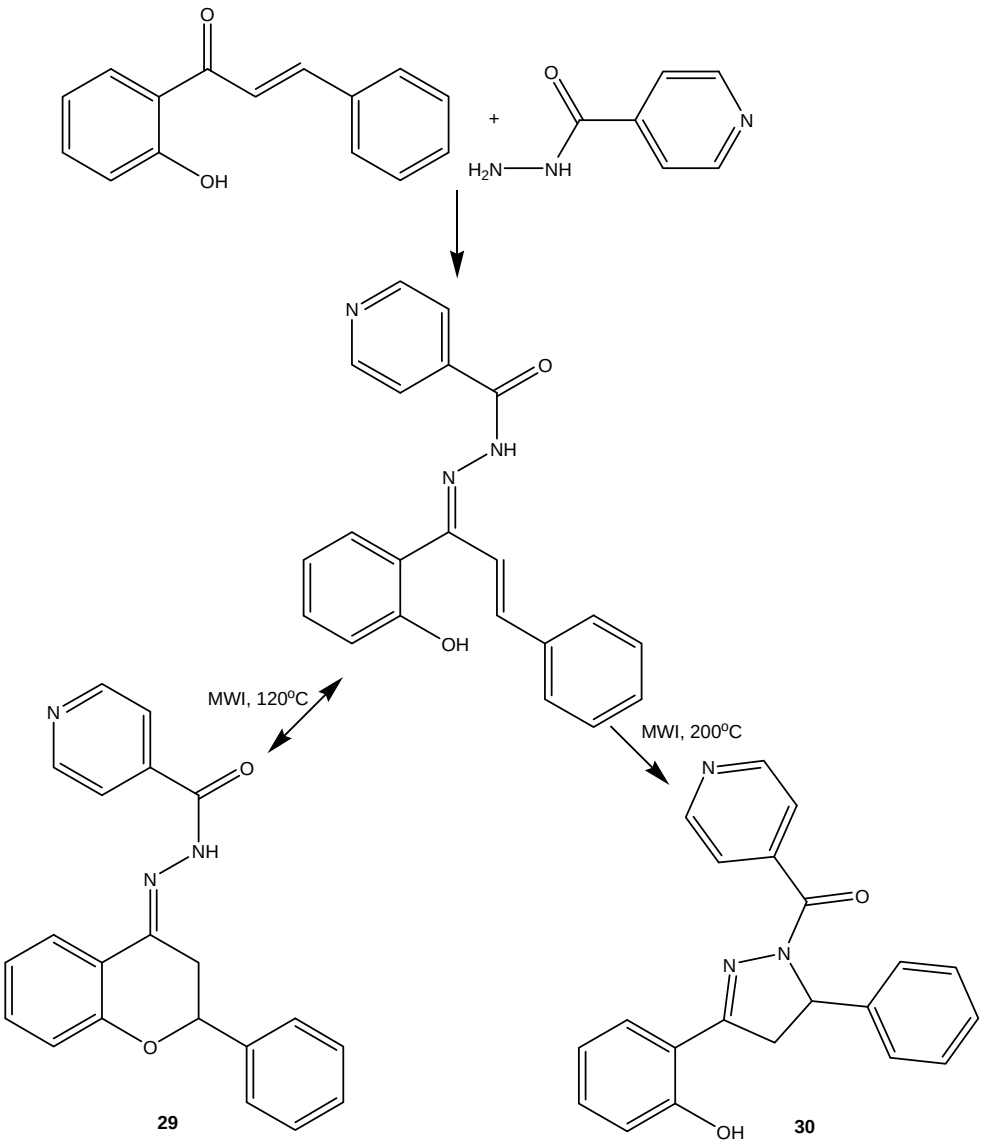
R^1= Ph, 4-ClPh *etc*
R^2= Ph



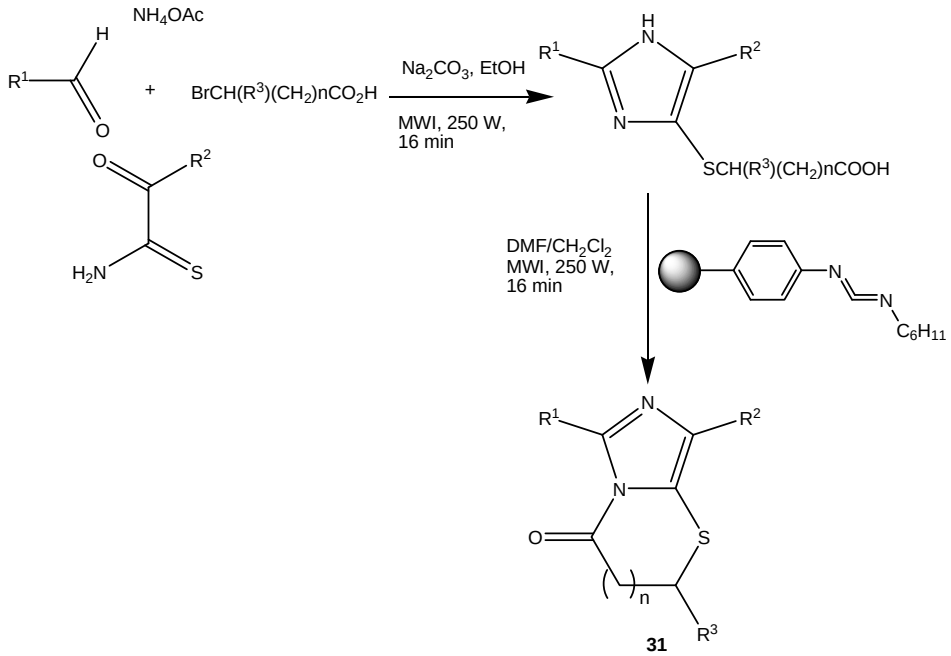
28

R^1= Ph, 4-C_2H_5Ph
R^2= Ph, 4-CH_3Ph
R^3= CH_2CH_2Ph, CH_2-3-ClPh

Nie and Huang[33] have demonstrated a method of solution-phase parallel synthesis coupled with microwave assisted synthesis for constructing two distinct combinatorial libraries of flavanone hydrazone **29** & 4,5-dihydropyrazole **30**, starting from the same reactants only by subtly changing the reaction temperature. Thus, two focused molecular libraries of >400 compounds were synthesized in high purity.



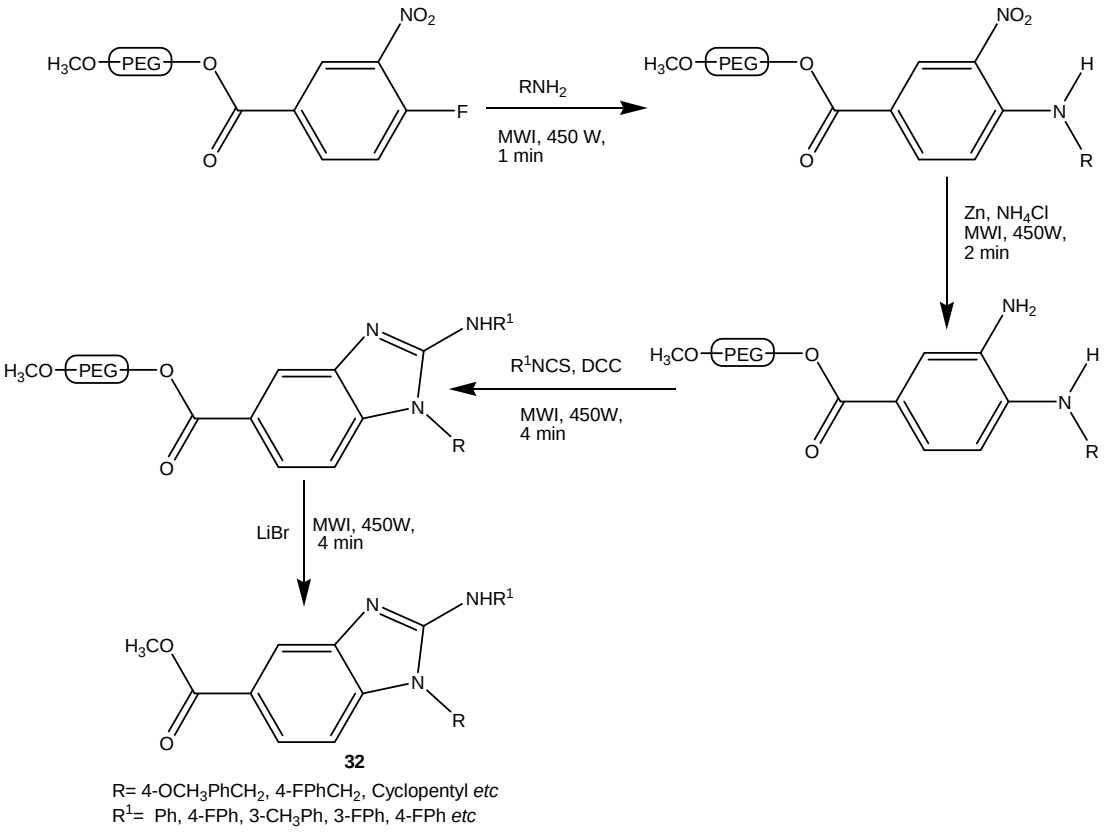
A methodology for the generation of a microwave-assisted parallel library and its conversion into a second library was described. A 24-membered library of substituted 4(5)-sulfanyl-1*H*-imidazoles was generated and subsequently converted into a second library of bicyclic imidazo[5,1-*b*]thiazol-3-ones and imidazo[5,1-*b*]-thiazin-4-ones **31**. The first library was generated using a multi-component reaction (MCR) and transformed into a daughter library with a polymer-supported coupling agent. The procedure involved microwave heating without loss of volatile solvents or reagents. Library generation time for each library was 16 min.[34]



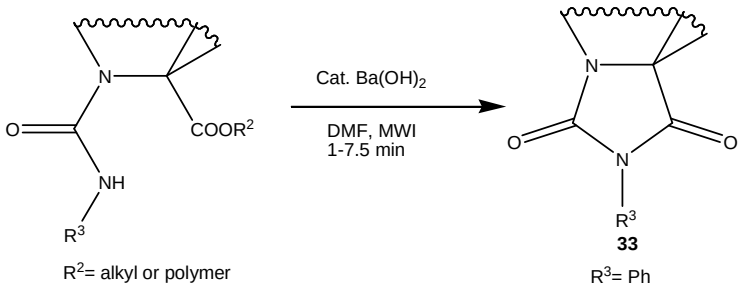
31

Substituted sulfanyl-1*H*-imidazole library and imidazo[5,1-*b*] thiazol-3-one, n = 0 and imidazo[5,1-*b*]-thiazin-4-one library n =1.

An efficient, facile, and practical liquid-phase combinatorial synthesis of benzimidazoles **32** under microwave irradiation has been described.[35] All reactions involving (S_NAr reaction, reduction, cyclization, and support cleavage) were performed completely within a few minutes under microwave irradiation. The coupling of microwave technology with liquid phase combinatorial synthesis constitutes a novel and particularly attractive avenue for the rapid generation of structurally diverse libraries of 23 compounds.

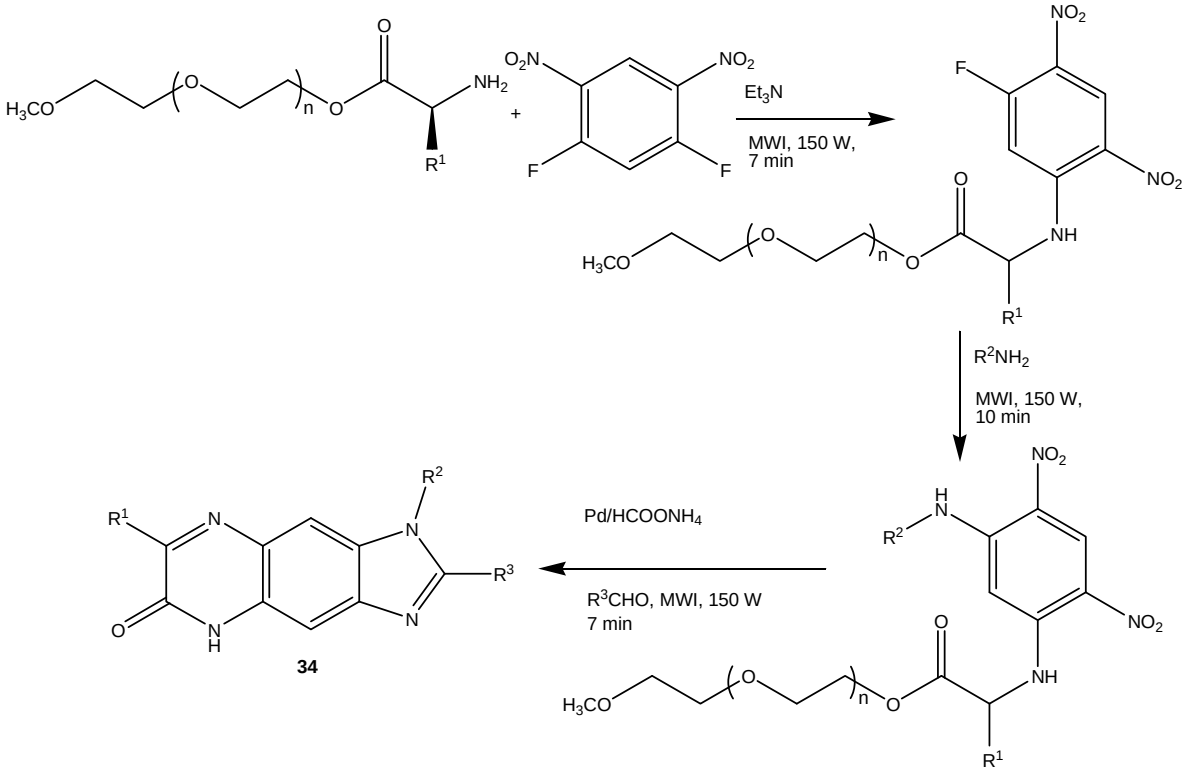


Microwave mediated intra molecular carbanilide cyclizations to condensed imidazolinediones **33** (17 compounds) have been reported[36] with significantly reduced reaction time (microwave minutes *vs* heating hours) for both solution and solid phase reactions. Catalytic barium hydroxide [$Ba(OH)_2$] in DMF was uniquely effective in this microwave mediated transformation and, in certain systems, can provide access to unepimerized products not available in thermal transformations.

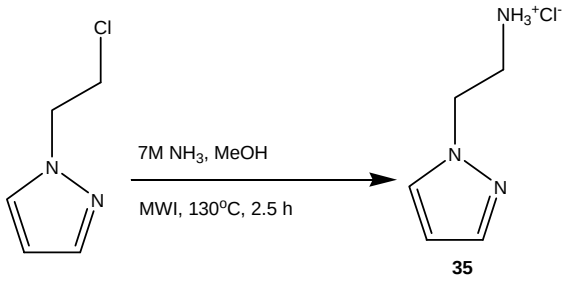


Lin and Sun[37] have explored a combination of microwave techniques and traceless polymer-supported strategies for the synthesis of tricyclic quinoxalinone imidazoles (13 compounds) **34** with three points of diversity. Simultaneous reduction of the two nitro groups led to the intramolecular cyclizative cleavage of polymer support and *N*-heterocyclization with aldehydes to the formation of imidazole ring in one pot. The

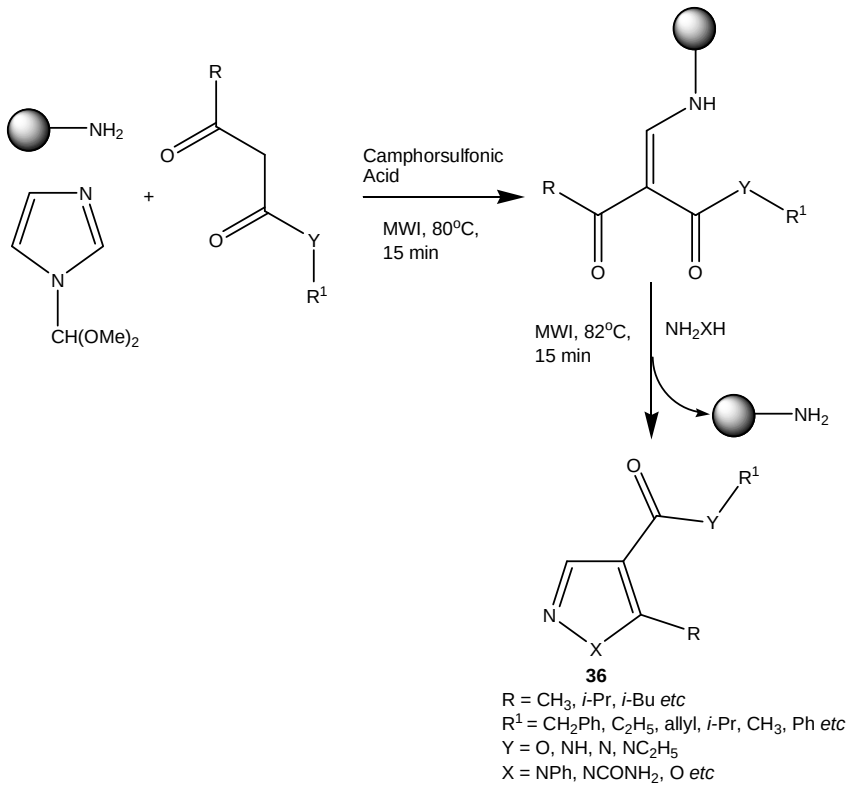
synthetic strategy constitutes a novel and attractive avenue for the rapid generation of structurally diverse libraries.



Microwave-assisted synthesis of hydrochloride salts of primary amines **35** from their corresponding halides and 7 M ammonia in methanol has been described by Saulnier *et al.*[38] It provides practically high yields, with even volatile primary amines for parallel synthesis.



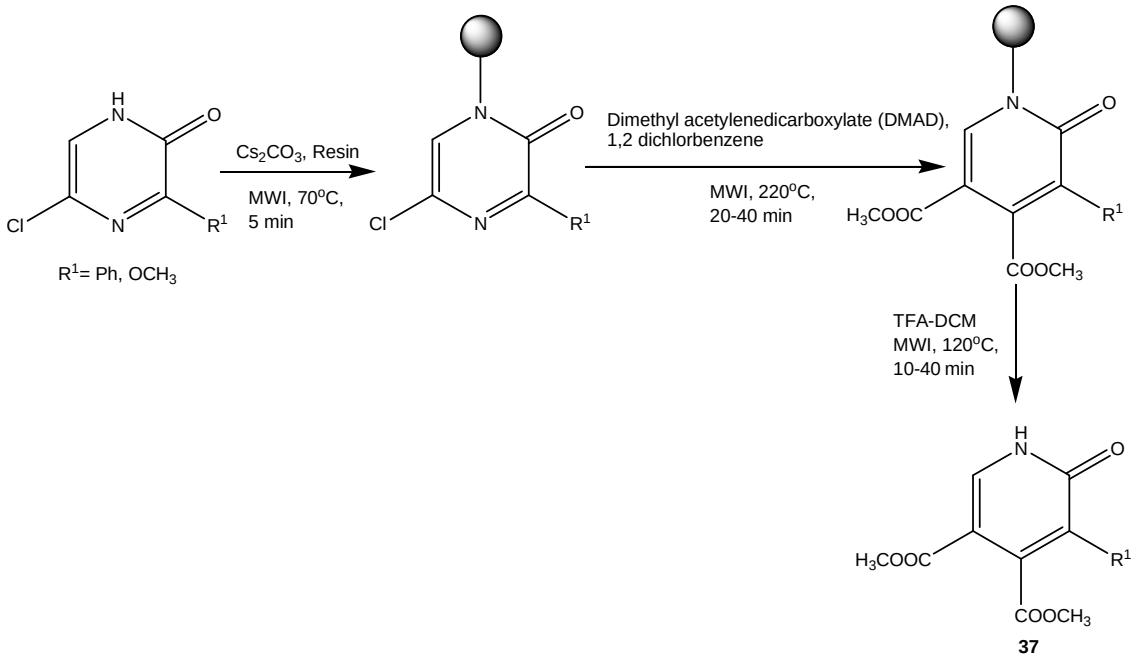
The library (34 compounds) synthesis of substituted pyrazoles and isoxazoles **36** has been developed *via* the *in situ* generation of polymer-bound enaminones. This new support allowed carrying out reactions in polar solvents under both conventional heating and MW irradiation without degradation of the polymer.[39]



36

R = CH_3, *i*-Pr, *i*-Bu *etc*
R^1 = CH_2Ph, C_2H_5, allyl, *i*-Pr, CH_3, Ph *etc*
Y = O, NH, N, NC_2H_5
X = NPh, $NCONH_2$, O *etc*

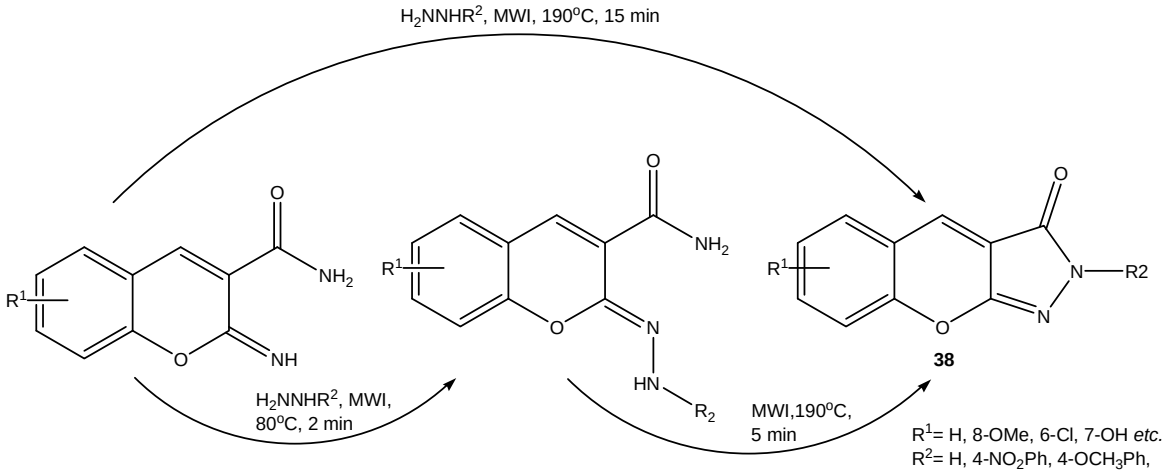
Microwave-assisted solid-phase Diels-Alder cycloaddition reactions of 2(1*H*)-pyrazinones with dienophiles to yield the products of general structure **37**, have been discussed by Kaval *et al.*[40] All steps in the solid-phase protocol (linking, cycloaddition, cleavage) were carried out under controlled microwave irradiation conditions. In general, significant rate enhancements were found along with the reduction of reaction times from hours or days to minutes.



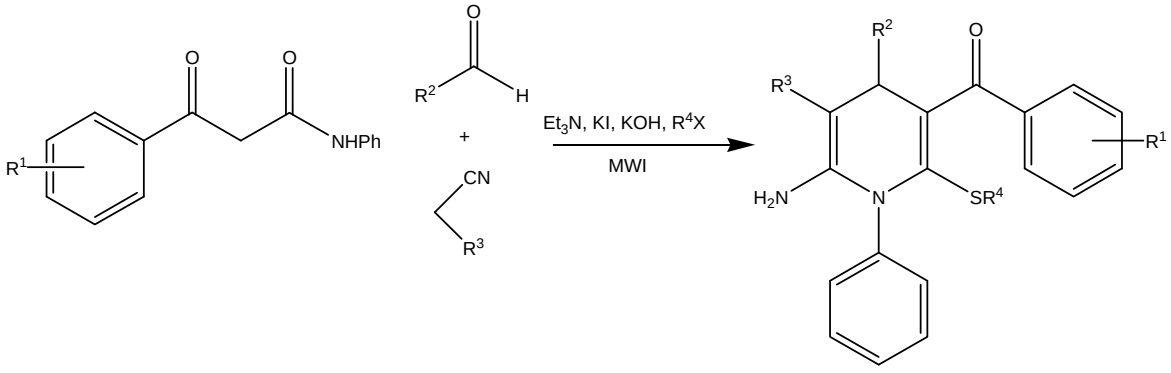
R^1= Ph, OCH_3

37

A facile microwave assisted protocol has been described for the fast generation of 2-arylbenzopyrano[2,3-*c*]pyrazol-3-one library, **38** of 144 compounds, utilizing highly reactive 2-iminocoumarines or the corresponding hydrazines, as starting materials. Microwave irradiation of the reaction mixture in the acetic acid led to one pot synthesis of the desired compounds in 43-87% yields and 90-100% purity.[41]



A sequential one-pot two-step protocol for microwave-assisted Hantzsch-type synthesis of hexa substituted 1,4-dihydropyridines **39** has been developed [42] (33 compounds). The three-component condensation of β-aroylthioamides, aldehydes and acetonitriles followed by the in situ *S*-alkylation of the intermediates afforded the hexasubstituted 1,4-dihydropyridines.

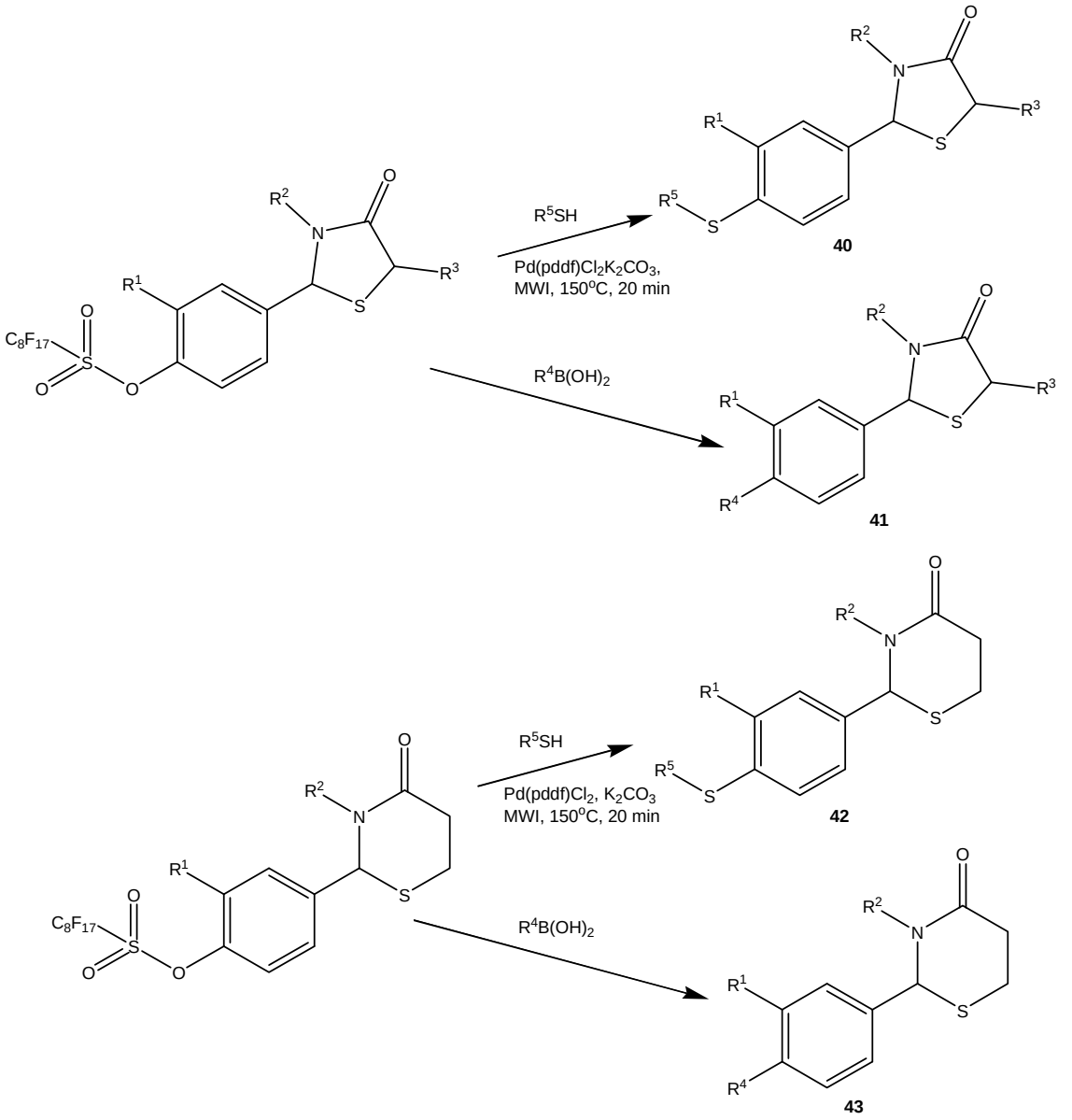


39

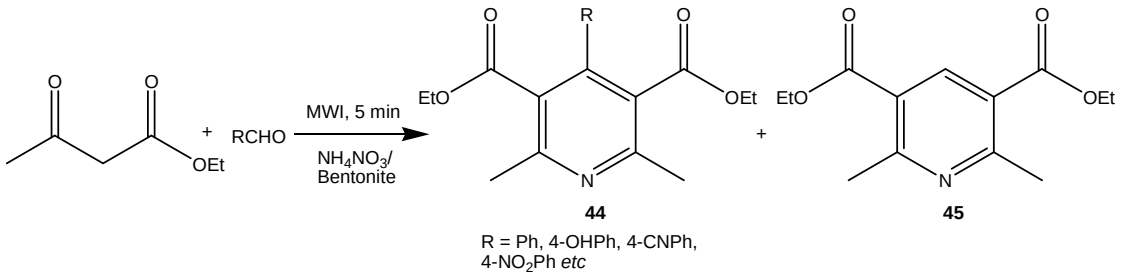
R^1= H, 4-Cl, 4-CH_3
R^2 = Ph, 4-ClPh, 4-OCH_{3P}h, 3-NO_2Ph, CH_3 *etc*
R^3 = CN, COOEt
R^4 =C_4H_9, CH_2Ph

Zhou *et al.*[43] have synthesized sixty compounds containing 4-thiazolidinone **40** & **41** and 4-thiazinanone **42** & **43** cores with biaryl and thioaryl substitutions using a microwave-

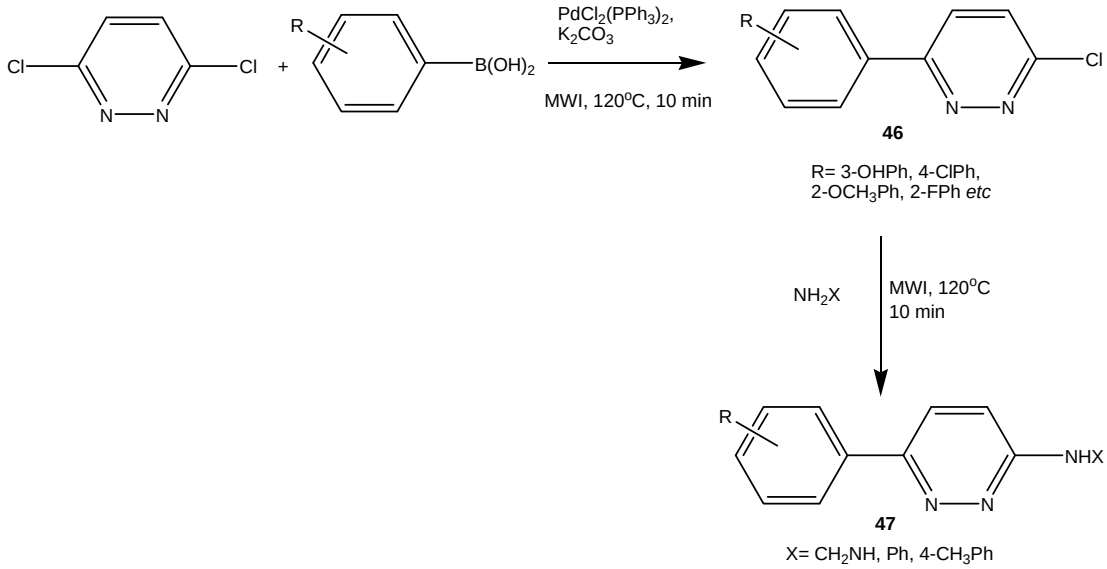
assisted fluorous synthesis protocol. Because of the favorable solution-phase reactions and the simple F-SPE for intermediate purifications, this protocol is expected to be easily adopted for the production of larger libraries.



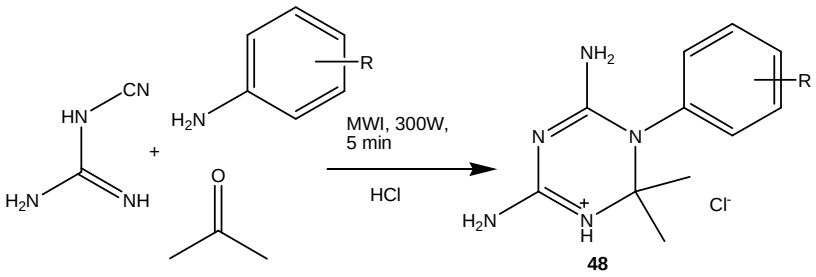
A new highly efficient microwave-assisted combinatorial synthesis for generating combinatorial libraries has been described by Cotterill *et al.*[44] The technology was applied to the high throughput, automated, one-step, parallel synthesis of diverse substituted pyridines **44** and **45** (108 derivatives) using the Hantzsch synthesis. The advantages of microwave-assisted chemistry for combinatorial synthesis included a broad range of available chemistries, simple reaction setup and product recovery readily amenable to automation, extremely short reaction times, and high product yields.



Lin *et al.*,[45] have developed an efficient method to generate the key intermediate 6-aryl-3-chloropyridazines **46** (8 compounds) by microwave-enhanced Suzuki coupling of 3,6-dichloropyrridazine with aryl boronic acids in moderate to good yields. Amination of **46** with various amines afforded 3-substitued-amino-6-arylpyridazines **47** in high yields under microwave irradiation. This approach could be used to rapidly construct the diverse pyridazine compound libraries for high-throughput biological screening.



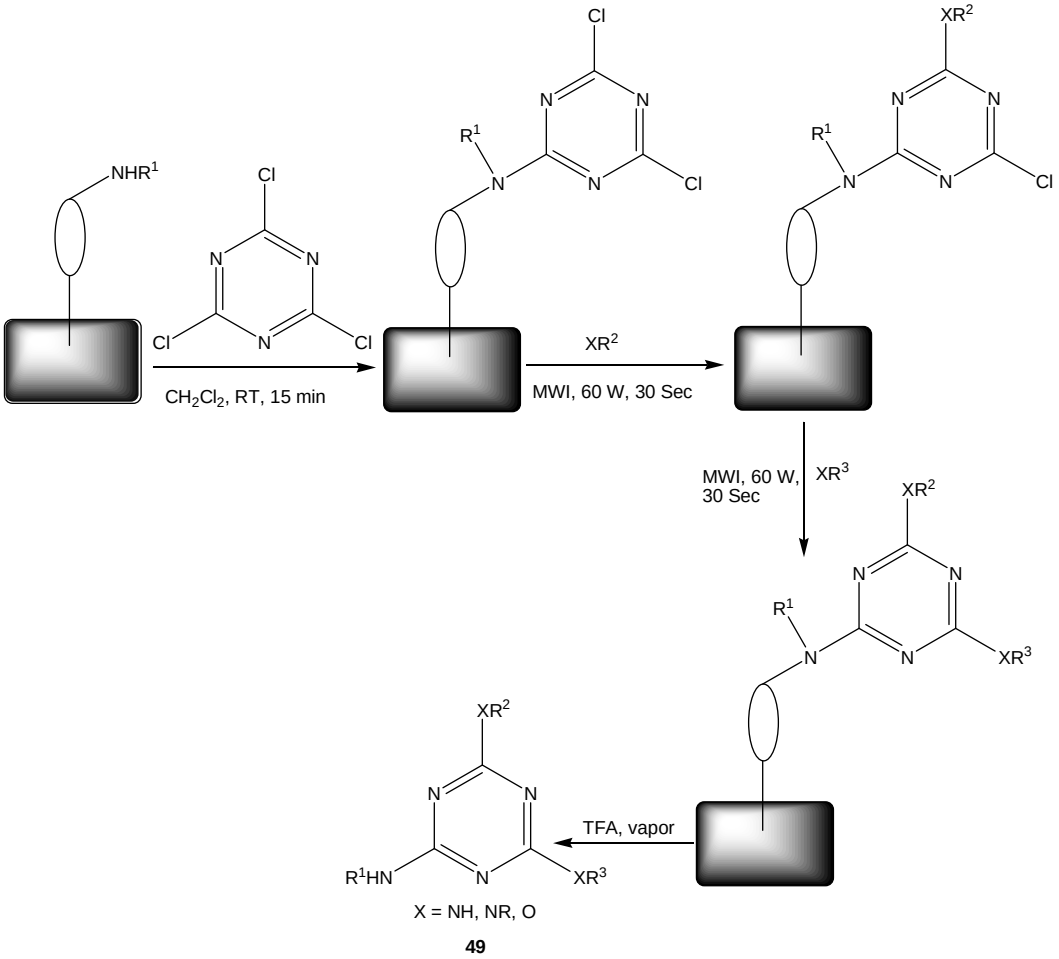
Microwave-assisted parallel synthesis of a library of 20 phenyl dihydrotriazines **48** was successfully achieved and compared to an identical library generated by conventional parallel synthesis. Microwave synthesis dramatically decreased reaction times from an average of 22 h to 35 min, and compounds generated using microwave irradiation were relatively pure.[46]



48

R = 2-Cl, 2-CH_3, 2-OCH_3, 3-Cl, 4-OCH_3, *etc*

Effective spatially addressed parallel assembly of trisamino- and amino-oxy-1,3,5-triazines **49** has been achieved by applying the SPOT-synthesis technique on cellulose and polypropylene membranes. In addition, a highly effective microwave-assisted nucleophilic substitution procedure at membrane-bound monochlorotriazines has been developed. The 1,3,5-triazines obtained could be cleaved in parallel from the solid support by TFA vapor to give compounds adsorbed on the membrane surface in a conserved spatially addressed format for analysis and screening. The reaction conditions developed were employed for the synthesis of 8000 cellulose bound 1,3,5-triazines which were probed in parallel for binding to the anti-transforming growth factor-R monoclonal antibody Tab2 in order to identify epitope mimics.[47]



49

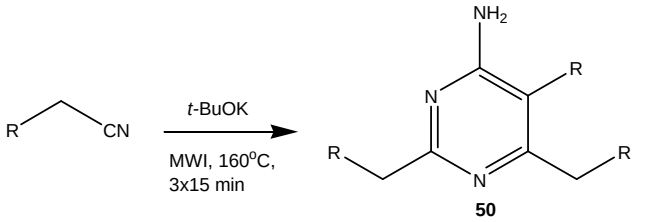
2.3.2 Library Syntheses of Mononuclear and Condensed Pyrimidines through MWI

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acid to their current use in the chemotherapy of AIDS. During last four decades, several pyrimidines and condensed pyrimidines have been developed as chemotherapeutic agents and have found wide clinical applications as anticancer, antiviral and anti-AIDS, antitubercular, sedative/hypnotic/antiepileptic, cardiac agents, as well as analgetics, diuretics, antibiotics and metabolic electrolytes *etc.*[48] There are some reports on the combinatorial as well as parallel library synthesis of pyrimidines and condensed pyrimidines through MWI that are reviewed here.

23.2.1 Mononuclear pyrimidine libraries through MWI

A series of substituted aliphatic nitriles have been trimerized to their corresponding pyrimidine structures **50** (32 analogues) under solvent-free conditions in the presence of catalytic quantities of potassium tert-butoxide using a focused microwave reactor.

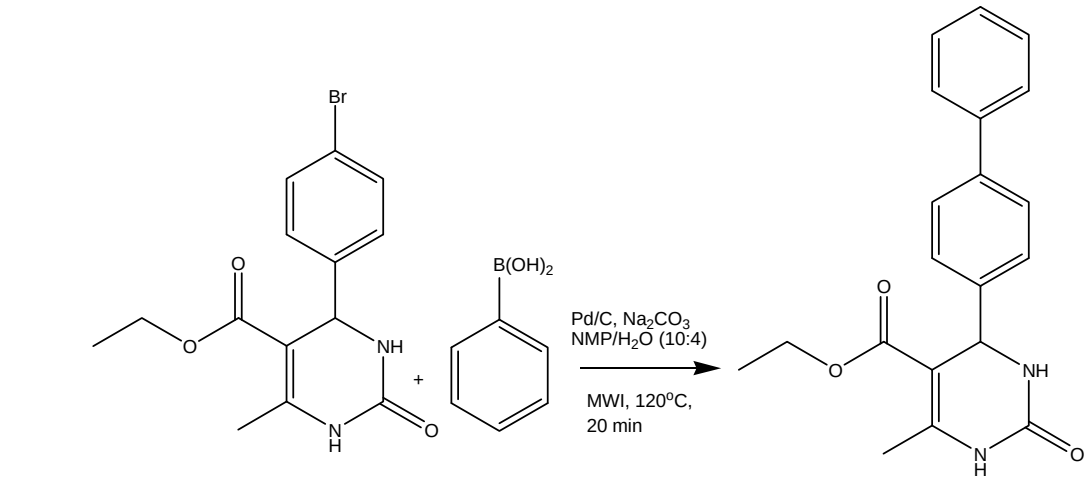
Multigram quantities of the corresponding 4-aminopyrimidines which are potential NCE's, have been prepared in high yields and purity following a simple and scaleable protocol.[49]



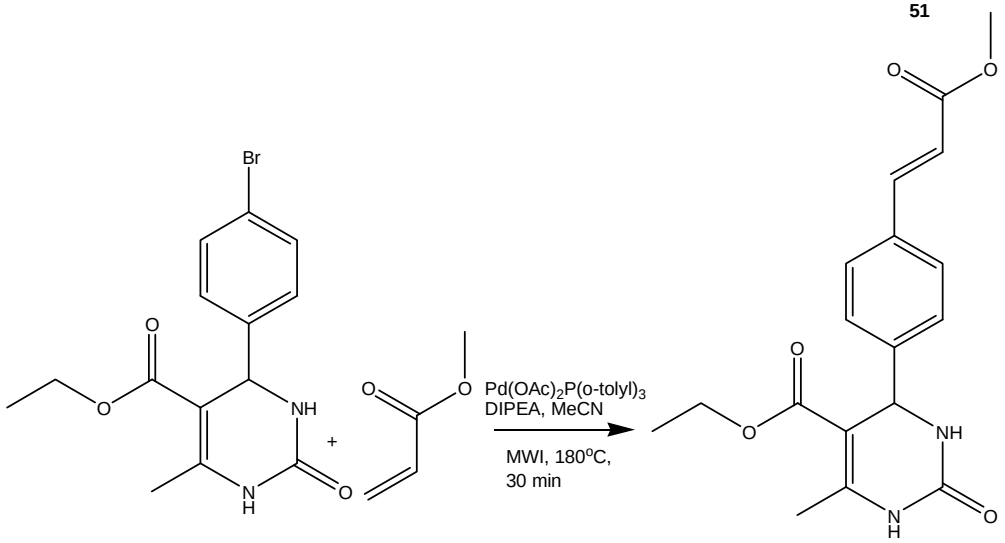
50

R = Ph, 4-OCH_3Ph, 4-ClPh, 4-BrPh, $(CH_2)_3$Ph, CH_2Me, H, $(CH_2)_3$Me *etc.*

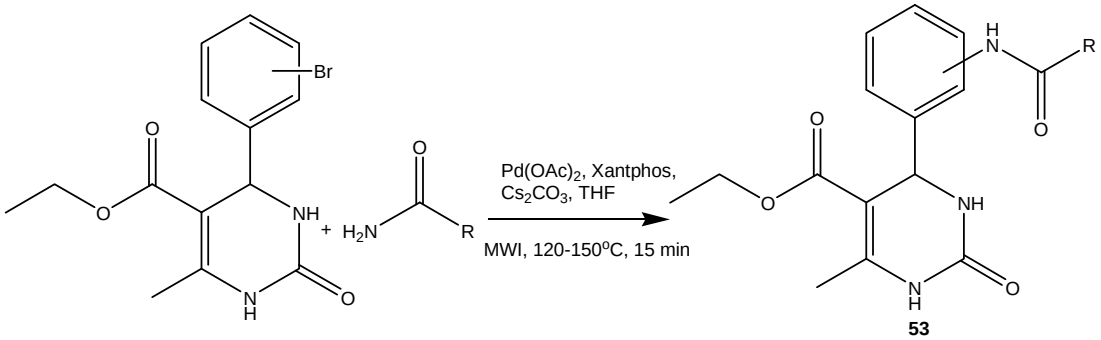
A fast method has been developed for transition-metal-catalyzed decoration of 4-aryl-dihydropyrimidones (29 compounds) using controlled microwave heating as the energy source. The palladium-catalyzed protocols allow facile installation of diversities into 4-(bromoaryl)-DHPMs as in **51** and **52**. Both in situ carbonylations, using different nitrogen and oxygen nucleophiles and direct *N*-arylations efficiently generated functionalized amides **53** or esters after only short periods of high-density microwave heating. Additionally, intramolecular seven-membered Heck-endocyclization using Harrmann's Palladacycle $\{Pd_2(Ac)_2[P(o\text{-tolyl})]_2\}$ to afford compounds **54** was successfully done under microwave irradiations.[50]



51

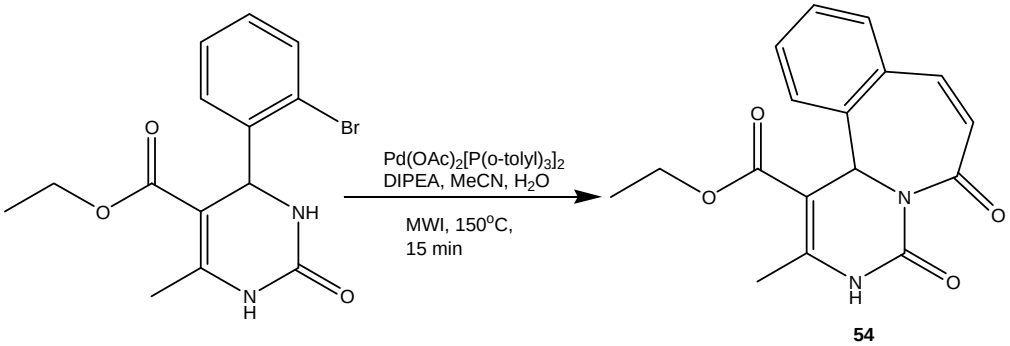


52



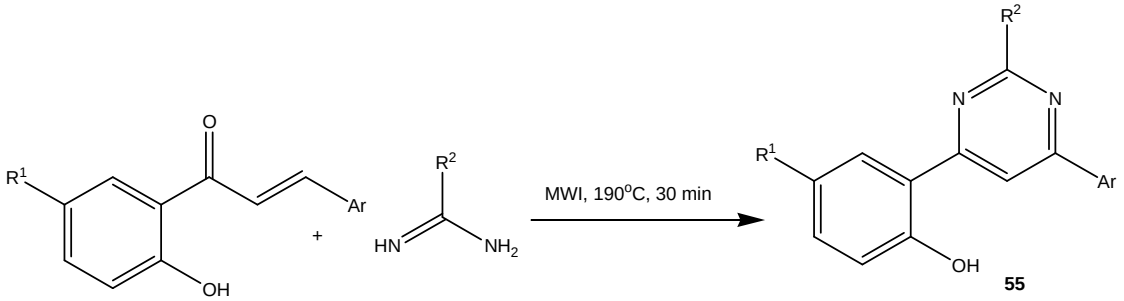
53

R= 3-NHCOCH$_3$, 4-NHCOCH$_3$ *etc*



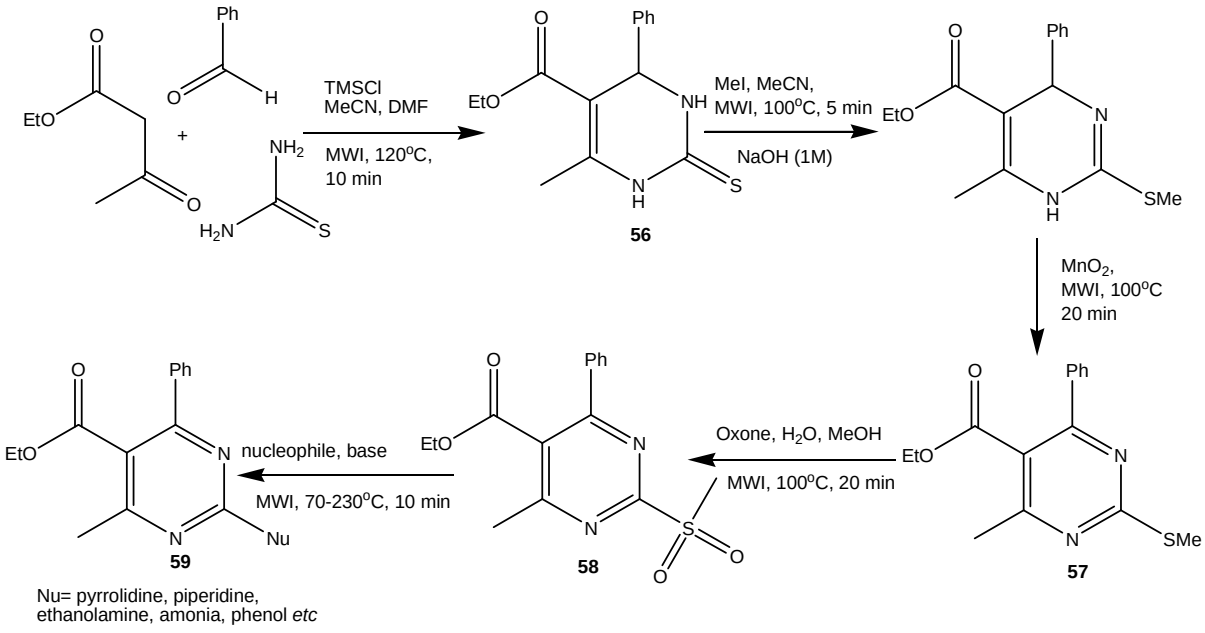
54

Nie *et al.*,[51] reported microwave-assisted reaction of 2'-hydroxychalcones with amidines or guanidines to synthesize 2,4,6-trisubstitutedpyrimidines **55** (100 compounds).



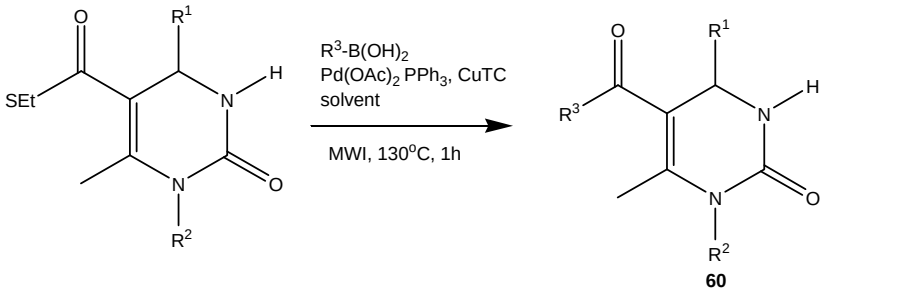
R^1= H, F, Cl, Br
R^2= Ph, 4-CH_3Ph, 4-ClPh, cyclopropyl, NH_2

An efficient and rapid microwave-assisted solution-phase method for the synthesis of 2-amino-4-arylpyrimidine 5-carboxylic acid derivatives has been developed. The five-step linear protocol involves an initial Biginelli multicomponent reaction leading to dihydropyrimidine-2-thiones **56** which are subsequently *S*-alkylated with methyl iodide. The resulting 2-methylthiodihydropyrimidines **57** are sequentially oxidized first with manganese dioxide and then with oxone to provide 2-methylsulfonyl-pyrimidines **58** which serve as excellent precursors for the generation of a variety of 2-substituted pyrimidines **59** *via* displacement of the reactive sulfonyl group with nitrogen, oxygen, sulfur, and carbon nucleophiles. The use of high-temperature sealed-vessel microwave irradiation allows the preparation of the desired target structures in high yields and comparatively short reaction times.[52]



Nu= pyrrolidine, piperidine,
ethanolamine, amonia, phenol *etc*

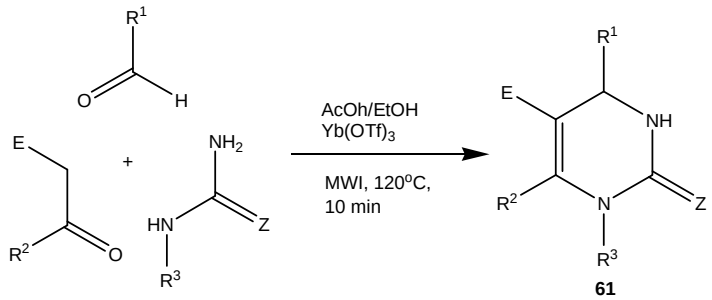
Pisani *et al.*,[53] have developed a two-step protocol for the synthesis of 5-aroyl-3,4-dihydropyrimidin-2-ones libraries (30 compounds) of type **60**, combining a trimethylsilyl chloride-mediated Biginelli multicomponent approach with the transition metalcatalyzed Liebeskind-Srogl ketone synthesis. Both reaction steps can be efficiently carried out with controlled microwave irradiation.



60

R^1= Ph, 3-BrPh, 2-CF_3Ph *etc.*
R^2=H, CH_3, C_2H_5
R^3= Ph, 3-ClPh,4-CH_3Ph, 3-OCH_3Ph *etc.*

A diverse set of 17 acidic carbonyl synthons, 25 aldehydes and 8 urea/thioureas was used in the preparation of a dihydropyrimidine library **61**. Out of the full set of 3400 possible DHPM derivatives, a representative subset of 48 analogues was prepared using automated addition of building blocks and subsequent sequential microwave irradiation of each process vial. For most building block combinations 10 min of microwave flash heating at 120°C using AcOH/EtOH (3:1) and 10 mol % ytterbium trifluoromethanesulfonate [$Yb(OTf)_3$] as solvent/catalyst system proved to be successful, leading to an average isolated yield of 52% of DHPMs with >90% purity.[54]

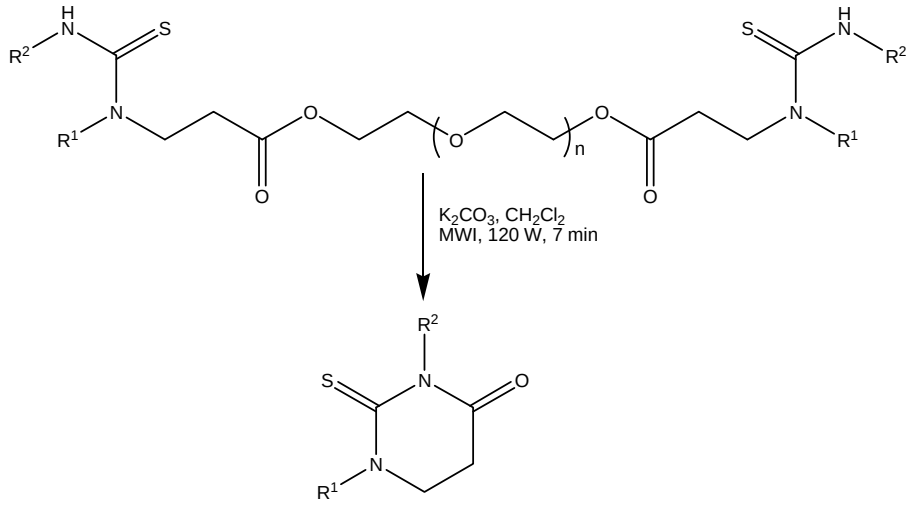


61

R^1=Ph, 2-NO_2Ph, 4-NO_2Ph, 3-OHPh,4-CH_3Ph *etc*
R^2= CH_3, C_2H_5, Ph *etc*
R^3= CH_3, C_2H_5, CH_2Ph, Ph *etc.*
E= $COOC_2H_5$, $COOCH_3$, $COCH_3$, $CONH_2$, *etc*

Yeh *et al.*,[55] have successfully combined the advantages of microwave technology with liquid phase combinatorial chemistry to facilitate thioxotetrahydropyrimidinone **62**

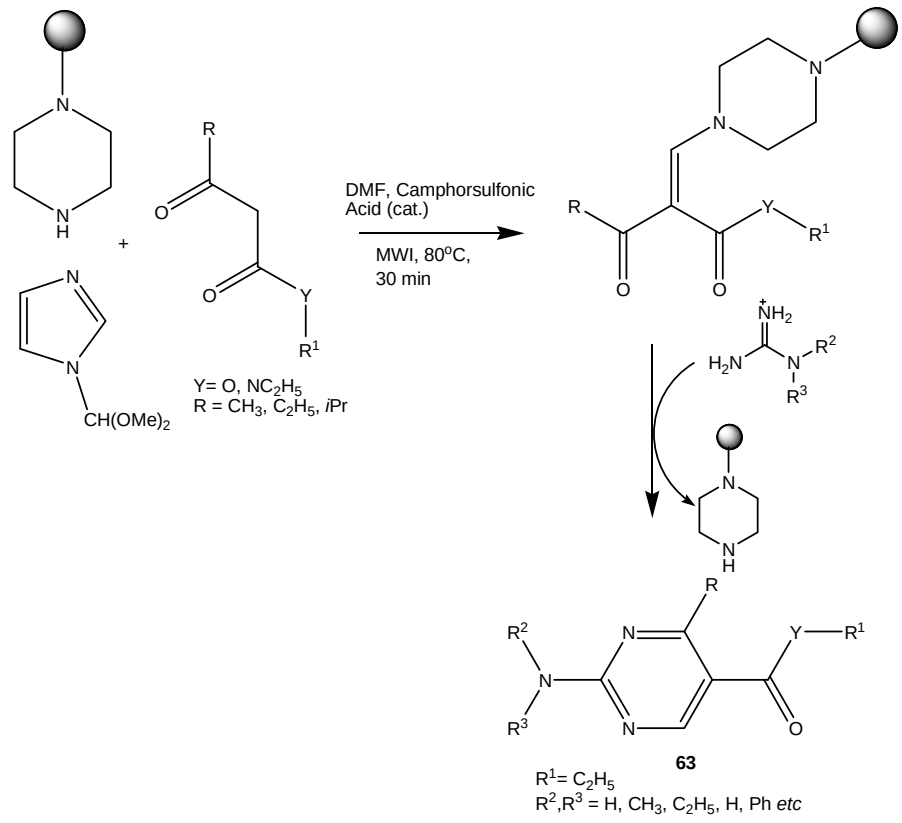
synthesis (15 derivatives). Purification steps were minimized, analytical methods were significantly simplified, and a much defined products were yielded.



62

R^1= Isopropyl, n-butane, *etc*
R^2= 4-F Ph, 4-NO_2Ph, 3-CH_3 Ph, 3-F Ph *etc*

Porcheddu *et al.*,[56] have described an efficient approach to synthesize the libraries (39 compounds) of substituted pyrimidines **63** starting from different β-keto-esters or β-keto-amides, using a low cost and high loading polymer, under very mild conditions using microwave irradiation.

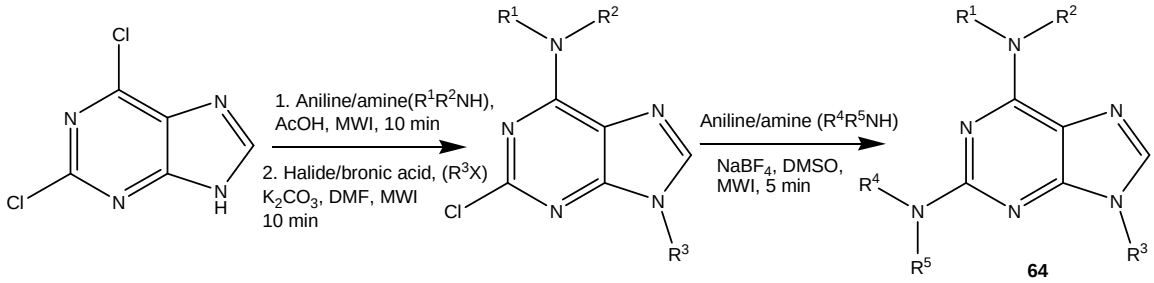


63

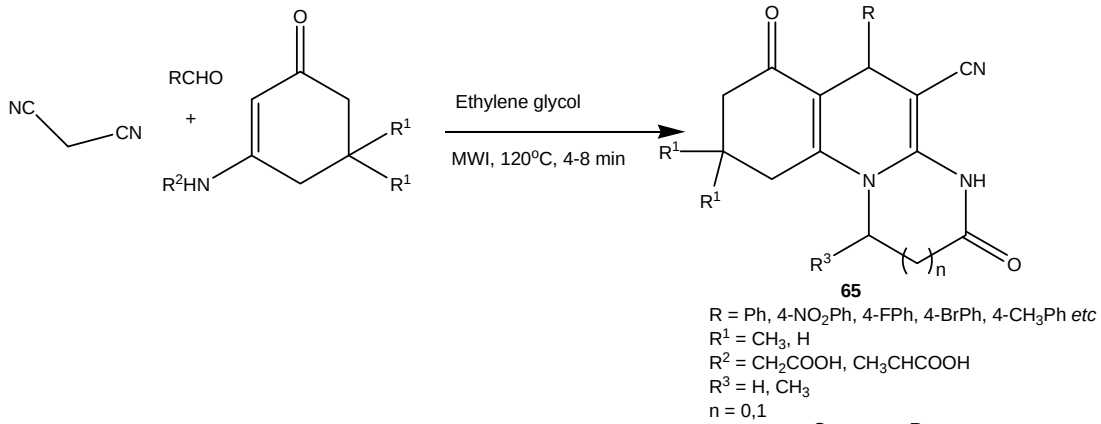
R^1= C_2H_5
R^2,R^3 = H, CH_3, C_2H_5, H, Ph *etc*

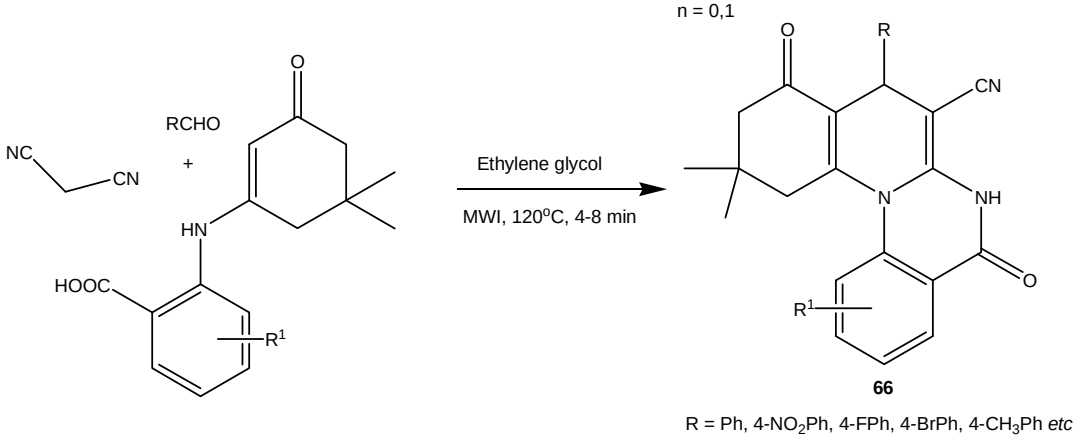
2.3.2.2 Condensed pyrimidine libraries through MWI

Huang *et al.*,[57] have reported an expeditious and efficient method to prepare 2,6,9-substituted purines **64** in a two-pot reaction using microwave assisted reactions. The 2-chloro-6,9-substituted purines were prepared via a one-pot two-step reaction, which involves a sequential S_NAr displacement of the C_6 chlorosubstituent with various anilines and amines, followed by *N*-alkylation and *N*-arylation at the N_9 position with different organic halides and boronic acids.

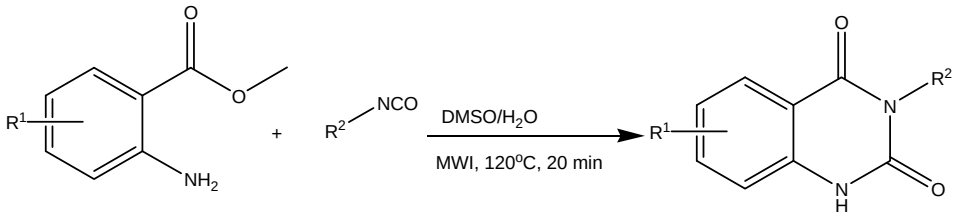


A high-speed, one-pot combinatorial method for synthesizing diverse sets of imidazo[1,2-*a*]quinolines, pyrimido[1,2-*a*]quinolines **65** and quinolino[1,2-*a*]quinazolines **66** from readily available starting materials (12 analogues) has been reported.[58]



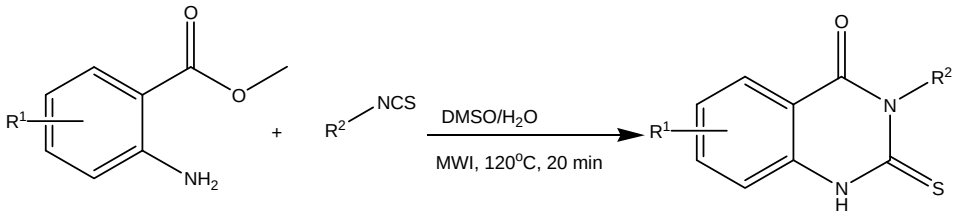


An efficient and convenient method has been developed for the preparation of 2,4(1*H*,3*H*)-quinazolinediones **67** and 2-thioxoquinazolinones **68** (35 compounds). Substituted methyl anthranilates have been reacted with various iso(thio)cyanates in DMSO/H_2O without any catalyst or base by using microwave irradiation to generate diversity on the 2,4(1*H*,3*H*)-quinazolinediones or 2-thioxoquinazolinones. A variety of substrates can participate in the process to yield products in good yields and high purity, making this methodology suitable for library synthesis of NCE's in drug discovery efforts.[59]



67

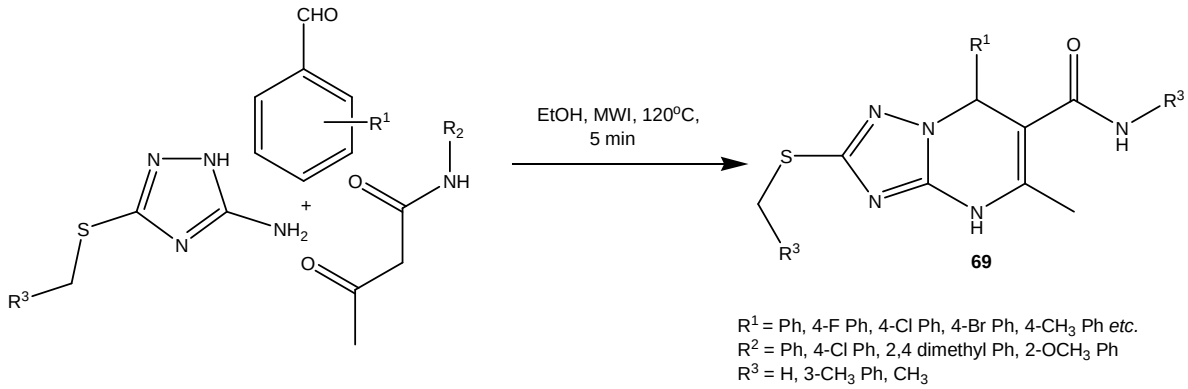
R^1= H, 5-F
R^2= 2-F_3CPh, 3-F_3CPh, 4-FPh, 4-BrPh *etc*



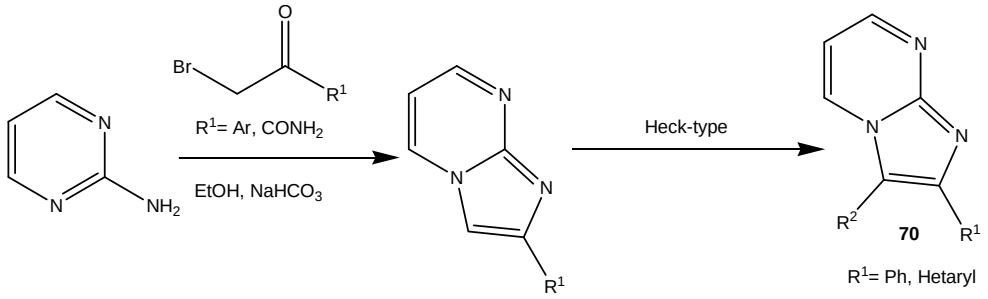
68

R^1= H, 5-F, 4,5-dimethoxy Ph
R^2= Ph, 3-F_3CPh, 4-F_3CPh, 2-BrPh, ethyl, bytyl *etc*

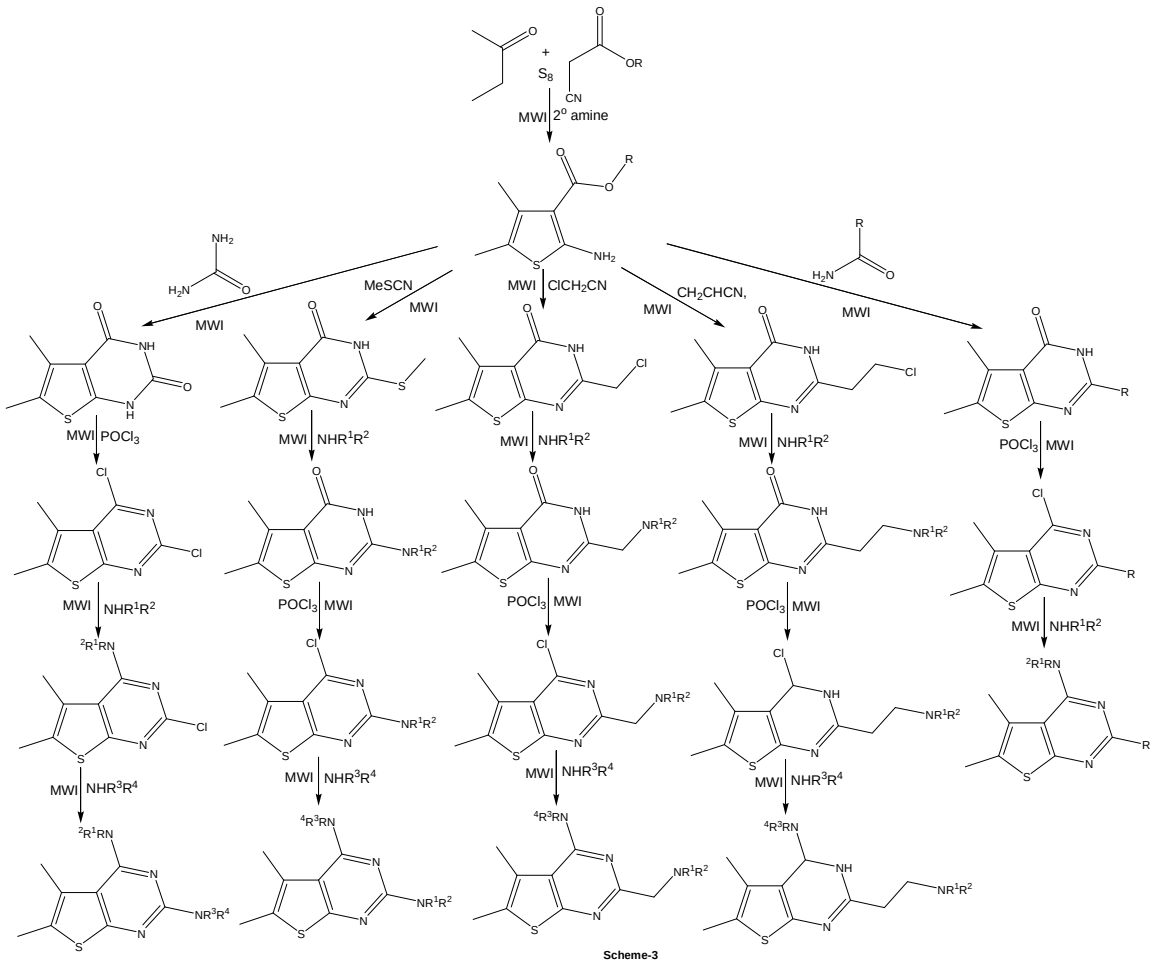
Some Russian workers[60] have developed a fast and convenient microwave assisted procedure for the rapid generation of 7-aryl-2-alkylthio-4,7-dihydro-1,2,4-triazolo[1,5-*a*]-pyrimidine-6-carboxamides **69** by three-component condensation of 3-amino-2-alkylthio-1,2,4-triazoles with aromatic aldehydes and acetoacetamides. All reactions were completed within 5 min of microwave irradition at 120°C and provided the desired products in high yields and excellent purity. Total 60 compounds have been reported.



A short and practical synthesis of 2,3-substituted imidazo[1,2-*a*]pyrimidines **70**, based on microwave-assisted Heck-type arylation of 2-substituted imidazo[1,2-*a*]pyrimidines, has been developed. A 45-membered library of 2,3-substituted imidazo[1,2-*a*]pyrimidines has been obtained with good yields and purities using this optimized protocol.[61]



Recently, we have reported microwave assisted cyclocondensation, chlorination and amination (nucleophilic displacement) reactions to afford a variety of libraries of 2,4-diamino and 4-aminothieno[2,3-*d*]pyrimidines. The versatile synthons *i.e.*, 2-amino-3-carbethoxy-4,5-disubstitutedthiophenes were also generated through rapid one pot Gewald condensations under microwave irradiations.[62] They are cyclocondensed with urea or amides under MWI to their corresponding thienopyrimidin-2,4-diones and -4-ones. These and the other 2-substitutedthieno[2,3-*d*]pyrimidin-4(3*H*)-ones have been subsequently chlorinated with $POCl_3$ under MWI. This chlorination under MWI, is indeed interesting, high yielding and less reported procedure to the best of our knowledge. Subsequent aminations involving nucleophilic displacements under MWI are also novel.[63] (Scheme-3).



Scheme-3

2.4. Conclusions

There are more than 5,000 documented examples of Microwave Assisted Organic Synthesis (MAOS) reported by both academic and industrial laboratories, which suggests that most chemical transformations can be carried out successfully under microwave conditions. This does not necessarily imply that dramatic rate-enhancements compared with a classical, thermal process will be observed in all cases, but the simple convenience of using microwave technology will make this non-classical heating method a standard tool in the laboratory within a few years The recently reported incorporation of real time, in situ monitoring of microwave-assisted reactions by Raman spectroscopy will facilitate a further increase in efficiency and speed.

For the production of New Chemical Entities (NCE's) in pharmaceutical industry today, microwave is an essential tool and several laboratories have already incorporated microwave reactors into in-house 'synthesis stations' for producing small- and medium-sized compound libraries in a high-throughput format.

2.5. Drawbacks associated with MWI

1. One of the major drawbacks of this relatively new technology is equipment. Although prices for dedicated microwave reactors for organic synthesis have dropped considerably since their first introduction in the late 1990s, the current price range for microwave reactors is still many times higher than that of conventional heating equipment cost, ranging from US$15,000-100,000.
2. An even bigger problem, especially for the drug discovery industry, is scalability. It has to be noted that with few exceptions most of the examples of microwave-assisted synthesis published so far were carried out on a small scale (<1 gm; typically 1-5-ml reaction volume).
3. There is a need to develop larger-scale microwave reaction techniques that can routinely provide products for lead development and ultimately for production on a large scale (multi 100 kg, or even higher).
4. Two different approaches that address these issues have emerged. Some groups have experimented with larger batch-type reactors, whereas others have used continuous-flow techniques to overcome the inherent problems associated with microwave irradiation scale-up.
5. Currently, there are no documented published examples of the use of microwave technology for organic synthesis on a production scale level (>1,000 kg), which is a clear limitation of this otherwise so successful technology.

Despite these limitations, microwave chemistry has opened up several new avenues in organic synthesis. Many reactions that previously were not possible, or resulted in a low yield, can now often be performed quickly, safely and efficiently in a few minutes. In summary, Microwave Assisted Organic Synthesis has changed the world of organic chemistry and drug discovery, and it would be wise to embrace this new technology or be left lagging behind with conventional heating methodologies.

2.6. References

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3. Aim of the Present Work

3. Aim of the Present Work

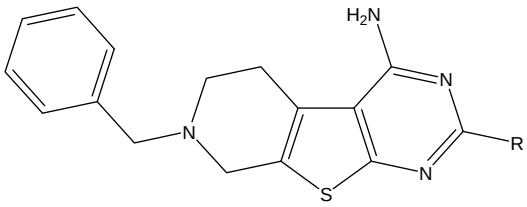
3.1 Introduction

Pyrimidines and condensed pyrimidines have a long and distinguished history of their immense biological and medicinal significance.[1] The synthesis and biological evaluation of condensed pyrimidines, appropriately functionalized, especially at 2- & 4- positions has attracted considerable attention of medicinal chemists worldwide, as they are potentially bioactive molecules.[2]

3.2 Biological and Pharmacological Significance of Thienopyrimidines

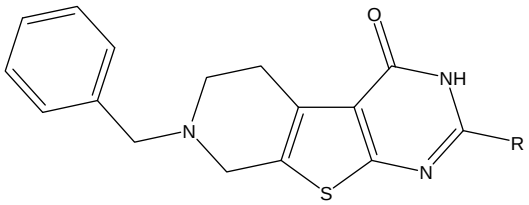
Condensed pyrimidines and quinazolines have shown a wide spectrum of biological activities and have been exhaustively reviewed.[1] Thieno[2,3-*d*]pyrimidines are considered to be bioisosteres of quinazolines. The concept of bioisosterism[3] has been exploited by medicinal chemists as an approach to the drug design. This has lead to the synthesis of various types of condensed pyrimidines, which show a wide range of biological activities.

4-Amino and 4-oxo-5,6,7,8-tetrahydro-7-benzylpyrido[4'.3':4,5]thieno[2,3-*d*]pyrimidines **1** and **2**[4,5], some derivatives of thieno[2,3-*d*]pyrimidin-4-(3*H*)-ones **3**[6], 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine carboxylates **4**[7] and **5**[8] and thieno[2,3-*d*]pyrimidin-4-(3*H*)-ones **6**[9] have exhibited potent anti-allergic activity.



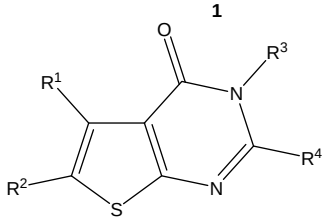
R= alkyl, Ar-CH_2-, alkylthio, pyridyl

1



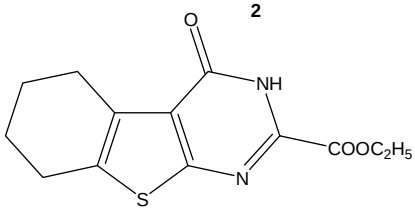
R= alkyl, Ar-CH_2-, alkylthio, pyridyl

2



R^1, R^2 = -(CH_2)n-; n=4, 6;
R^3 = H, alkyl;
R^4= COOH, CH_2OH.

3



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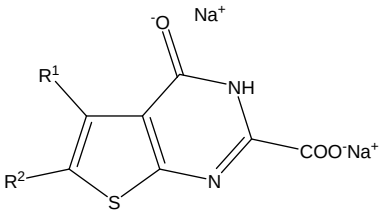
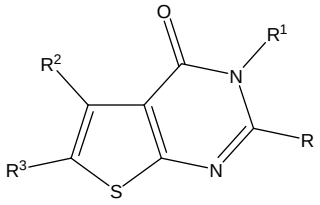


R^1, R^2 = -(CH_2)n;
R^1 = H, CH_3, *i*-But, NH_2, NO_2 ;
R^2 = H, C_2H_5, *i*-But, $COCH_3$, n-Hexyl *etc*.

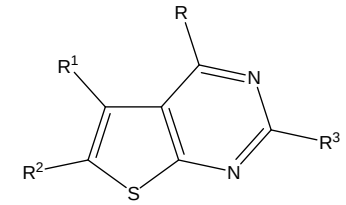
5



R = $COOC_2H_5$, COONa;
R^1 = H;
R^2 = H, CH_3;
R^3 = C_2H_5, Hexyl

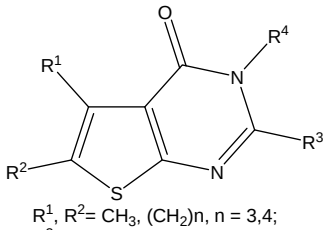
6

Some thienopyrimidines derivatives such as thieno[2,3-*d*]pyrimidine 2-mercaptoacetic acids **7**[10-12], 2-alkyl-3-arylthieno[2,3-*d*]pyrimidin-4-(3*H*)-ones **8**[13,14], 2-ethoxy-4-oxo-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidine **9**[15], thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **10**[16,17], 2-(substituted)styrylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones **11**[18], 3-arylamino-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidines **12**[19], 5,6-disubstitutedthieno[2,3-*d*]-pyrimidines **13** and **14**[20], derivatives of general structure **15**[21], [1,3,4]thiadiazolo[3,2-*a*]-thieno[2,3-*d*]pyrimidin-5-ones **16**[22], thieno[2,3-*d*]pyrimidin-4-ones **17**[23], thiadiazolo-thieno[2,3-*d*]pyrimidines **18**[24] and 2-substituted-4-oxo-5,6,7,8-tetrahydrobenzo(*b*)thieno-[2,3-*d*]pyrimidines **19**[25] have been discussed for their potent analgesic, anti-inflammatory and CNS depressant activities.



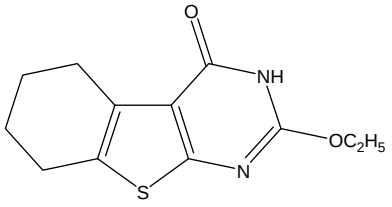
R= C_6H_5, ClC_6H_4, OH, alkyl, alkoxyl, aralkyl;
R^1, R^2= H, alkyl, (-CH_2-)n, n = 3,4 - halo, nitro;
R^3 = $SCH_2COOC_2H_5$, SCH_2COOH, SCH_2CH_2COOH.

7



R^1, R^2= CH_3, (CH_2)n, n = 3,4;
R^3= H, alkyl;
R^4= C_6H_5, 2-F-C_6H_4, $CH_2CH_2C_6H_5$

8



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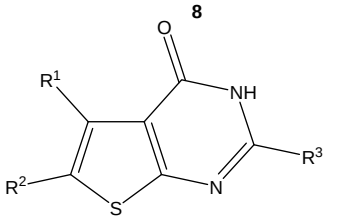
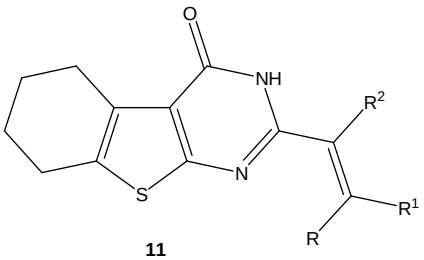


R^1 = NH_2;
R^2 = $COCH_3$, $CONH_2$, $COOC_2H_5$;
R^1, R^2 = $(CH_2)_4$, $CH_2CH_2CH_2$;
R^3 = H, CH_3, Ph.

10



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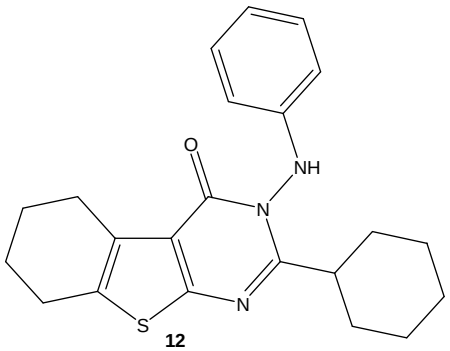

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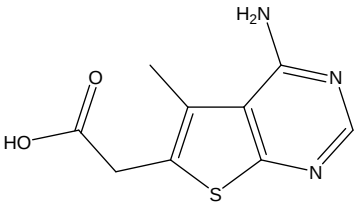

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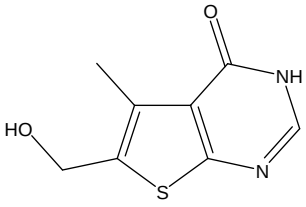

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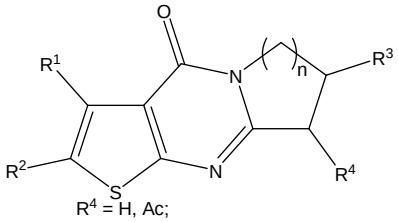
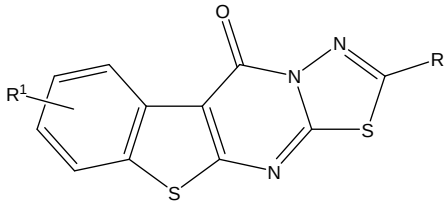


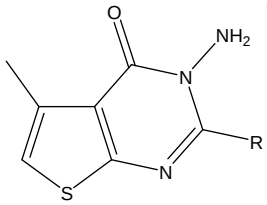
R^4 = H, Ac;
R^1,R^2 = CH_3, (-CH_2-)n, n = 1, 2;

15



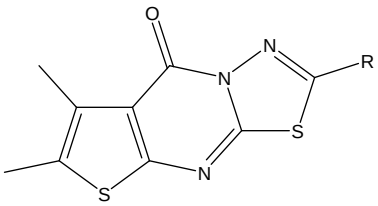
R = H, Me, Et
R^1 = alkyl, alkoxyl, OH

16



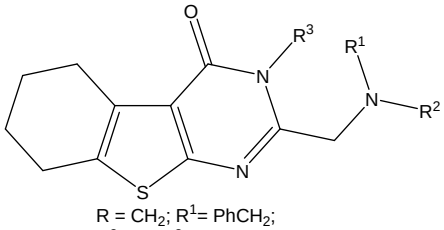
R = CH_3, CH_2Ph

17



R = Me, Et, Ph, SMe

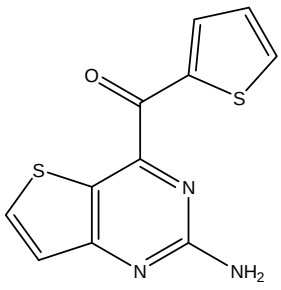
18



R = CH_2; R^1= $PhCH_2$;
R^2 = H; R^3 = H
R^1, R_2 = piperidine, morpholine,
N-methyl piperazine.

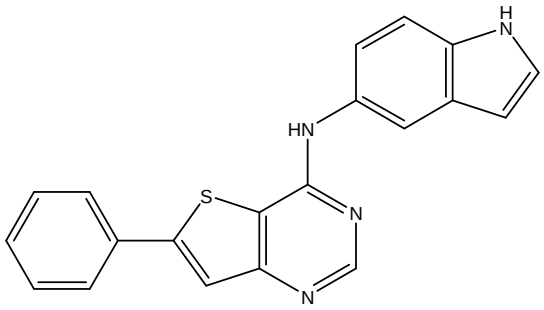
19

Recently, a series of 4-arylthieno[3,2-*d*]pyrimidines was reported as potent adenosine A_{2A} receptor antagonist. These novel compounds showed high degrees of selectivity against the human A_1, A_{2B} and A_3 receptor sub-types. Compounds **20** showed promising activity *in vivo*, suggesting potential utility in the treatment of Parkinson's disease.[26]



20

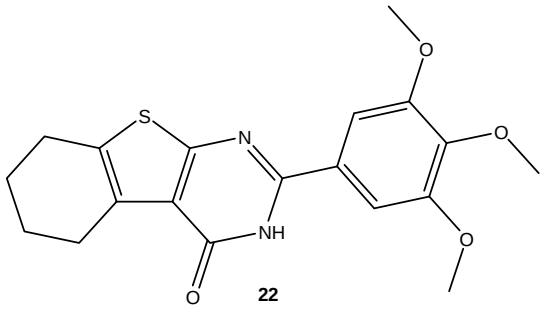
Another novel series of thienopyrimidines and thienopyridines have been identified as potent inhibitors of VEGFR-2 kinase. Compound **21** was found most potent with IC_{50} values of 80 nM and 3 nM for VGFR and EGFR respectively.[27]



VGFR IC_{50} = 80 nM
EGFR IC_{50} = 3 nM

21

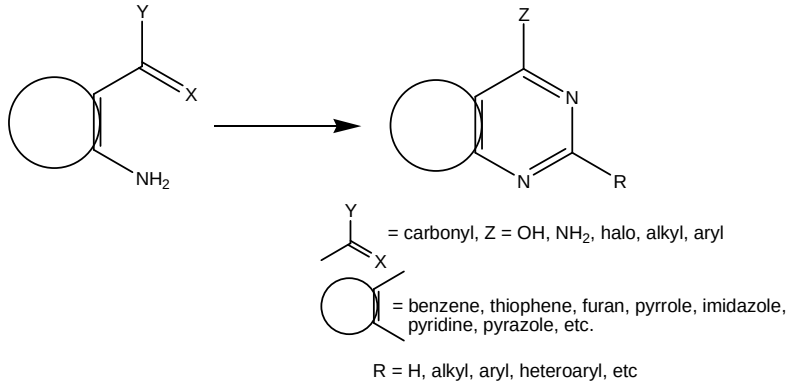
Interestingly, a series of novel thieno[2,3-*d*]pyrimidin-4(1*H*)-one based analogs were found potent inhibitor of the growth of human colon tumors. Compound **22** was the most potent inhibitor of the tumor cell growth.[28]



22

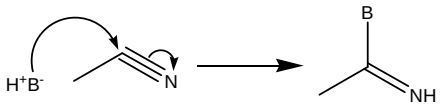
3.3 Synthesis of Pyrimidines:

The synthesis of condensed pyrimidines is a very important process which is subject to improvement, routinely. The regularly employed synthetic methodology involves annealation of the pyrimidine ring on an appropriately substituted heterocycles in which a variety of *o*-aminocarbonyl heterocycles have been cyclocondensed with a host of reagents namely amides, thioamodes, imidates, amidines, *etc.*, mostly under basic conditions to afford variety of condensed pyrimidines, quinazolines, thienopyrimidines, furanopyrimidines, purines, pteridines, pyridopyrimidines, pyrrolopyrimidines, pyrazolopyrimidines, *etc.*,[29] (Scheme 1).



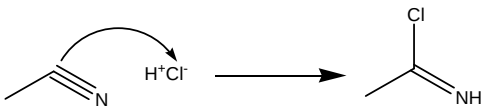
Scheme-1

There are also a few reports on the direct use of 'R-CN' as the reagent to be cyclocondensed with *o*-aminocarbonyl substrates, under basic conditions to prepare condensed 2-substitutedpyrimidin-4-ones, mostly 2-substitutedquinazolin-4-ones, albiet in low yields.[30,31] A large number of heterocyclic structures have been successfully synthesized through the reactions of nitriles mainly under such basic conditions.[32,33] All these syntheses involve the nucleophilic attack of the reagent on the nitrile function (Scheme 2).[34] The direct use of the electrophillic properties of the nitrile in such syntheses, though has received relatively less attention, is however not new.[35]



Scheme-2

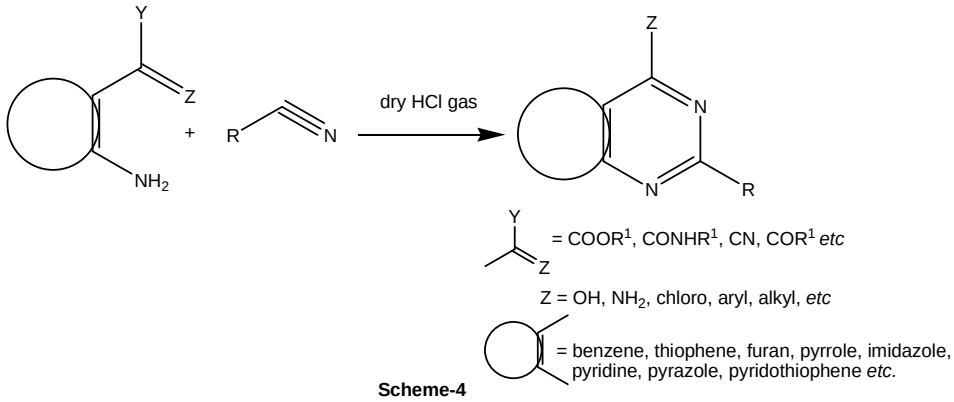
Shishoo and co-workers[36-44] have exploited the reactions of a variety of nitriles with a host of *o*-aminocarbonyl substrates, under the influence of dry HCl gas to obtain a wide range of 2-substituted-4-oxo/4-amino/4-chloro & 4-aryl condensedpyrimidines. The scope of this work is subject to a review.[45] These reaction proceeds *via* the imidoyl halide intermediates (Scheme 3).



Scheme-3

Interestingly, the nitriles 'RCN', possessing strongly electron withdrawing substituents (R) are more reactive under this conditions.[39] The main limitation of this interesting reaction is the failure of *o*-aminocarbonyl substrates possessing azaheterocyclic nucleus.

In spite of this limitation, the reaction is indeed a facile, high yielding one pot synthetic procedure for a variety of condensed pyrimidines (Scheme 4).

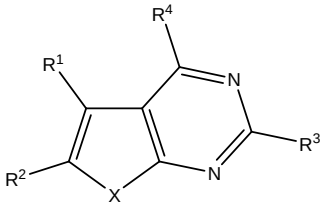


Scheme-4

3.4 Thienopyrimidines as an Important Scaffold for Parallel Synthesis of Compound Library for NDDR

Reactions that are adaptable for high speed and throughput syntheses have become an important component of the modern medicinal chemist's library, as a great number of compounds can be produced through such rapid parallel synthetic programs.[46] Synthetic methods that enable the rapid production of an array of heterocycles, useful for the identification of new lead structures are of critical importance to the pharmacological activity. Thienopyrimidine and other condensed pyrimidines scaffolds and their derivatives are important heterocyclic building blocks and have been shown to possess significant pharmacological activity against a variety of molecular targets.[47]

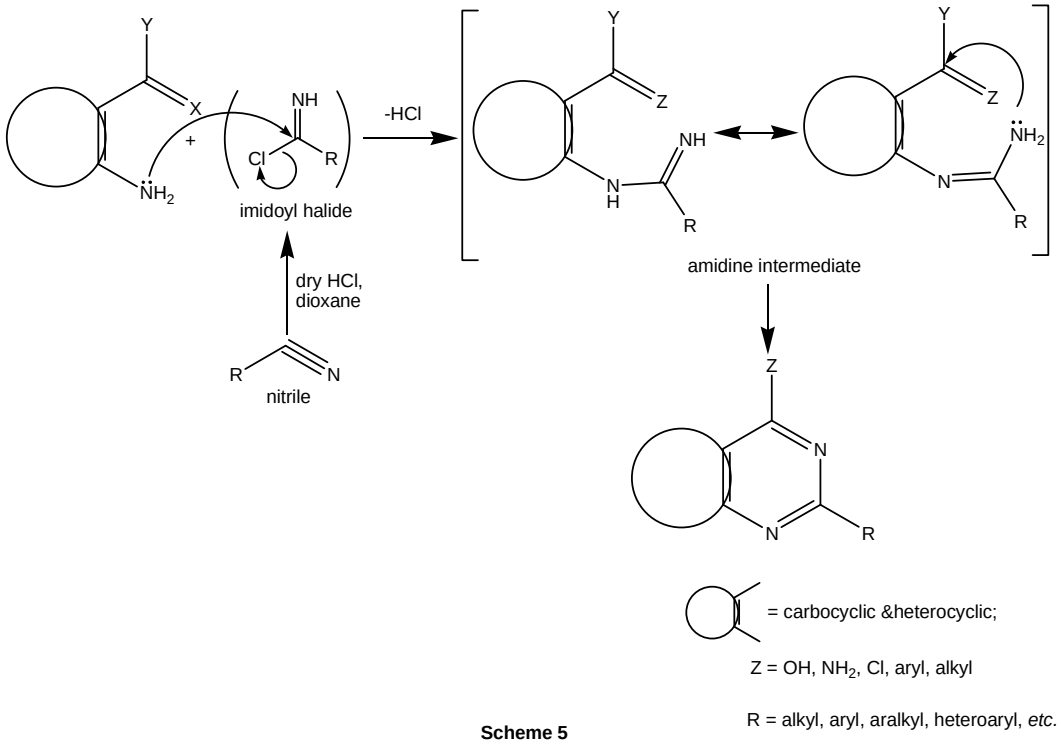
Extensive work on condensed 2-substitutedpyrimidin-4(3*H*)-ones, **23**, especially thieno[2,3-*d*]pyrimidin-4(3*H*)-ones by Shishoo *et al.*, as discussed above involve the reaction of nitriles under acidic conditions using dry HCl gas.[36-44] These condensedpyrimidines have four diversity points.



23

X = S, -CH=CH-, N, O, *etc*
R^1, R^2 = H, alkyl, aryl, cycloalkyl, carboalkoxy, carbocyclic, heterocyclic, *etc*
R^3 = alkyl, aryl, arylalkyl, heteroaryl, substituted amino, heteroalkyl,aryl, *etc*
R^4 = OH, alkyl, aryl, Cl, NH_2

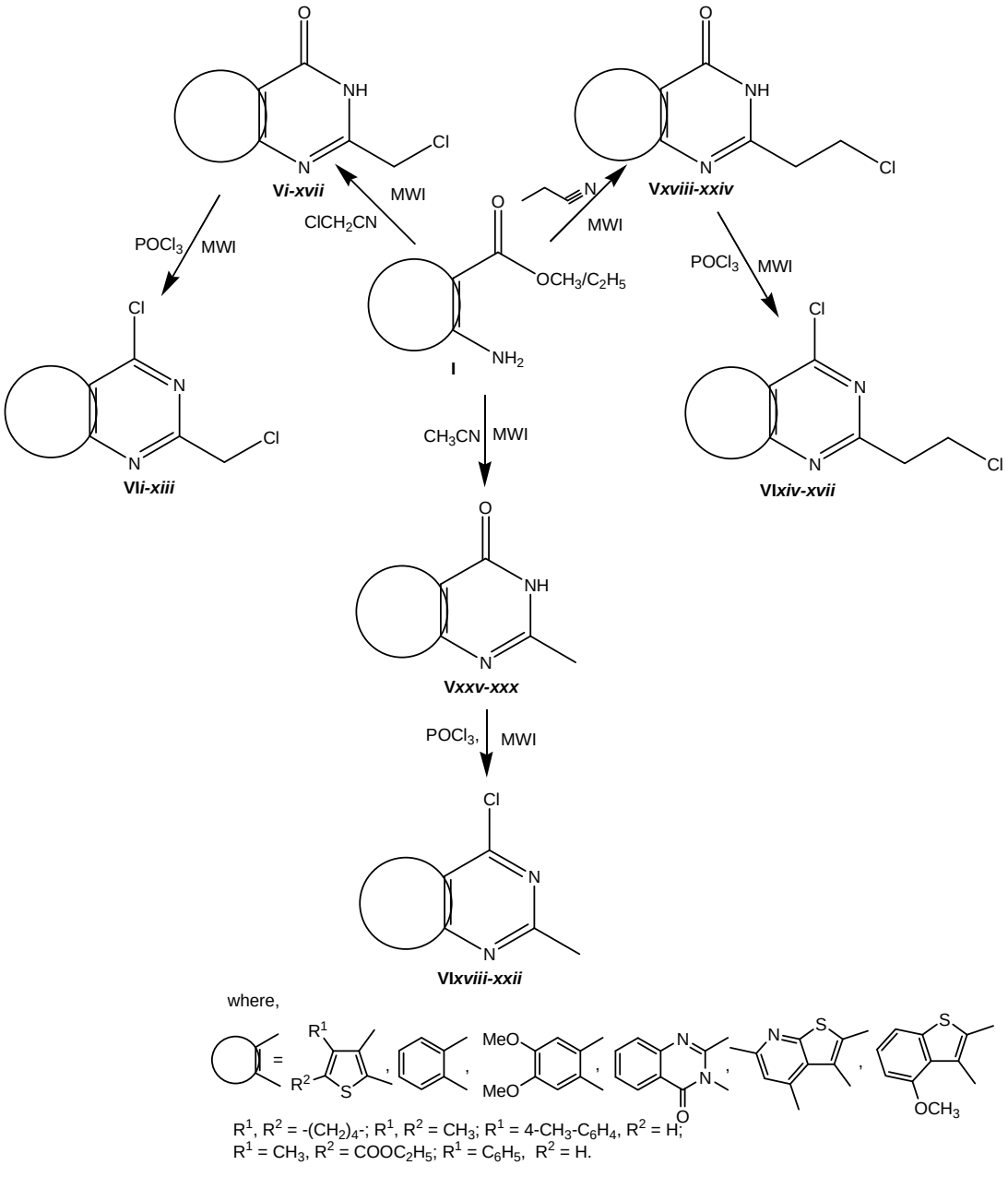
The cyclization reactions involving their synthesis proceed *via* the formation of transient amidine intermediates resulting from the reaction of the *o*-aminocarbonyl compounds with protonated nitrile or imidoyl chloride intermediate. The imodyl chloride intermediate possess the nitrile carbon with enhanced electrophilicity towards the amino function of the thiophene *o*-aminocarbonyl substrates leading to their facile condensation to the amide intermediate to form the amidine intermediate (Scheme 5). This is followed by intramolecular cyclisation of these transient amidine intermediates.



Scheme 5

Recently, encouraging results in the MWI based syntheses of thiophene *o*-aminoesters involving Gewald reaction,[48] as well as, thienopyrimidine bioisosteres of gefitinib[49] under microwave conditions from this laboratory gave an impetus to assess whether these could be extended to the single pot cyclocondensation of the acetonitriles with various *o*-aminoester substrates under solvent free conditions using MWI. This was particularly of interest, especially for quickly generating compound libraries of increasing molecular diversity, through the development of synthetic methods that could combine the expediency of microwave energy.

Thus, **the Aim of the Present Work** was to use microwave irradiation for the synthesis of condensed 2-substitutedpyrimidin-4(3*H*)-ones (**V** and **VI)** involving the condensation of variety of nitriles with *o*-aminoesters of thiophene, benzene, dimethoxybenzene and quinazolinone in the presence of catalytic amount of HCl alone or with the Lewis acid, $AlCl_3$ under solvent free conditions for the first time. Further, it was decided to synthesize 4-chloro derivatives of these condensed 2-substituted pyrimidines-4-ones through MWI assisted facile and rapid chlorination method (Scheme 6), and also evaluated them for some biological activity.



Scheme 6

3.5 References:

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4. Results and Discussion

4.1 Synthesis of Starting Materials:

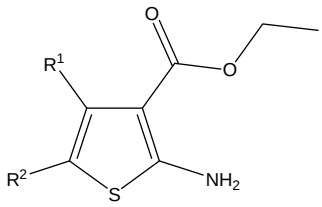
4.1.1 Synthesis of *o*-aminothiophenes (The Gewald reaction)

4.1.2 Synthesis of other *o*-amino esters substrate

4.1.1 Synthesis of *o*-Aminothiophenes (The Gewald reaction)

The starting material used for the synthesis of 2-substituted condensed pyrimidines was synthesized using classical Gewald reaction, which is explained in Part-1 of this thesis.

Table-36: Physical data of 2-amino-3-carbethoxythiophenes (I*i-xii*)



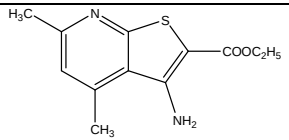
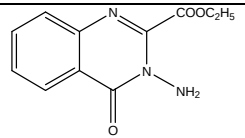
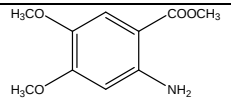
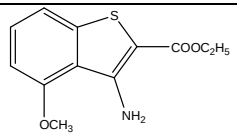
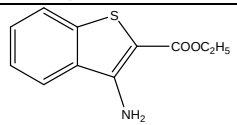
Sr. No	R^1	R^2	Mol.For. Sol. of recryst	Yield (%)	M.P. °C	Time (hrs.)	Route
I*i*	$-(CH_2)_4-$		$C_{11}H_{15}NO_2S$ (E)	80	110-112	3	A
I*ii*	$-CH_3$	$-COOCH_3$	$C_{10}H_{13}NO_4S$ (E)	70	80-82	2-3	A
I*iii*	$-CH_3$	$-COOC_2H_5$	$C_{11}H_{15}NO_4S$ (B)	50	103-105	2	A
I*iv*	$-CH_3$	$-CH_3$	$C_9H_{13}NO_2S$ (E)	50	92-93	3	A
I*v*	$-C_6H_5$	H	$C_{13}H_{13}NO_2S$ (E)	75	95-97	15-18	B
I*vi*	$4\text{-}CH_3OC_6H_4$	H	$C_{14}H_{15}NO_3S$ (E)	73	96-99	15-18	B
I*vii*	$4\text{-}CH_3C_6H_4$	H	$C_{14}H_{15}NO_2S$ (E)	89	102-104	15-18	B
I*viii*	$4\text{-}BrC_6H_4$	H	$C_{13}H_{12}BrNO_2S$ (E)	76	78-80	15-18	B
I*ix*	$4\text{-}ClC_6H_4$	H	$C_{13}H_{12}ClNO_2S$ (E)	80	102-104	15-18	B
I*xi*	$-(CH_2)_5-$		$C_{12}H_{17}NO_2S$ (E)	71	75-77	15-18	B
I*xii*	$-(CH_2)_2-N-(CH_2C_6H_5)CH_2-$		$C_{17}H_{16}ClN_3OS$ (E-C)	78	232-234	15-18	B

E =Ethanol, B = Benzene

4.1.2 Synthesis of other *o*-amino esters substrates

The other *o*-amino ester substrates used were synthesized by using the methods reported in Part-1 of this thesis.

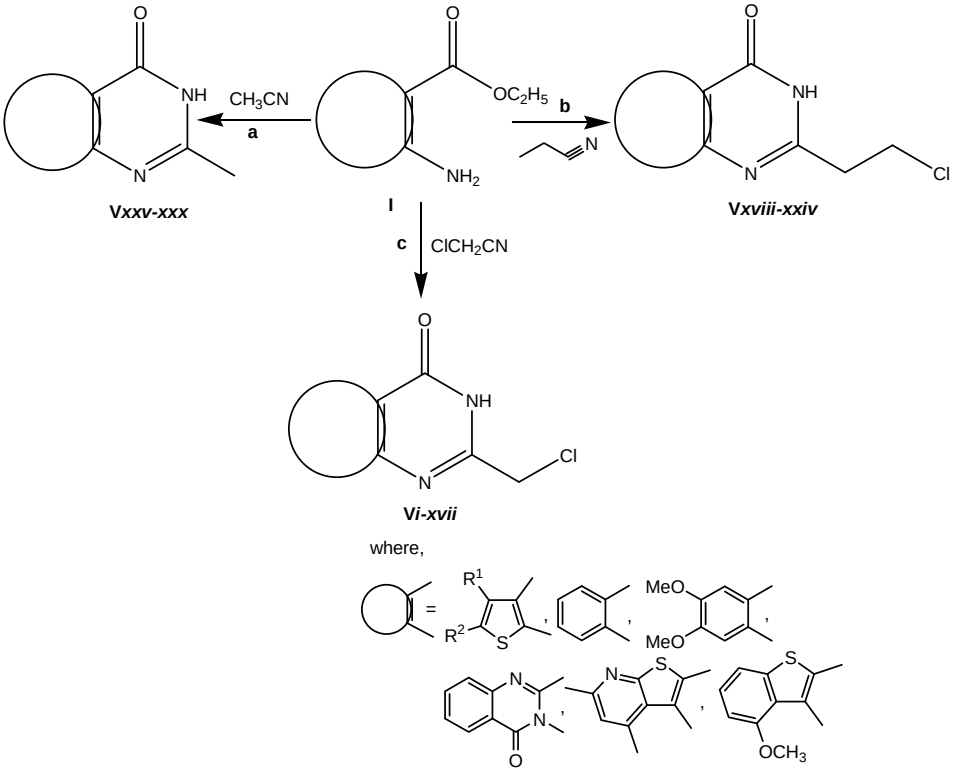
Table 37: Physical data of other *o*-aminoesters (I*xiii-xvii*) synthesized

Compd No.	Compound	M.P (^oC)	Yield (%)	Mol. Formula (Solv. of Crystn.)	IR (cm^{-1})	Mass (m/e)	NMR (δppm)
I*xiii*		152-156	90	$C_{12}H_{14}N_2O_2S$ (E)	3435, 3332(γ_{NH}), 2979(γ_{C-H}), 1668($\gamma_{C=O}$)	250(M^+), 222, 204, 176, 149, 132	1.38 (3H, t, $COOCH_2CH_3$, *J* = 5.1 & 6.9), 2.57 (3H, s, CH_3), 2.71 (3H, s, CH_3), 4.32 (2H, q, $COOCH_2CH_3$, *J* = 6.9 & 7.2), 6.14 (2H, s, NH_2), 6.82 (1H, s, Ar-*H*)
I*xiv*		137-138	44	$C_{11}H_{11}N_3O_3$ (E)	3476, 3334(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$), 1687(γ_{CONH})	218(M^+), 204, 161, 144, 218, 204, 161, 144	1.45 (3H, t, $COOCH_2CH_3$, *J* = 7.2), 4.50 (2H, q, $COOCH_2CH_3$, *J* = 6.9 & 7.2), 5.15 (2H, s, br, NH_2), 7.48-8.29 (4H, m, Ar-*H*)
I*xv*		120-122	47	$C_{11}H_{15}NO_4$ (E)	3476, 3373(γ_{NH}), 2998(γ_{C-H}), 1739($\gamma_{C=O}$).	--	--
I*xvi*		145-147	80	$C_{12}H_{13}NO_3S$ (E)	3484, 3376(γ_{NH}), 2947(γ_{C-H}), 1670($\gamma_{C=O}$)	--	--
I*xviii*		106-108	60	$C_{11}H_{11}NO_2S$ (E)	3452, 3397(γ_{NH}), 3130(γ_{C-H}), 1686(γ_{CONH})	--	--

E= Ethanol

4.2 Synthesis of condensed 2-substitutedpyrimidines

For the first time a rapid, microwave assisted green chemical synthesis of condensed 2-substitutedpyrimidin-4(3*H*)-ones involving the condensation of a variety of nitriles with *o*-aminoesters of thiophene, benzene, dimethoxybenzene, pyridothiophene, 4-methoxy-benzothiophene and quinazolinone in the presence of catalytic amount of HCl alone or with the Lewis acid, $AlCl_3$ under solvent free conditions is reported, herein (Scheme 1).



V*xxv-xxx* **I** **V*xviii-xxiv***

V*i-xvii*

where,

R^1, R^2 = -$(CH_2)_4$-; R^1, R^2 = CH_3; R^1 = 4-CH_3-C_6H_4, R^2 = H; R^1 = CH_3, R^2 = $COOC_2H_5$; R^1 = C_6H_5, R^2 = H.

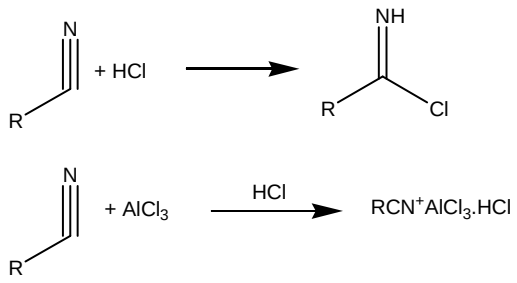
Scheme 1: Reaction conditions a: Under microwave irradiation; 350W, 20-75 min (60-94%), b: Under microwave irradiation; 350W, 20-50 min (83-99%), c: Under microwave irradiation; 350W, 10-40 min (66-95%).

These reactions under microwave irradiation at 350W were accomplished by using catalytic amount of concentrated HCl (33%w/v) in very short time periods. The reaction time varied depending upon the type of the nitrile used. The reactions with acetonitrile were completed in 40-75 min to obtain the condensed 2-methylpyrimidin-4(3*H*)-ones (**V*xxv-xxx*)** with isolated yields ranging from 68-94%. The reactions with acrylonitrile were completed in 20-50 min time and afforded the condensed 2-chloroethylpyrimidin-

4(3*H*)-ones (**V*xviii-xxiv*)** excellent isolated yields (85-96%), as well as, purity (Scheme 1).

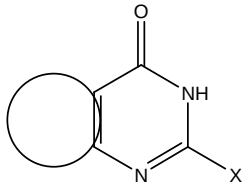
Interestingly, when the reactive nitrile, chloroacetonitrile, was used the reaction went to completion in just 10-40 min and afforded the corresponding 2-chloromethylpyrimidin-4(3*H*)-ones (**V*i-xvii*)** in excellent isolated yields (>90%). Thus, in all the above cases, there is considerable reduction in the reaction times, when conventional method is replaced by microwave assisted heating, *i.e*., from 6-12 hrs to 10-75 min, respectively. Considerable improvement in yields was also observed.

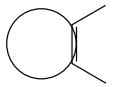
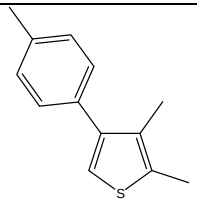
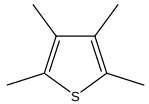
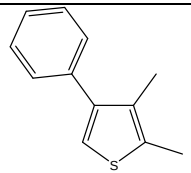
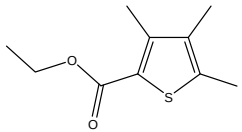
A very important and noteworthy fact is that, all the reactions, depicted in Scheme 3, failed to proceed in the absence of HCl. This indicates that these reactions under MWI, may also be involving the imidoyl chloride intermediates.[8,23] Further, in a few typical cases, only catalytic amount of HCl failed to bring about the completion of the reaction. However, addition of catalytic amount of a Lewis acid, anhydrous $AlCl_3$ along with conc. HCl, accomplished the successful completion of the above reactions to afford the target condensed 2-substitutedpyrimidin-4(3*H*)-ones in excellent isolated yields. Thus, the Lewis acid $AlCl_3$, has forwarded the reactions, probably by way of forming the electrophilic nitrile-metal halide, hydrohalide complex as shown below.[24]

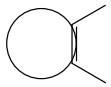
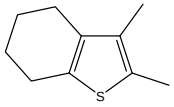
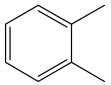
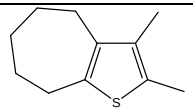
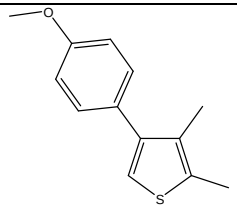
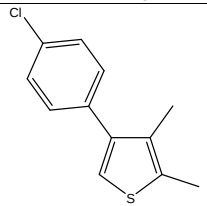
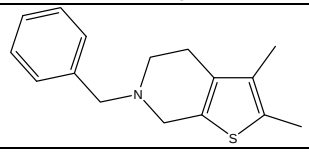


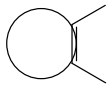
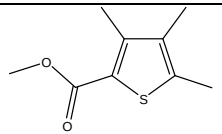
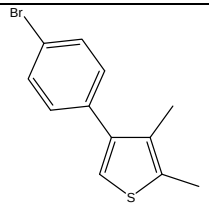
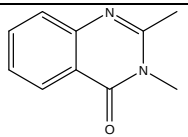
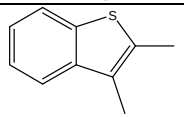
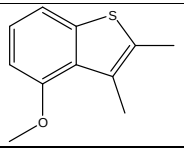
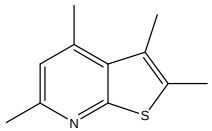
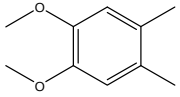
Using this novel microwave assisted green synthesis, following 2-substituted-thieno[2,3-*d*]pyrimidin-4(3*H*)-ones and other 2-substitutedpyrimidin-4(3*H*)-ones were synthesized:

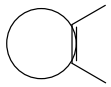
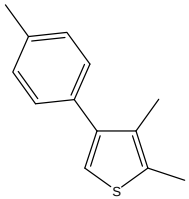
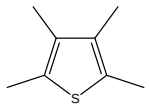
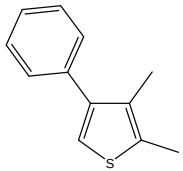
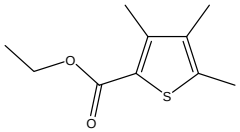
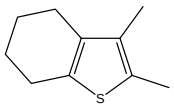
Table 38: Physical data of condensed 2-substitutedpyrimidin-4(3*H*)-ones synthesized using MWI irradiation (V*i-xxx*)

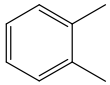
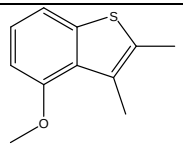
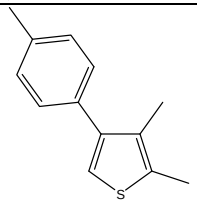
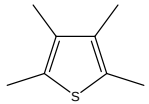
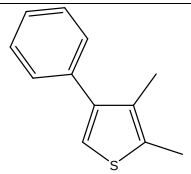
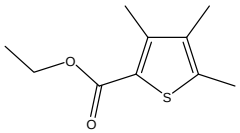


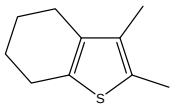
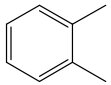
S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*i*		$-CH_2Cl$	25	85	258-260	$C_{14}H_{11}ClN_2OS$ (E-C)	3438, (γ_{NH}), 2919(γ_{C-H}), 1650($\gamma_{C=O}$), 712(γ_{C-Cl})	--	2.39 (3H, s, C*H*$_3$), 4.53 (2H, s, CH_2), 7.13 (1H, s, C*H*), 7.19-7.46 (4H, m, Ar-*H*), 10.43 (1H,s, N*H*)
V*ii*		$-CH_2Cl$	30	92	252-254	$C_9H_9ClN_2OS$ (D)	2917 (γ_{C-H}), 1662($\gamma_{C=O}$), 769(γ_{C-Cl})	--	2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 4.51 (2H, s, C*H*$_2$), 10.03 (1H, s, br, N*H*)
V*iii*		$-CH_2Cl$	20	75	220-222	$C_{13}H_9ClN_2OS$ (D)	2855(γ_{C-H}), 1660($\gamma_{C=O}$), 748(γ_{C-Cl})	--	4.58 (2H, s, C*H*$_2$), 7.31-7.52 (5H, m, Ar-*H* and 1*H* at 6 position), 12.69 (1H, s, br, N*H*)
V*iv*		$-CH_2Cl$	27	90	241-243	$C_{11}H_{11}ClN_2O_3S$ (T-M)	2864(γ_{C-H}), 1725($\gamma_{C=O}$), 1670(γ_{CONH}), 763(γ_{C-Cl})	--	1.41 (3H, t, *J* = 7, C*H*$_3$), 2.95 (3H, s, C*H*$_3$), 4.38 (2H, quartlet, *J* = 7, C*H*$_2$), 4.57 (2H, s, C*H*$_2$), 10.62 (1H, s, N*H*)

S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*v*		$-CH_2Cl$	10	86	273-276	$C_{11}H_{11}ClN_2OS$ (D)	2931(γ_{C-H}), 1663(γ_{CONH}), 754(γ_{C-Cl})	255(M^+), 221, 149	1.86 (4H, s, C*H*$_2$ at 6 and 7), 2.79 (2H, s, C*H*$_2$ at 5), 3.02 (2H, s, C*H*$_2$ at 8), 4.55 (2H, s, C*H*$_2$), 10.65 (1H, s, br, N*H*)
V*vi*		$-CH_2Cl$	20	84	257-258	$C_9H_7ClN_2O$ (E-C)	2981(γ_{C-H}), 1697(γ_{CONH}), 776(γ_{C-Cl})	--	4.53 (2H, s, C*H*$_2$), 7.49-7.82 (4H, m, Ar-*H*), 12.56 (1H, s, br, N*H*)
V*vii*		$-CH_2Cl$	12	74	188-190	$C_{12}H_{13}ClN_2OS$ (M-C)	2925(γ_{C-H}), 1660(γ_{CONH}), 755(γ_{C-Cl})	--	--
V*viii*		$-CH_2Cl$	25	84	205-207	$C_{14}H_{11}ClN_2O_2S$ (T-M)	2990(γ_{C-H}), 1680($\gamma_{C=O}$), 746(γ_{C-Cl})	--	3.85 (3H, s, OC*H*$_3$), 4.48 (2H, s, *CH*$_2$Cl), 6.94-7.54 (5H, m, 4H, Ar-*H* and 1H at 6), 12.02 (1H, s, N*H*)
V*ix*		$-CH_2Cl$	20	71	233-234	$C_{13}H_8Cl_2N_2OS$ (T-M)	3107(γ_{NH}), 1649(γ_{CONH}), 756(γ_{C-Cl})	--	4.54 (2H, s, *CH*$_2$Cl), 7.23-7.57 (5H, m, 4H, Ar-*H* and 1*H* at 6), 11.5 (1H, s, N*H*)
V*x*		$-CH_2Cl$	15	78	232-234	$C_{18}H_{17}ClN_2OS$ (T-M)	3016(γ_{C-H}), 1669(γ_{CONH}), 743(γ_{C-Cl})	--	--

S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*xi*		$-CH_2Cl$	30	86	250-254	$C_{10}H_9ClN_2O_3S$ (M-C)	2863(γ_{C-H}), 1724($\gamma_{C=O}$), 1664(γ_{CONH}), 763(γ_{C-Cl})	--	--
V*xii*		$-CH_2Cl$	20	68	247-249	$C_{13}H_8BrClN_2OS$ (E-C)	2980(γ_{C-H}), 1655($\gamma_{C=O}$), 775(γ_{C-Cl})	--	--
V*xiii*		$-CH_2Cl$	30	60	240-242	$C_{11}H_7ClN_4O_2$ (E-C)	2896(γ_{C-H}), 1686(γ_{CONH}), 778(γ_{C-Cl}).	--	--
V*xiv*		$-CH_2Cl$	45	62	150-152	$C_{11}H_7ClN_2OS$ (E-C)	2980(γ_{C-H}), 1680(γ_{CONH}), 740(γ_{C-Cl})	--	--
V*xv*		$-CH_2Cl$	40	83	265-267	$C_{12}H_9ClN_2O_2S$ (E-C)	2978(γ_{C-H}), 1676(γ_{CONH}), 736(γ_{C-Cl})	--	--
V*xvi*		$-CH_2Cl$	50	76	275-277 (dec.)	$C_{12}H_{10}ClN_3OS$ (E-C)	3443, 3338 (γ_{NH}), 2946(γ_{C-H}), 1672(γ_{CONH}), 760(γ_{C-Cl}).	279(M^+), 253, 244, 230, 216	--
V*xvii*		$-CH_2Cl$	40	70	240-245	$C_{11}H_{11}ClN_2O_3$ (E-C)	3012(γ_{ArH}), 2888(γ_{CH2}), 1666(γ_{CONH}), 754(γ_{C-Cl})	254(M^+), 239, 219	--

S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*xviii*		$-CH_2CH_2Cl$	40	81	166-168	$C_{15}H_{13}ClN_2OS$ (E-C)	2837(γ_{C-H}), 1672(γ_{CONH}), 762(γ_{C-Cl})	--	2.40 (3H, s, C*H*$_3$), 3.06 (2H, t, *J* = 7, C*H*$_2$), 3.87 (2H, t, *J* = 7, C*H*$_2$), 7.06 (1H, s, C*H*), 7.15-7.45 (4H, m, Ar-*H*), 12.99 (1H, s, N*H*)
V*xix*		$-CH_2CH_2Cl$	60	86	200-202	$C_{10}H_{11}ClN_2OS$ (E-C)	2922(γ_{C-H}), 1666(γ_{CONH}), 758(γ_{C-Cl})	--	2.38 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 3.19 (2H, t, C*H*$_2$, *J* = 7.5), 3.97 (2H, t, C*H*$_2$, *J* = 7.2), 12.34 (1H, s, br, N*H*).
V*xx*		$-CH_2CH_2Cl$	45	90	268-270	$C_{14}H_{11}ClN_2OS$ (E-C)	2848(γ_{C-H}), 1670($\gamma_{C=O}$), 748(γ_{C-Cl})	--	3.14 (2H, t, C*H*$_2$, *J* = 7.2), 4.02 (2H, t, C*H*$_2$, *J* = 7.5), 7.30-7.50 (6H, m, 5*H* Ar-*H* and 1*H* at 6 position), 12.40 (1H, s, br, N*H*)
V*xxi*		$-CH_2CH_2Cl$	70	72	250-252	$C_{12}H_{13}ClN_2O_3S$ (M-C)	2960(γ_{C-H}), 1718($\gamma_{C=O}$) 1667(γ_{CONH})	--	1.40 (3H, t, *J* = 7.3, C*H*$_3$), 2.55 (3H, s, C*H*$_3$), 2.94 (3H, s, C*H*$_3$), 4.36 (2H, quartlet, *J* = 7.1, C*H*$_2$); 10.95 (1H, s, N*H*)
V*xxii*		$-CH_2CH_2Cl$	55	83	156-158	$C_{12}H_{13}ClN_2OS$ (D)	2920(γ_{C-H}), 1661(γ_{CONH})	--	1.77 (4H, s, 2 X C*H*$_2$ at 6 and 7), 2.30 (3H, s, C*H*$_3$ at 2), 2.70 (2H, s, C*H*$_2$ at 5), 2.83 (2H, s, C*H*$_2$ at 8), 7.07 (br, s, 1H, N*H* at 3)

S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*xxiii*		$-CH_2CH_2Cl$	40	91	200-202	$C_{10}H_9ClN_2O$ (M-C)	2979(γ_{C-H}), 1665(γ_{CONH}), 771(γ_{C-Cl})	--	3.18 (2H, t, C*H*$_2$, *J* = 6.3, 7.2), 4.06 (2H, t, C*H*$_2$ at 2, *J* = 6.3, 7.2), 7.44-8.07 (4H, m, Ar-*H*).
V*xxiv*		$-CH_2CH_2Cl$	75	63	260-262	$C_{13}H_{11}ClN_2O_2S$ (E-C)	2978(γ_{C-H}), 1676(γ_{CONH}), 736(γ_{C-Cl})	--	--
V*xxv*		$-CH_3$	40	80	97-99	$C_{14}H_{12}N_2OS$ (E-C)	2898(γ_{C-H}), 1667(γ_{CONH})	--	2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 7.04 (1H, s, *H*), 7.16-7.48 (4H, m, Ar-*H*), 11.90 (1H, s, N*H*).
V*xxvi*		$-CH_3$	55	70	102-104	$C_9H_{10}N_2OS$ (E-C)	2918(γ_{C-H}), 1665(γ_{CONH})	--	2.37 (3H, s, C*H*$_3$), 2.46 (3H, s, C*H*$_3$), 2.51 (3H, s, C*H*$_3$), 12.04 (br, s, 1H, N*H*)
V*xxvii*		$-CH_3$	45	77	235-237	$C_{13}H_{10}N_2OS$ (E-C)	2998(γ_{C-H}), 1667(γ_{CONH})	--	3.36 (3H, s, C*H*$_3$), 7.31-7.50 (5H, m, Ar-*H* and 1H at 6), 12.28 (1H, s, N*H*)
V*xxviii*		$-CH_3$	30	99	278-280	$C_{11}H_{12}N_2O_3S$ (E-C)	2960(γ_{C-H}), 1718($\gamma_{C=O}$) 1667(γ_{CONH})	--	1.40 (3H, t, *J* = 7.3, C*H*$_3$), 2.55 (3H, s, C*H*$_3$), 2.94 (3H, s, C*H*$_3$), 4.36 (2H, quartlet, *J* = 7.1, C*H*$_2$), 10.95 (1H, s, N*H*)

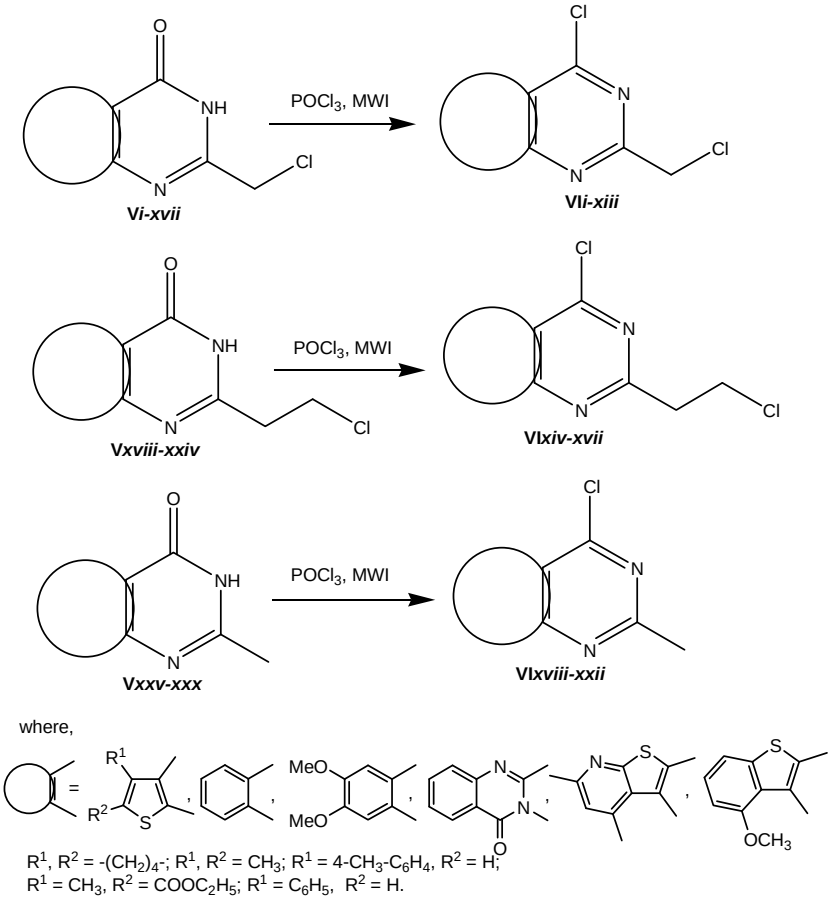
S. No.		X	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
V*xxix*		$-CH_3$	30	91	155-158	$C_{11}H_{12}N_2S$ (D)	2920(γ_{C-H}), 1661(γ_{CONH})	--	1.77 (4H, s, 2 X C*H*$_2$ at 6 and 7), 2.30 (3H, s, C*H*$_3$ at 2), 2.70 (2H, s, C*H*$_2$ at 5), 2.83 (2H, s, C*H*$_2$ at 8), 7.07 (br, s, 1H, N*H* at 3)
V*xxx*		$-CH_3$	40	71	237-239	$C_9H_8N_2O$ (E-C)	2918(γ_{C-H}), 1666(γ_{CONH})	--	2.50 (2H, s, C*H*$_3$), 7.38-7.74 (4H, m, Ar-*H*), 12.13 (1H, br, s, N*H*)

4.3 Synthesis of condensed 4-chloro-2-substitutedpyrimidines using MWI Irradiation

Heterocycles and especially pyrimidines and condensed pyrimidines are potentially bioactive compounds. The halogeno heterocycles have wide synthetic applicability, as important intermediates of metatheses. These halogeno substituents are mostly active towards nucleophillic displacements akin to those of aliphatic halogeno compounds or halogeno of nitro substituted aromatic compounds.

The chloro derivatives of various heterocycles are most widely employed compared to the corresponding bromo or iodo derivatives as there is not much difference in their reactivity and they are easily accessible.

Though, there are many reported methods for chlorination of heterocycles conventionally[1-3] there are only a few reports on MWI assisted chlorination of heterocycles. Some of them involve side chain chlorination and some involve ring chlorination.[4] To the best of our knowledge there are just two reports[5,6] on the chlorination of 4-hydroxypyrimidines to 4-chloropyrimidines. Thus, it was decided to use MWI assisted methodology for the conversion of condensed-4-hydroxypyrimidines to condensed-4-chloropyrimidines, which is one pot, solvent free, facile, eco-friendly and highly productive as well (Scheme 2).



Scheme 2

Conventionally, the chlorination of the above type of condensed 2-substituted-4-hydroxypyrimidines involves refluxing with excess of $POCl_3$ or PCl_3 alone or in combination with excess of PCl_5. Use of catalytic amount of 2^o amines or DMAP is also well known. The reaction time required generally ranges on an average from 2 to 12 hrs. The work up of the reaction mixture involves, removal of excess of chlorinating agent under reduced pressure almost to the last traces and neutralization of the HCl and H_3PO_4 formed. This involves excess of water which may sometimes render the chlorination product partially soluble in it and may require extraction of products using solvents like methylene dichloride. The neutralization needs to be under ice cold conditions to avoid the decomposition or the conversion of condensed-4-chloropyrimidines back to condensed-4-hydroxypyrimidines.

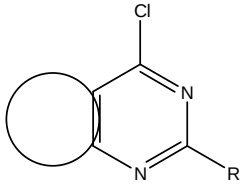
Herein, we are reporting remarkable improvisation over the conventional methodology wherein we have mainly circumvented,

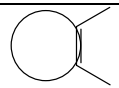
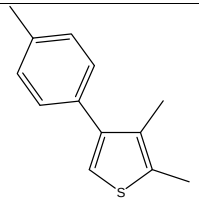
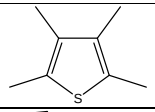
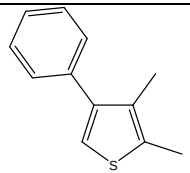
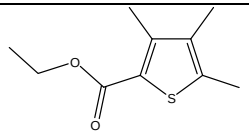
I. Prolonged reaction time from 2-12 hrs to just few minutes,
II. Usage of excess of chlorinating reagent required to form a homogenous reaction mixture (just 2-3 times excess of volumes of starting material).

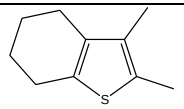
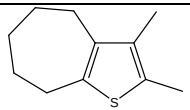
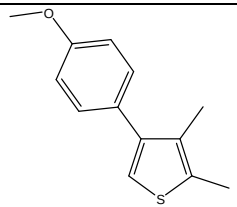
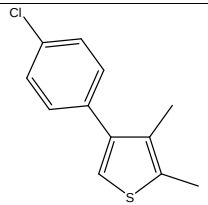
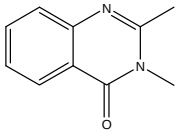
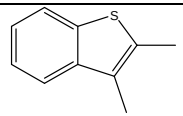
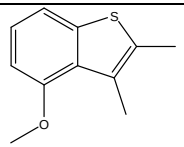
Further, the reaction is conducted under very mild conditions (80W). Usage of minimal quantity of the chlorinating agent helps in very quick and simpler workup like pouring the reaction mixture over crushed ice and using of minimum quantity of neutralizing agent (pinch full of solid sodium bicarbonate). The resultant product, condensed 4-chloro-2-substitutedpyrimidine, separates out in high yield (90-99%) and purity more than (>95% by TLC). Thus, this methodology offers a very simple rapid high yielding, eco-friendly process for the chlorination of condensed-4-hydroxypyrimidines to condensed-4-chloropyrimidines.

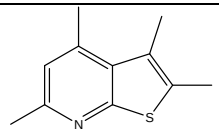
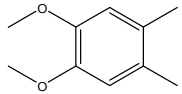
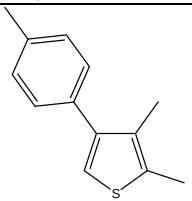
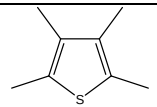
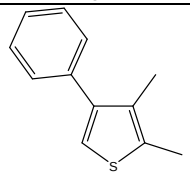
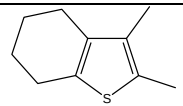
Using this novel microwave assisted green synthesis, following 4-chloro 2-substituted condensed pyrimidine were synthesized.

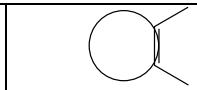
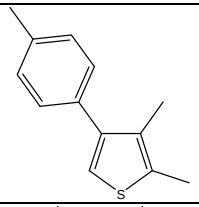
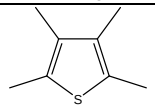
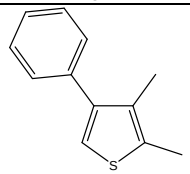
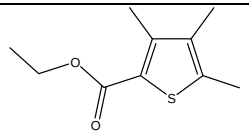
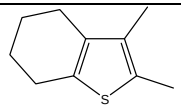
Table 39: Physical data of condensed 4-chloro 2-substitutedpyrimidines synthesized using MWI irradiation (VI*i-xxii*)



S. No.		R	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
VI*i*		$-CH_2Cl$	4	90	85-87	$C_{14}H_{10}Cl_2N_2S$	2979(γ_{C-H}), 1458(γ), 722(γ_{C-Cl})	--	2.42 (3H, s, C*H*$_3$), 4.82 (2H, s, C*H*$_2$Cl), 7.25-7.51 (5H, m, Ar-*H* and H at 6)
VI*ii*		$-CH_2Cl$	5	92	116-118	$C_9H_8Cl_2N_2S$	2980(γ_{C-H}), 677(γ_{C-Cl})	--	2.45 (3H, s, C*H*$_3$), 2.50 (3H, s, CH_3), 4.82 (2H, s, *CH*$_2$Cl)
VI*iii*		$-CH_2Cl$	4	77	68-70	$C_{13}H_8Cl_2N_2S$	2932(γ_{C-H}), 1510(γ_{C-C})	--	4.82 (2H, s, *CH*$_2$Cl), 7.25-7.62 (4H, m, Ar-*H* and 1H at 6)
VI*iv*		$-CH_2Cl$	6	75	135-137	$C_{11}H_{10}Cl_2N_2O_2S$	1718($\gamma_{C=O}$), 1534(γ_{C-C}), 762(γ_{C-Cl})	305(M^+), 289, 276, 259, 241, 232, 244, 197	1.43 (3H, t, CH_2CH_3, *J* = 7.2 & 6.9), 3.06 (3H, s, C*H*$_3$), 4.42 (2H, q, *CH*$_2$$CH_3$, *J* = 6.9 & 7.2), 4.78 (2H, s, *CH*$_2$Cl)

S. No.		R	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
VI*v*		$-CH_2Cl$	4	70	80-82	$C_{11}H_{10}Cl_2N_2S$	2941(γ_{C-H}), 1447(γ_{C-C}), 736(γ_{C-Cl})	273(M^+), 244, 239, 209, 174, 140	1.99 (4H, s, C*H*$_2$ at 6 & 7), 2.57 (3H, s, C*H*$_2$ at 5), 2.95 (2H, s, C*H*$_2$ at 8), 4.80 (2H, s, *CH*$_2$Cl)
VI*vi*		$-CH_2Cl$	5	85	75-77	$C_{12}H_{12}Cl_2N_2S$	2923(γ_{C-H}), 1658(γ_{C-C}), 755 (γ_{C-Cl})	--	--
VI*vii*		$-CH_2Cl$	6	90	122-124	$C_{14}H_{10}Cl_2N_2OS$	3001(γ_{C-H}), 1608(γ_{C-C}), 787(γ_{C-Cl})	--	3.90 (3H, s, OC*H*$_3$), 4.92 (2H, s, C*H*$_2$ at 2), 6.95-7.55 (5H, m, Ar-*H* & 1*H* at 6)
VI*viii*		$-CH_2Cl$	4	70	175-177	$C_{13}H_7Cl_3N_2S$	3030(γ_{C-H}), 1543(γ_{C-C}), 787 (γ_{C-Cl})	--	2.71 (3H, s, C*H*$_3$), 4.90 (2H, s, C*H*$_2$Cl), 7.20-7.57 (5H, m, Ar-*H* & 1*H* at 6)
VI*ix*		$-CH_2Cl$	10	88	90-92	$C_{11}H_6Cl_2N_4O$	3046(γ_{C-H}), 1608(γ_{C-C}), 756(γ_{C-Cl})	--	--
VI*x*		$-CH_2Cl$	12	76	220-222	$C_{11}H_6Cl_2N_2S$	3040(γ_{C-H}), 1547(γ_{C-C}), 768(γ_{C-Cl})	--	--
VI*xi*		$-CH_2Cl$	8	80	180-182	$C_{12}H_8Cl_2N_2OS$	2980(γ_{C-H}), 1534(γ_{C-C}), 718(γ_{C-Cl})	--	--

S. No.		R	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
VI*xii*		$-CH_2Cl$	10	86	116-118	$C_{12}H_9Cl_2N_3S$	2990(γ_{C-H}), 1629(γ_{C-C}), 730(γ_{C-Cl})	--	--
VI*xiii*		$-CH_2Cl$	6	90	162-164	$C_{11}H_{10}Cl_2N_2O_2$	2919 (γ_{C-H}), 1506(γ_{C-C}), 743(γ_{C-Cl})	--	--
VI*xiv*		$-CH_2CH_2Cl$	5	81	160-162	$C_{15}H_{12}Cl_2N_2S$	2923(γ_{C-H}), 1496(γ_{C-C}), 794(γ_{C-Cl})	--	--
VI*xv*		$-CH_2CH_2Cl$	4	70	40-42	$C_{10}H_{10}Cl_2N_2S$	1478(γ_{C-C}), 841(γ_{C-Cl})	--	--
VI*xvi*		$-CH_2CH_2Cl$	6	67	>300	$C_{14}H_{10}Cl_2N_2S$	2940 (γ_{C-H}), 1553(γ_{C-C}), 759(γ_{C-Cl})	--	--
VI*xvii*		$-CH_2CH_2Cl$	4	90	62-64	$C_{12}H_{12}Cl_2N_2S$	2939 (γ_{C-H}), 1528(γ_{C-C}), 735(γ_{C-Cl})	287(M^+), 286(M-1), 253, 251, 225, 209	1.94 (4H, s, CH_2 at 6 & 7), 2.92 (2H, s, CH_2 at 5), 3.05 (2H, s, CH_2 at 8), 3.42 (2H, t, CH_2CH_2Cl, J = 6.9 & 7.1), 4.05 (2H, t, CH_2CH_2Cl, J = 6.9 & 7.1)

S. No.		R	Time of MWI Heating (Min)	Yield (%)	M.P.	Mol. formula (Solv. of crystn.)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) ($CDCl_3$)
VI*xviii*		$-CH_3$	3	65	97-99	$C_{14}H_{11}ClN_2S$	1553(γ_{C-C}), 791(γ_{C-Cl})	--	2.47 (3H, s, C*H*$_3$), 4.82 (2H, s, C*H*$_2$ at 2), 7.18-7.50 (5H, m, 4-Ar-*H* and 1*H* at 6)
VI*xix*		$-CH_3$	4	90	102-104	$C_9H_9ClN_2S$	1560(γ_{C-C}), 841(γ_{C-Cl})	--	--
VI*xx*		$-CH_3$	6	90	235-237	$C_{13}H_9ClN_2S$	2932(γ_{C-H}), 1510(γ_{C-C}), 818(γ_{C-Cl})	--	--
VI*xxi*		$-CH_3$	4	90	280-282	$C_{11}H_{11}ClN_2O_2S$	1718(γ_{C-O}), 1534(γ_{C-C})	--	2.43 (3H, t, C*H*$_3$CH$_2$COO, *J* = 6.9 & 7.1), 3.05 (3H, s, C*H*$_3$ at 5), 4.35 (2H, q, CH$_3$C*H*$_2$COO, *J* = 6.9, 7.2), 4.82 (2H, s, C*H*$_2$ at 2)
VI*xxii*		$-CH_3$	5	90	157-159	$C_{11}H_{11}ClN_2S$	2939(γ_{C-H}), 1413($\gamma_{C=C}$)	--	--

4.4 Spectral Discussion:

4.4.1. 2-Substituted condensed pyrimidin-4(3*H*)-ones

The 2-chloromethylthienopyrimidine are colorless to buff white colored solid, with high melting points generally above 240^{o}C. These compounds are soluble in mixture of chloroform and methanol and hot DMF and insoluble in methanol, hexane or ethanol. The 2-chloroethylthienopyrimidine and 2-methylthienopyrimidine are buff white to slight yellow coloured solids with gernally high melting point.

Infra Red (IR) spectra

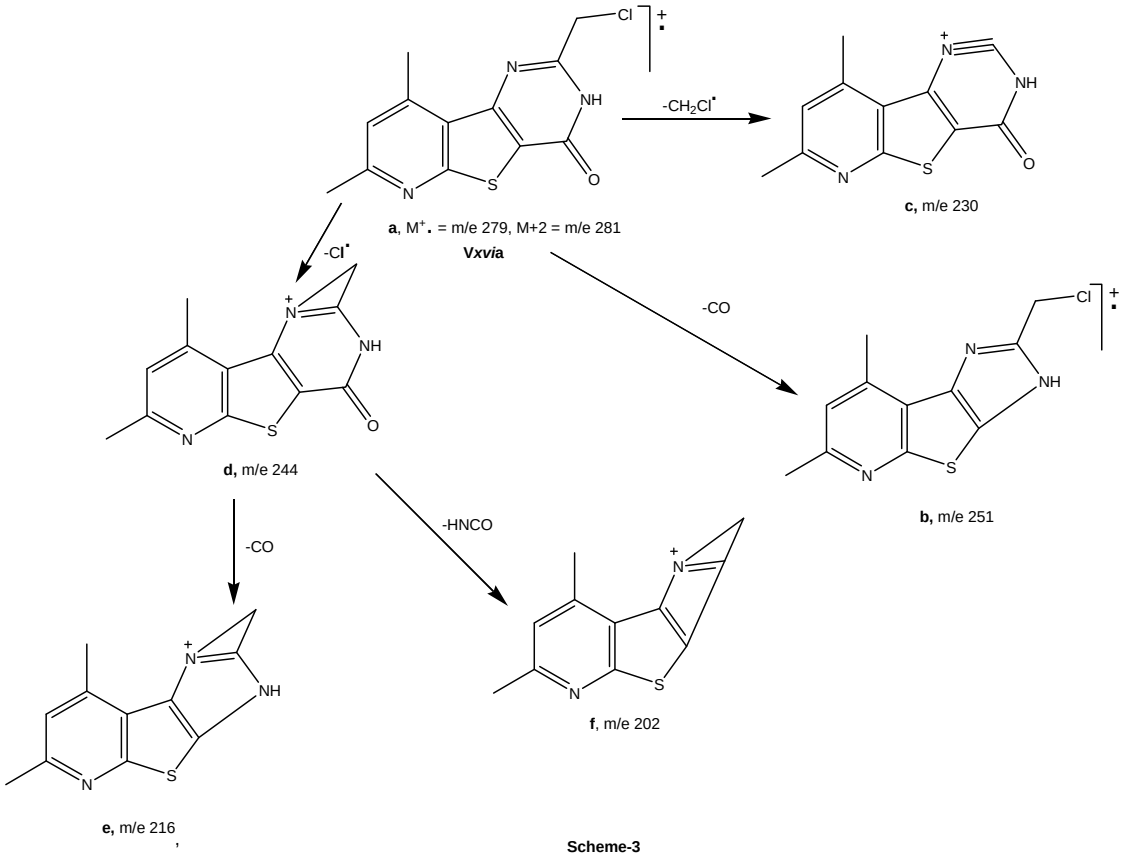
IR spectra of 2-chloromethylthienopyrimidines, 2-chloroethylthienopyrimidine and 2-methylthienopyrimidines exhibit multiple bands, of medium intensity around 3200-3100 cm^{-1} due to asymmetric and symmetric N-H stretching vibrations. Intense absorption bands observed in all these spectras around 1680-1650 may be due to N-H deformation vibrations. The IR spectra of ethyl 2-substituted-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine-6-carboxylates exhibited a strong absorption band around 1730-1720 cm^{-1} due to C=O stretching.

The ^{1}H NMR spectra

The ^{1}H NMR spectra of 2-chloromethylthienopyrimidines, 2-chloroethylthieno-pyrimidine and 2-methylthienopyrimidine were taken in $CDCl_3$. All the compounds showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons of the chloromethyl linkage appear as a singlet at around 4.4 to 4.6 ppm. Since this methylene group is attached to electronegative atom, the proton signal appear downfield then the normal position. In 2-chloroethylpyrimidines, characteristic triplets were observed at 3 to 4 ppm. The 2-methyl protons were observed above 2 ppm in the spectra due to presence of two nitrogen atoms of the pyrimidine ring system. The NH proton present in all the compounds at the 3 position of the pyrimidine ring is observed as a singlet between 10 to 13 ppm. The aromatic protons were observed as a multiplet at around 7-8 ppm.

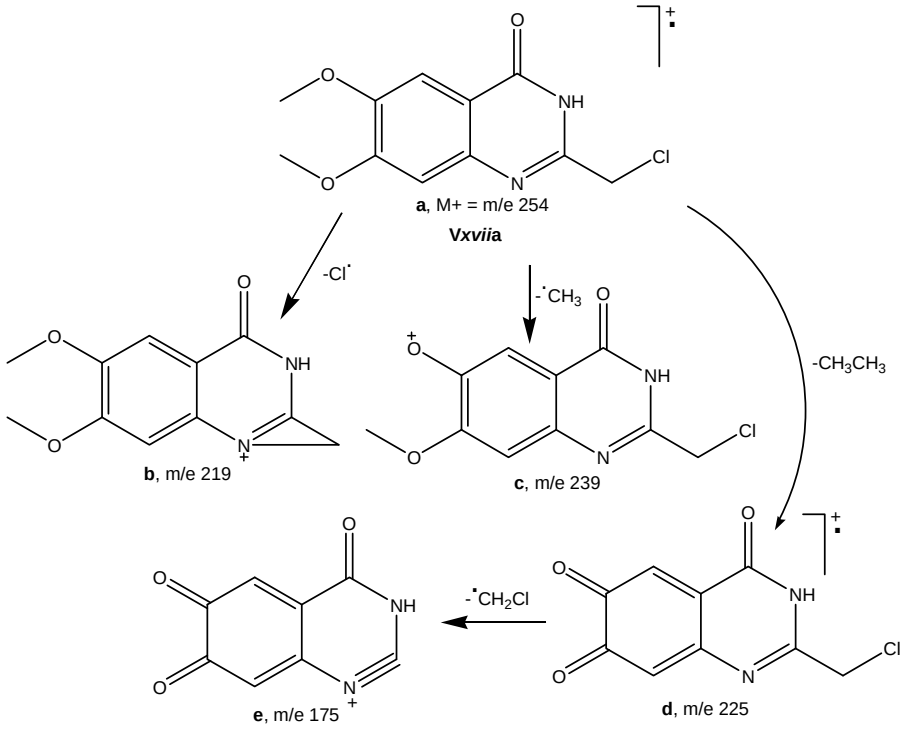
The Mass spectra

The fragmentation pattern of the synthesized compounds 2-chloromethylthieno-pyrimidines, under electron impact ionization has been studied. Many prominent fragment ion peaks were revealed in the mass spectra of these compounds. The mass spectrum of compound **V*xvi*** showed the molecular ion ($M^{+\cdot}$) peak (**a)** corresponding to the molecular weight and (M+2) due to presence of ^{37}Cl isotope. The major mode of fragmentation is loss of chloride ion from the molecular ion (**a)** to give daughter ion (**d)** m/e 244. The daughter ion (**d**) loses neutral CO molecule to give fragment (**e**) at m/e 216. The daughter ion (**d**) also loses neutral HNCO fragment to give fragment (**f**) at m/e 202. The molecular ion (**a**) also loses neutral CO and CH_2Cl radical to give daughter ion (**b**) m/e 251 and (**c**) m/e 230, respectively. The fragmentation pattern of the compound **V*xvi*** is given in the Scheme 3.



Scheme-3

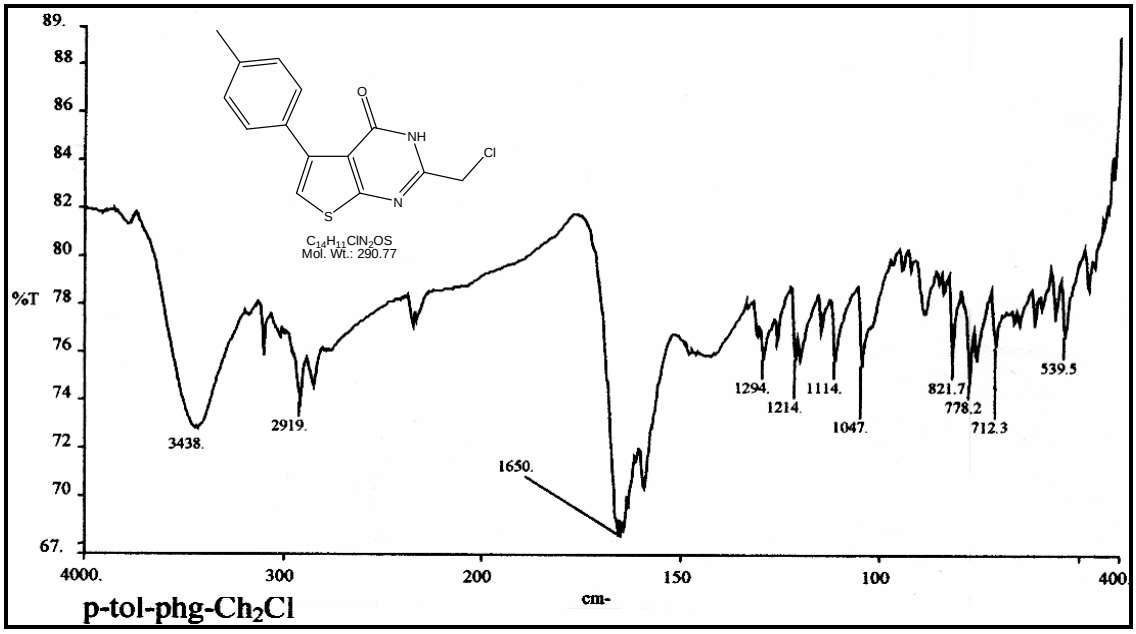
The mass spectrum of compound **V*xvii*** showed the molecular ion ($M^{+\cdot}$) peak (**a**), m/e 254, is corresponding to the molecular weight of the compound. The major mode of fragmentation is loss of chloride ion from the molecular ion (**a)** to give daughter ion (**b)** m/e 219. The second daughter ion (**c**) m/e 239 was obtained by the loss of methyl radical from the molecular ion (**a**). Alternatively, a neutral ethyl molecule is ejected out from the molecular ion to yield the fragment (**d**) m/e 225. This further loses CH_3Cl as a neutral molecule to afford fragment ion (**e**) at m/e 175. The fragmentation pattern of the compound **V*xvii*** is given in the Scheme 4.



Scheme-4

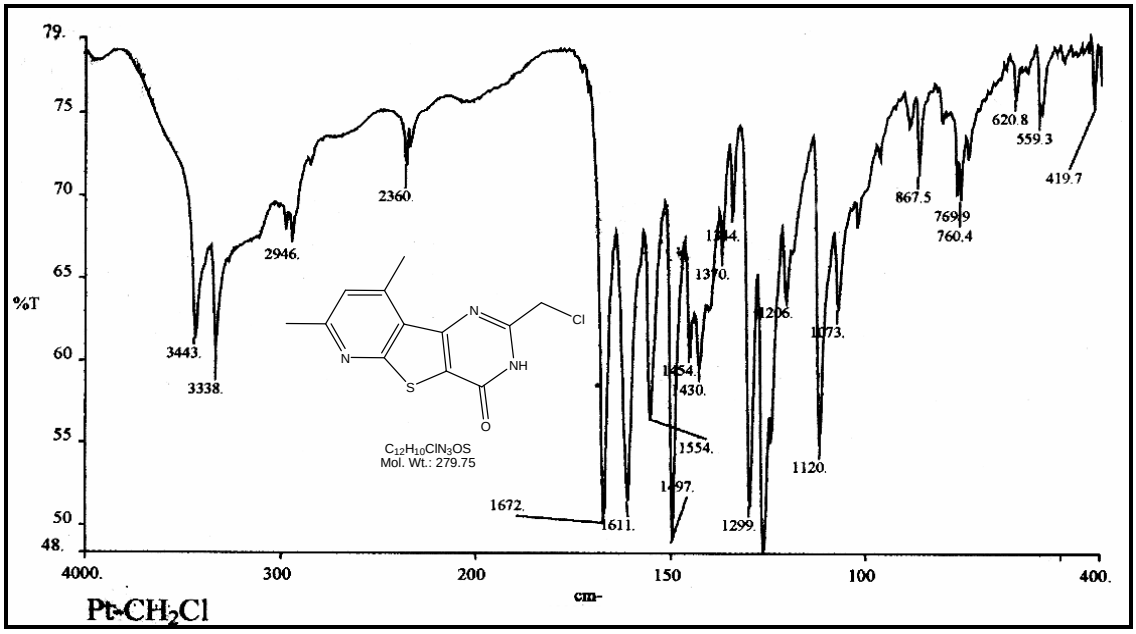
Specimen IR spectra of some 2-chloromethylthienopyrimidines

1. IR spectrum of 2-(chloromethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*i*)**



IR (KBr) cm^{-1}: 3438, (γ_{NH}), 2919(γ_{C-H}), 1650($\gamma_{C=O}$), 712(γ_{C-Cl})

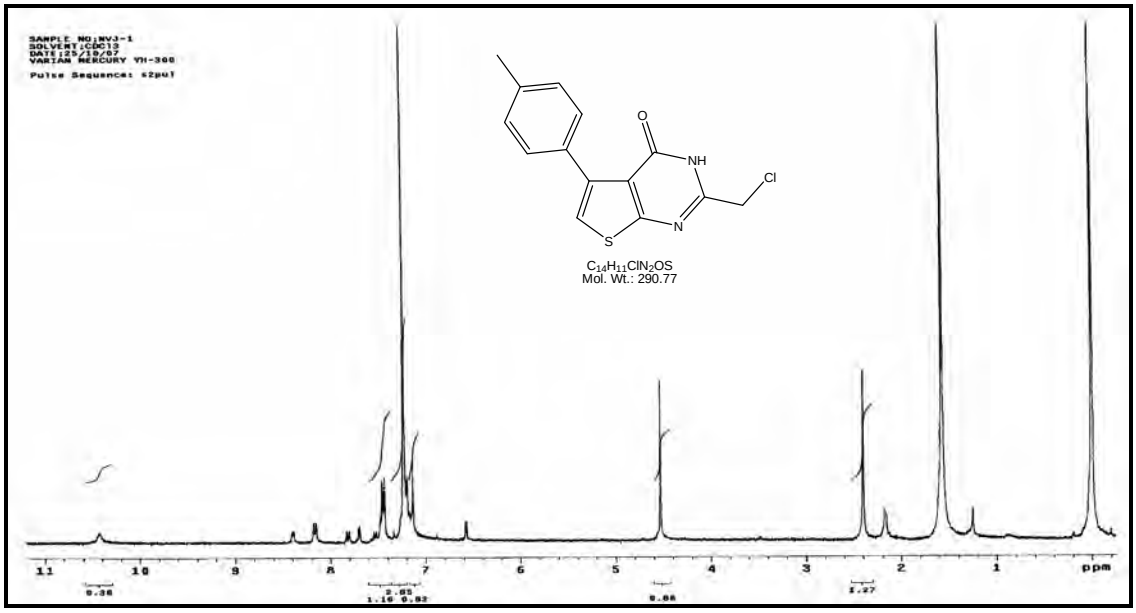
2. IR spectrum of 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triaza-fluoren-8-one **(V*xvi*)**



IR (KBr) cm^{-1}: 3443, 3338 (γ_{NH}), 2946(γ_{C-H}), 1672(γ_{CONH}), 760(γ_{C-Cl}).

Specimen ^{1}H NMR spectra of some 2-chloromethylthienopyrimidines

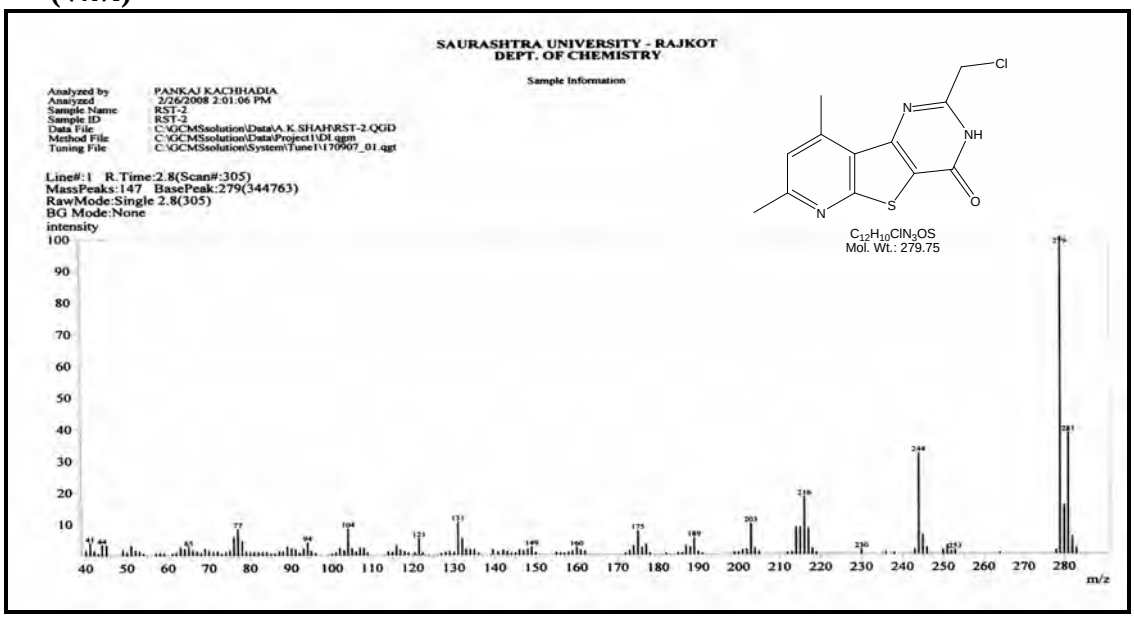
3. ^{1}H NMR spectrum of 2-(chloromethyl)-5-(4-methylphenyl)thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(V*i*)**



^{1}H NMR ($CDCl_3$)δppm: 2.39 (3H, s, C*H*$_3$), 4.53 (2H, s, CH_2), 7.13 (1H, s, C*H*), 7.19-7.46 (4H, m, Ar-*H*), 10.43 (1H,s, N*H*).

Specimen Mass spectra of some 2-chloromethylthienopyrimidines

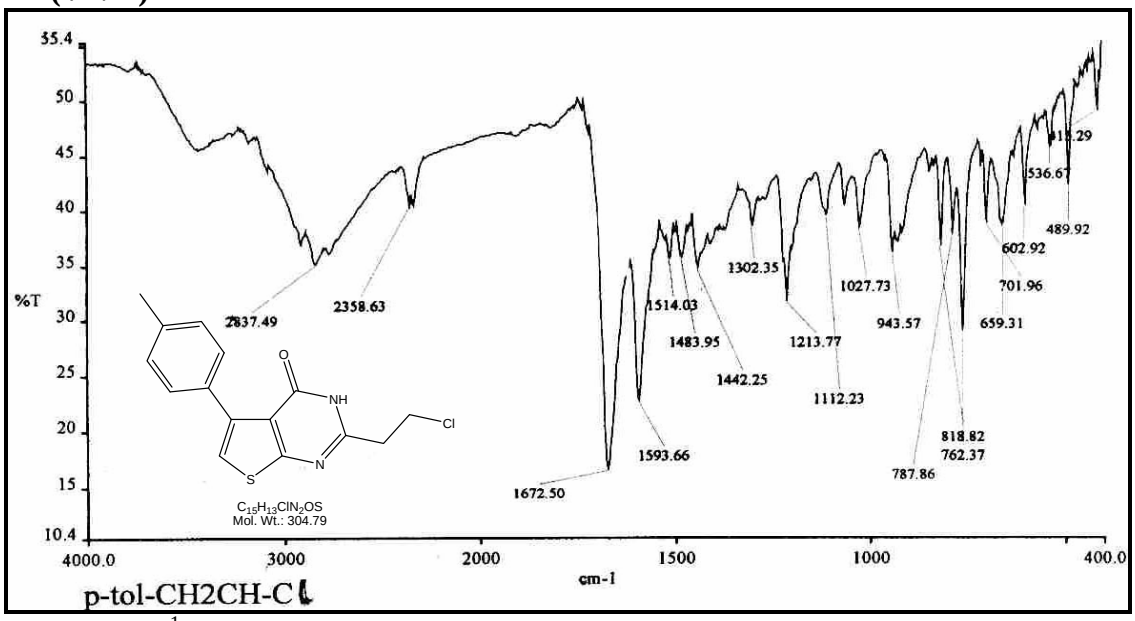
4. Mass spectrum of 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triaza-fluoren-8-one **(V*xvi*)**



MS m/e: 281(M+1), 279(M^+), 244, 216.

Specimen IR spectra of some 2-chloroethylthienopyrimidine

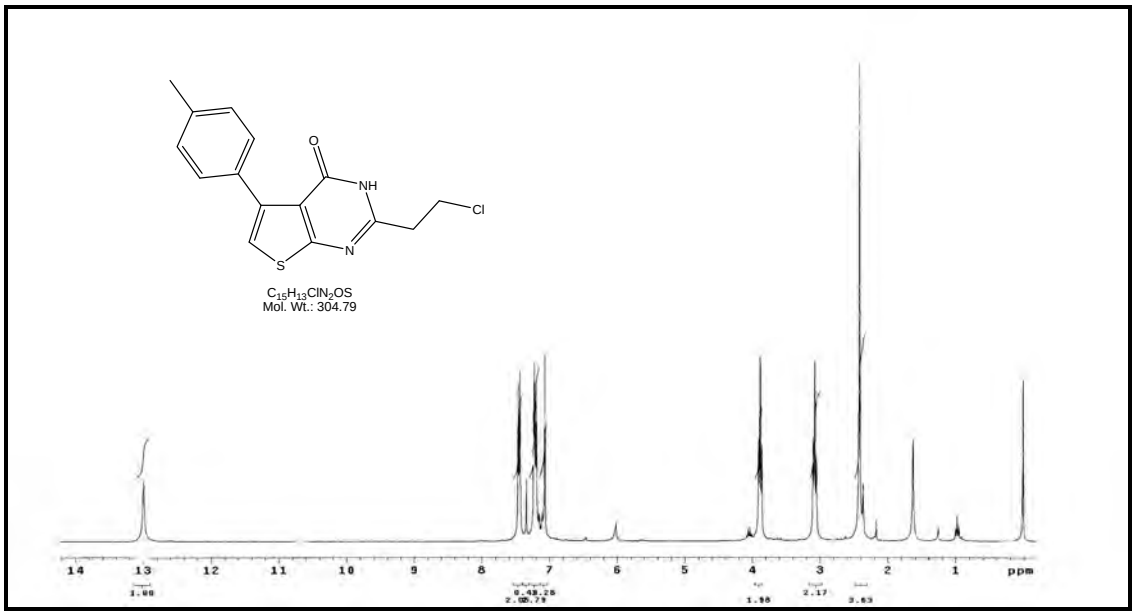
5. IR spectrum of 2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xviii*)**



IR (KBr) cm^{-1}: 2837(γ_{C-H}), 1672(γ_{CONH}), 762(γ_{C-Cl})

Specimen IR spectra of some 2-chloroethylthienopyrimidine

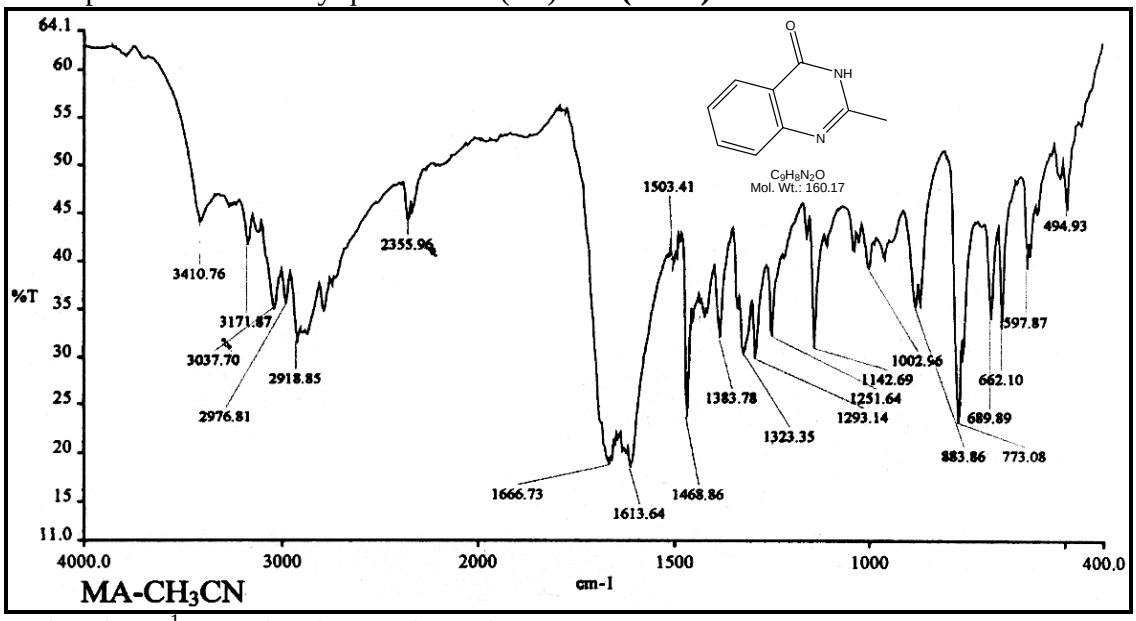
6. ^{1}H NMR spectrum of 2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **(V*xviii*)**



^{1}H NMR ($CDCl_3$)δppm: 2.40 (3H, s, C*H*$_3$), 3.06 (2H, t, *J* = 7, C*H*$_2$), 3.87 (2H, t, *J* = 7, C*H*$_2$), 7.06 (1H, s, C*H*), 7.15-7.45 (4H, m, Ar-*H*), 12.99 (1H, s, N*H*).

Specimen IR spectra of some 2-methyl condensed pyrimidine

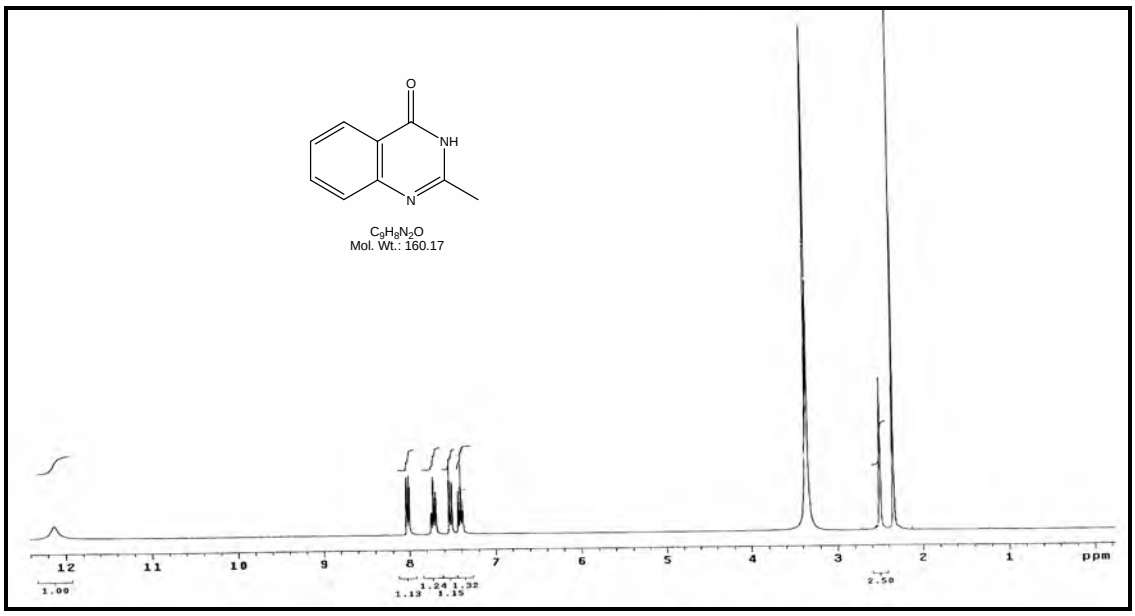
7. IR spectrum of 2-methylquinazolin-4(3*H*)-one **(V*xxx*)**



IR (KBr) cm^{-1}: 2918($\gamma_{C\text{-}H}$), 1666(γ_{CONH}).

Specimen ^{1}H NMR spectra of some 2-methylthienopyrimidine

8. ^{1}H NMR spectrum of 2-methylquinazolin-4(3*H*)-one **(V*xxx*)**



^{1}H NMR ($CDCl_3$)δppm: 2.50 (2H, s, C*H*$_3$), 7.38-7.74 (4H, m, Ar-*H*), 12.13 (1H, br, s, N*H*).

4.4.2. Condensed 4-chloro-2-substitutedpyrimidines

The condensed 4-chloro-2-substitutedpyrimidines are brown or slightly dark colored solid, with low melting points generally below 200^{o}C. These compounds are soluble in chloroform and insoluble in ethanol, methanol or hexane.

Infra Red (IR) spectra

IR spectra of 4-chloro-2-chloromethylthienopyrimidines, 4-chloro-2-chloroethylthieno-pyrimidine and 4-chloro-2-methylthienopyrimidine did not exhibits bands due to asymmetric and symmetric N-H stretching vibrations along with absence of absorption bands around 1680-1650 due to N-H deformation vibrations. The IR spectra of ethyl 2-substituted-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine-6-carboxylates exhibited a strong absorption band around 1730-1720 cm^{-1} due to C=O stretching.

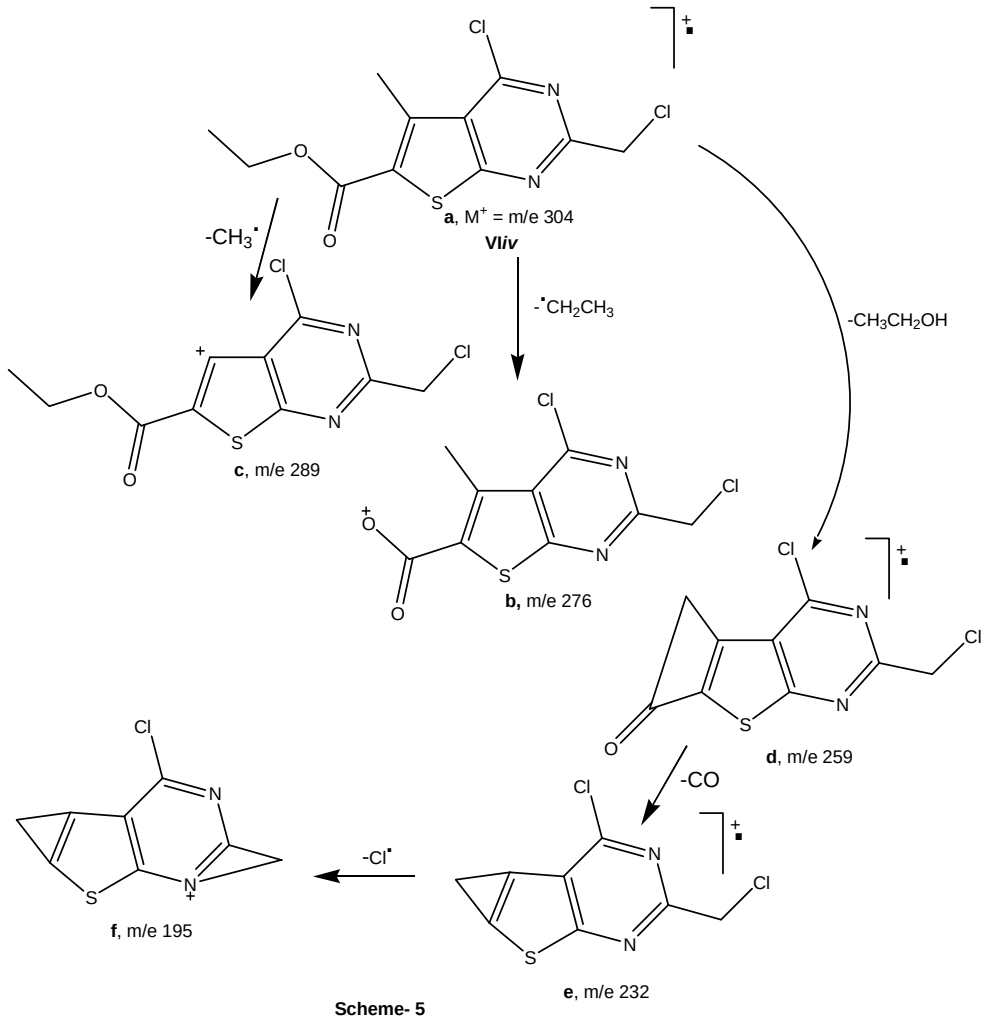
The ^{1}H NMR spectra

The ^{1}H NMR spectra of 4-chloro-2-chloromethylthienopyrimidines, 4-chloro-2-chloroethylthienopyrimidine and 4-chloro-2-methylthienopyrimidine were taken in $CDCl_3$. All the compounds showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons of the chloromethyl linkage appear as a singlet at around 4.4 to 4.6 ppm. Since this methylene group is attached to electronegative atom, the proton signal appear downfield then the normal position. In 2-chloroethylpyrimidines, characteristic triplets were observed at 3 to 4 ppm. The 2-methyl protons were observed above 2 ppm in the spectra due to presence of two nitrogen atoms of the pyrimidine ring system. The aromatic protons were observed as a multiplet at around 7-8 ppm.

The Mass spectra

The fragmentation pattern of the synthesized compounds (4-chloro-2-chloromethyl-thienopyrimidines), under electron impact ionization has also been studied. Many prominent fragment ion peaks are revealed in the mass spectra of these compounds. The

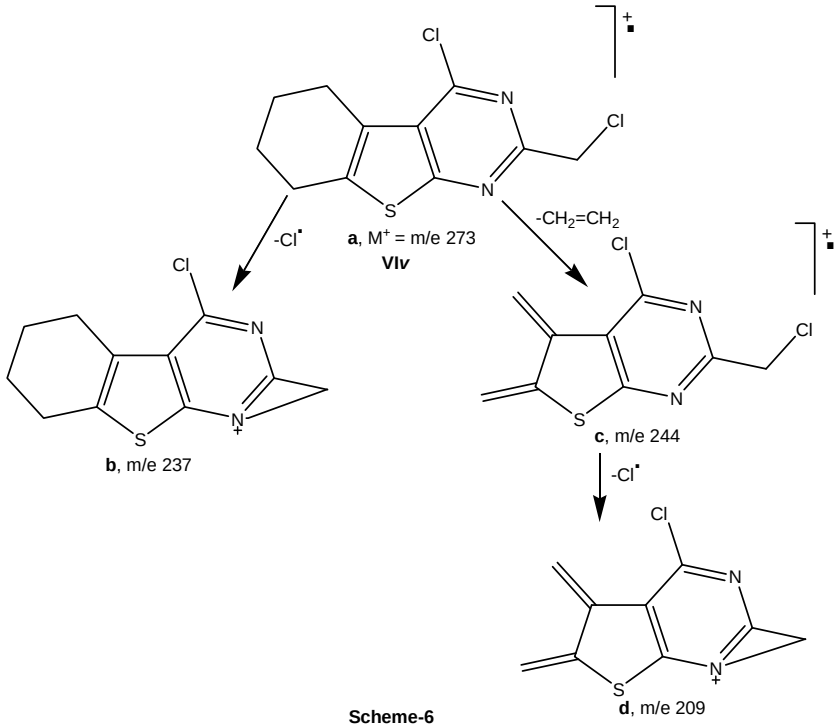
mass spectrum of compound, **VI*iv*** showing the molecular ion (**a**) at m/e 304, is corresponding to its molecular weight. The major mode of fragmentation is loss of ethyl fragment from the molecular ion (**a**) to give the daughter ion (**b**) m/e 276. The molecular ion (**a**) also loses 5-methyl group to give second daughter ion (**c**), m/e 289. The molecular ion (**a**) loses a neutral molecule of ethanol to give a third daughter ion (**d**) m/e 259. The fragment (**d**) loses neutral molecule of CO to give fragment (**e**) m/e 232, which further ejects one of the chloride ion to give fragment (**f**) m/e 195. The fragmentation pattern of the compound **VI*iv***, is given in the Scheme 5.



Scheme- 5

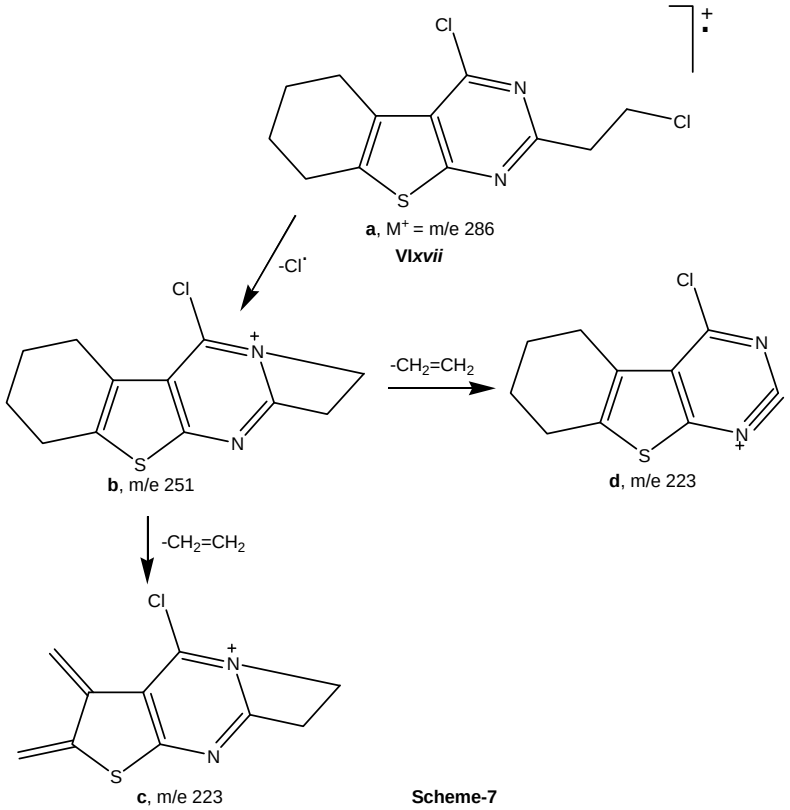
The mass spectrum of compound, **VI*v*** showing the molecular ion (**a**) at m/e 273 and is corresponding to its molecular weight. The major mode of fragmentation is loss of ethyl fragment from the molecular ion (**a**) to give the daughter ion (**c**) m/e 244. The daughter

ion (**c**) loses chloride ion to give the fragment (**d**) m/e 209. The molecular ion (**a**) also loses chloride ion to give second daughter ion (**b**) m/e 237. The fragmentation pattern of the compound **VI*v***, is given in the Scheme 6.



Scheme-6

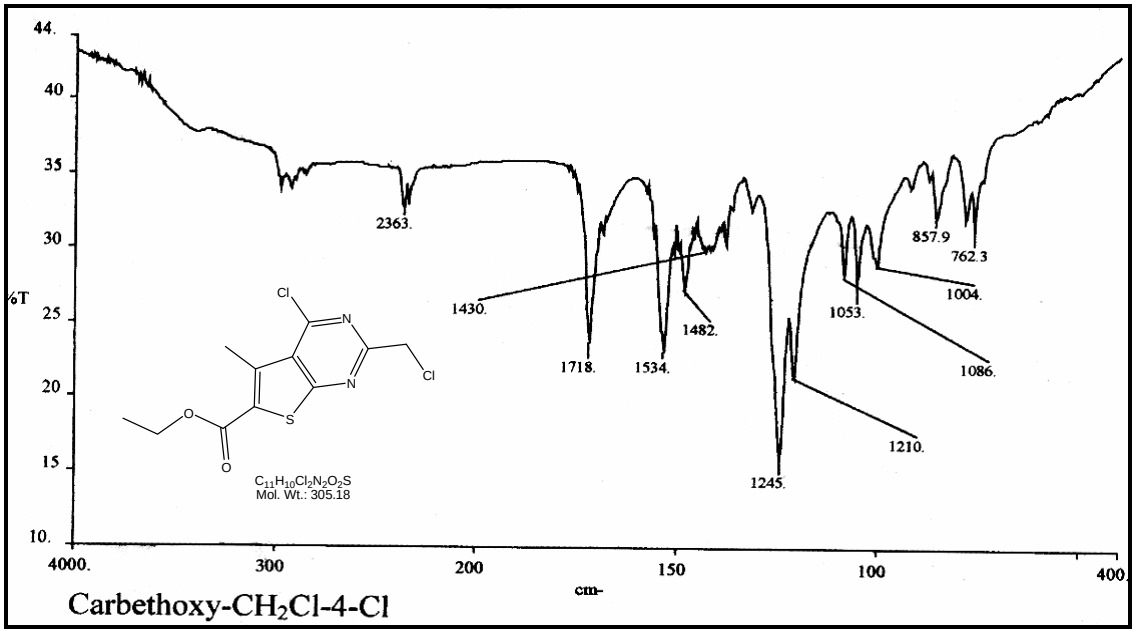
The mass spectrum of compound, **VI*xvii*** showing the molecular ion (**a**) at m/e 286 and is corresponding to its molecular weight. The major mode of fragmentation is loss of chloride ion from the molecular ion (**a**) to give the daughter ion (**b**) m/e 251. The daughter ion (**b**) loses ethyl fragment either from cyclohexane ring or from the C2 of the pyrimidine ring to give fragment (**c**) and (**d**), m/e 223 respectively. The fragmentation pattern of the compound **VI*xvii***, is given in the Scheme 7.


Cl
N
Cl
N
S
+
a, M+ = m/e 286
VIxvii
-Cl·
Cl
N
+
N
S
b, m/e 251
-CH2=CH2
Cl
N
+
N
S
c, m/e 223
-CH2=CH2
Cl
N
N
+
S
d, m/e 223

Scheme-7

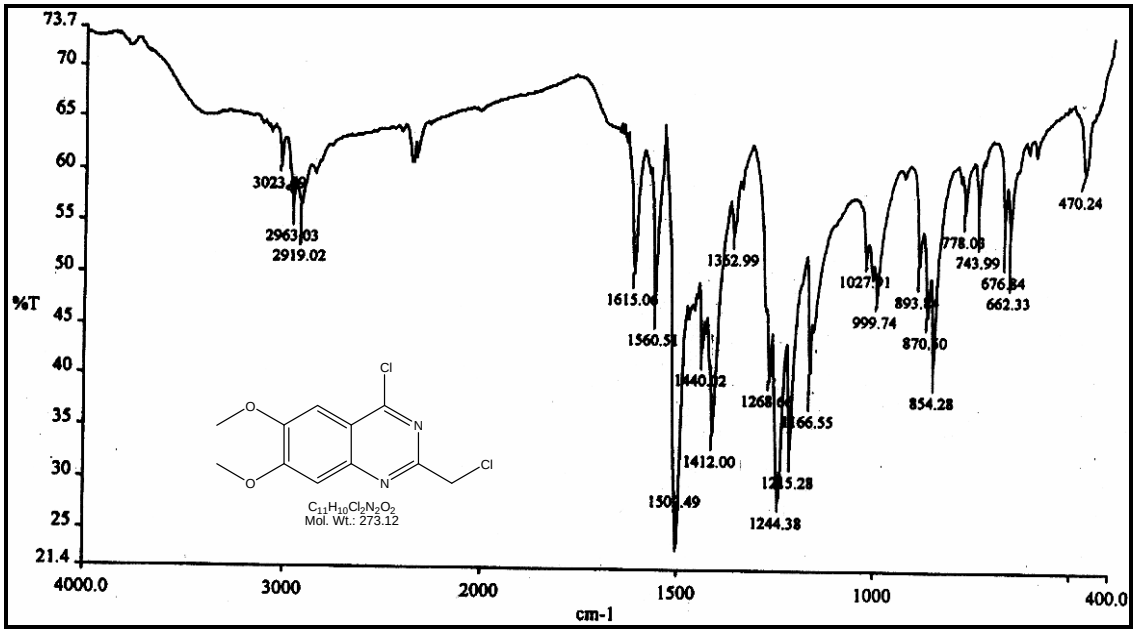
Specimen IR spectra of some 4-chloro-2-chloromethylthienopyrimidine

9. IR Spectra of ethyl 4-chloro-2-(chloromethyl)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **(VI*iv*)**



IR (KBr) cm^{-1}: 1718($\gamma_{C=O}$), 1534(γ_{C-C}), 762(γ_{C-Cl})

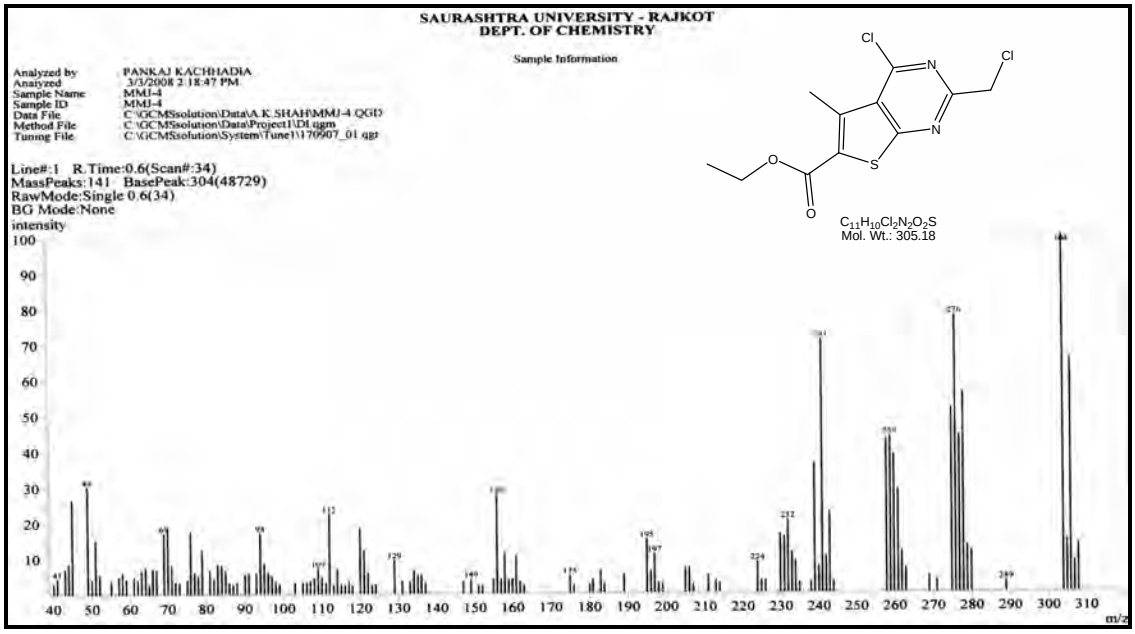
10. IR Spectra of 4-chloro-2-(chloromethyl)-6,7-dimethoxyquinazoline **(VI*xiii*)**



IR (KBr) cm^{-1}: 2919 (γ_{C-H}), 1506(γ_{C-C}), 743(γ_{C-Cl})

Specimen Mass spectra of some 4-chloro-2-chloromethylthienopyrimidines

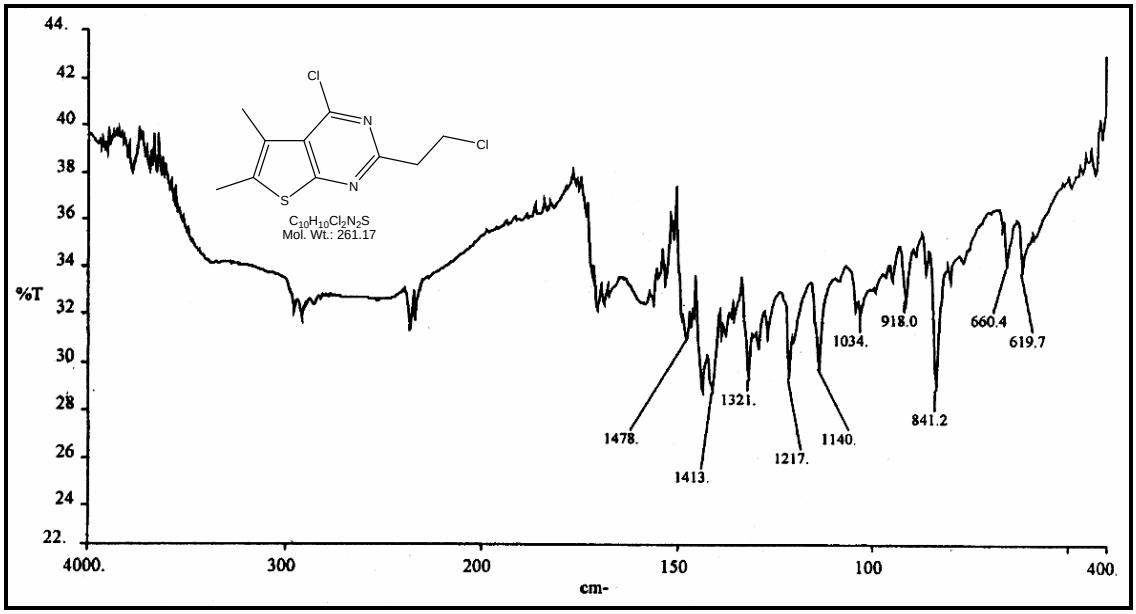
11. Mass spectrum of ethyl 4-chloro-2-(chloromethyl)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **(VI*iv*)**



MS m/e : 305 (M^+), 289, 276, 259, 241, 232, 244, 197.

Specimen IR spectra of some 4-chloro-2-(2-chloroethyl)-thienopyrimidines

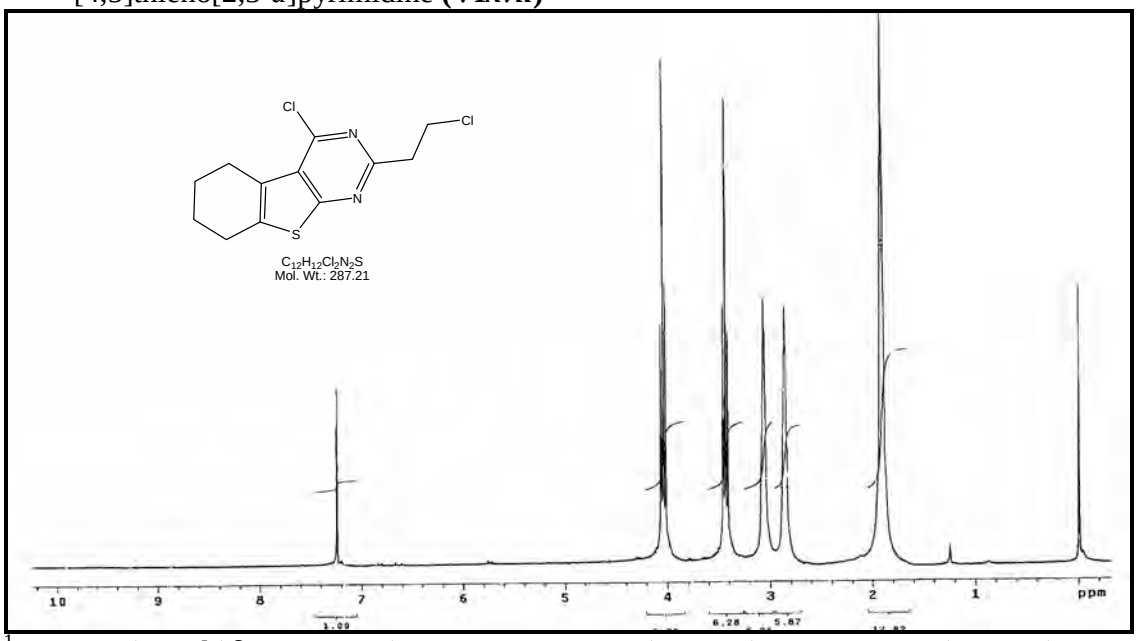
12. IR Spectra of 4-chloro-2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidine **(VI*xv*)**



IR (KBr) cm^{-1}: 1478(γ_{C-C}), 841(γ_{C-Cl}).

Specimen ^{1}H NMR spectra of 4-chloro-2-(2-chloroethyl)-thienopyrimidines

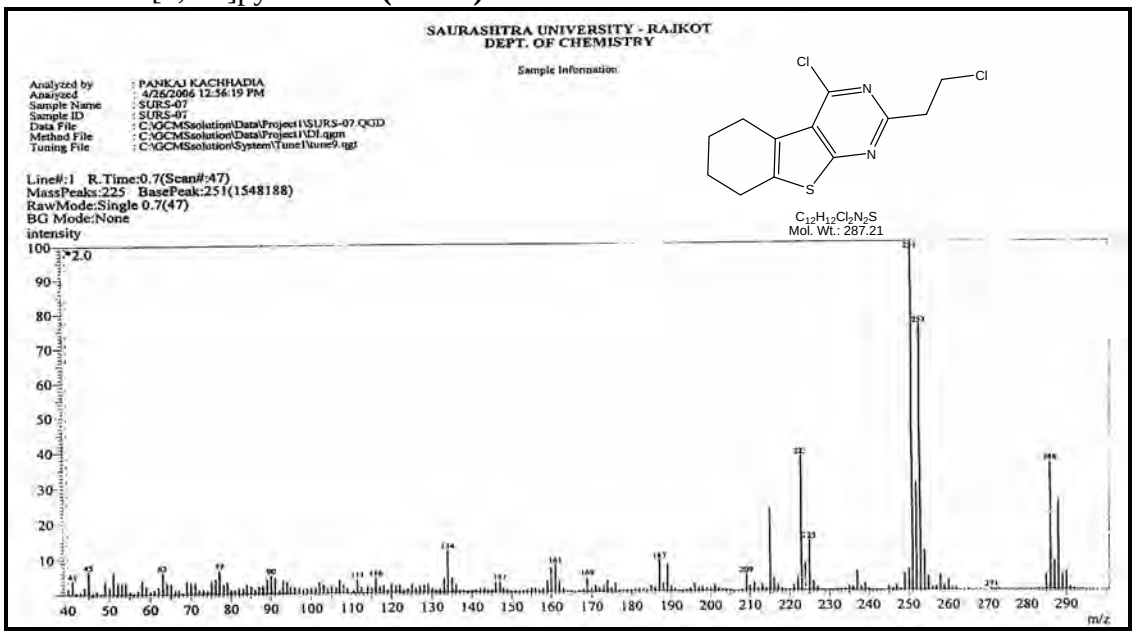
13. ^{1}H NMR spectrum of 4-chloro-2-(2-chloroethyl)-5,6,7,8-tetrahydro-benzo-[4,5]thieno[2,3-*d*]pyrimidine **(VI*xvii*)**



^{1}H NMR ($CDCl_3$)δppm: 1.94 (4H, s, CH_2 at 6 & 7), 2.92 (2H, s, CH_2 at 5), 3.05 (2H, s, CH_2 at 8), 3.42 (2H, 7, CH_2CH_2Cl, J = 6.9 & 7.1), 4.05 (2H, 7, CH_2CH_2Cl, J = 6.9 & 7.1).

Specimen Mass spectra of 4-chloro-2-(2-chloroethyl)-thienopyrimidine.

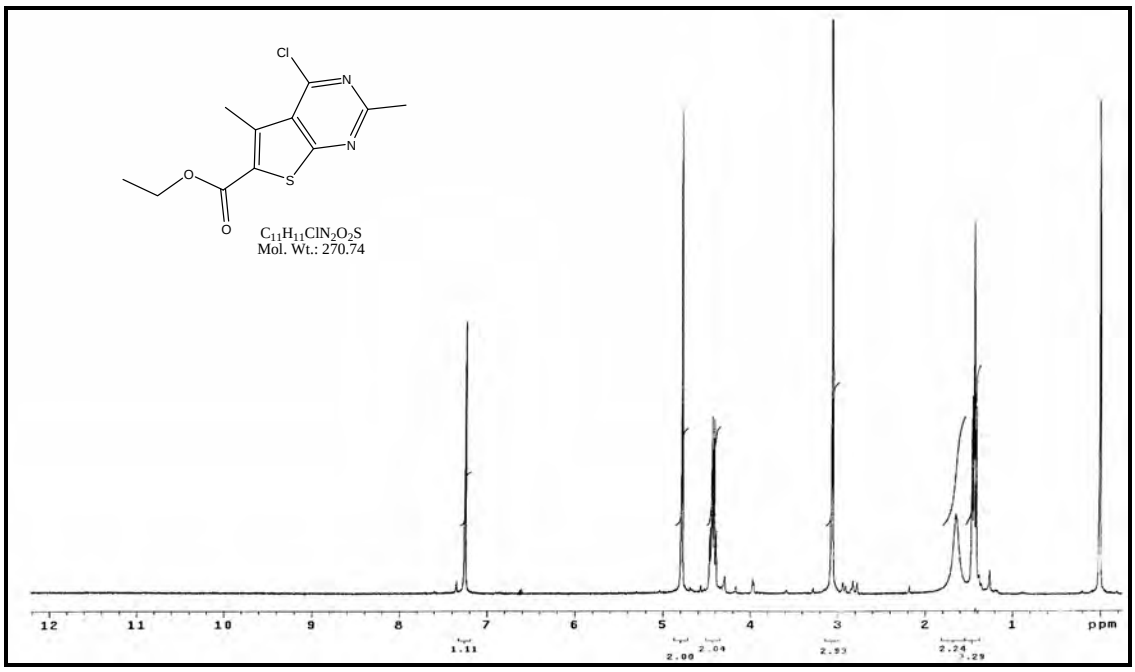
14. Mass spectrum of 4-chloro-2-(2-chloroethyl)-5,6,7,8-tetrahydro-benzo[4,5]-thieno[2,3-*d*]pyrimidine **(VI*xvii*)**



MS m/e: 287(M^+), 286(M-1), 253, 251, 225, 209.

Specimen ^{1}H NMR spectra of 4-chloro-2-methylthienopyrimidines

15. ^{1}H NMR spectrum of ethyl 4-chloro-2,5-dimethylthieno[2,3-*d*]pyrimidine-6-carboxylate **(VI*xxi*)**



^{1}H NMR ($CDCl_3$)δppm: 1.43 (3H, t, C*H*$_3$CH$_2$COO, *J* = 6.9), 3.05 (3H, s, C*H*$_3$ at 5), 4.35 (2H, q, CH$_3$C*H*$_2$COO, *J* = 6.9, 7.2), 4.82 (2H, s, CH$_2$ at 2).

4.5 MDR Reversal Activity of Condensed Pyrimidines synthesized:

MDR reversal effects of condensed pyrimidines on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) was carried out in the laboratory of Prof. Joseph Molnar at Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Hungary.

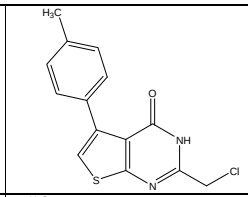
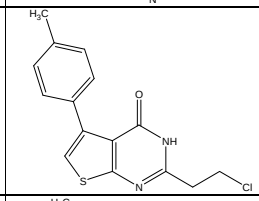
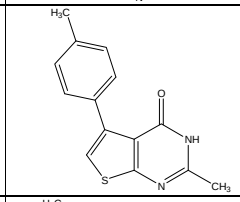
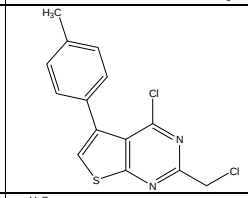
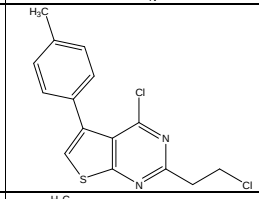
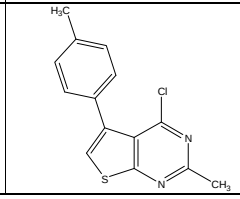
4.5.1 Assay for reversal of MDR in tumor cells[7,8]:

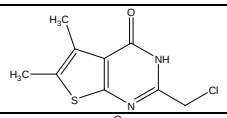
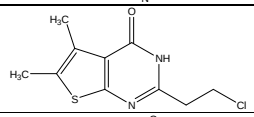
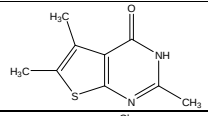
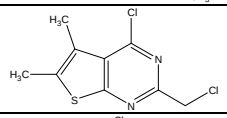
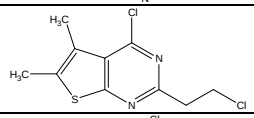
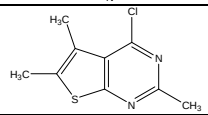
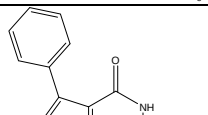
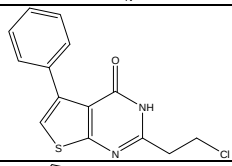
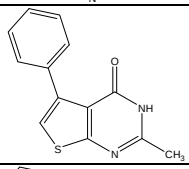
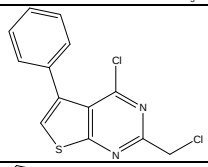
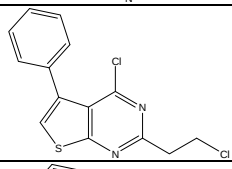
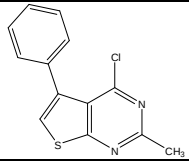
The cells were adjusted to a density of 2×10^6/ml, resuspended in serum-free McCoy's 5A medium and distributed in 0.5-ml aliquots into Eppendorf centrifuge tubes. The tested compounds were added at various concentrations in different volumes (2.0-20.0 µl) of the 1.0-10.0 mg/ml stock solutions, and the samples were incubated for 10 min at room temperature. Next, 10 µl (5.2 µM final concentration) of the indicator rhodamine 123 was added to the samples and the cells were incubated for a further 20 min at 37°C, washed twice and resuspended in 0.5 ml PBS for analysis. The fluorescence of the cell population was measured with a Beckton Dickinson FAC Scan flow cytometer. Verapamil was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared with the untreated cells. An activity ratio R was calculated via the following equation, on the basis of the measured fluorescence values:

$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\ treated \,/\ parental\,control}$$

The fluorescence activity ratio (FAR) is calculated based on the mean fluorescence intensities. That is ratio of FL^{-1} value of treated sample to that of the untreated sample. The ratio of parentral treated/parentral control is taken as 1, as the MDR efflux protein does not exist in them, therefore they cannot modify the uptake or efflux of drug.

Table-40. MDR reversal effects of newly synthesized compounds on *mdr1*-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry

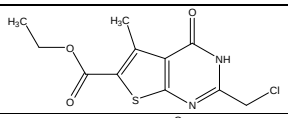
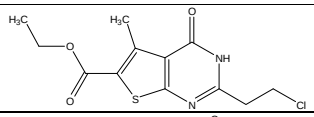
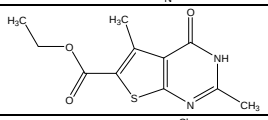
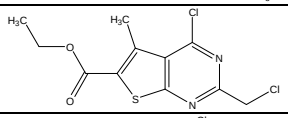
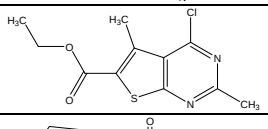
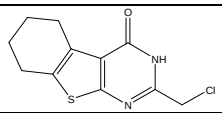
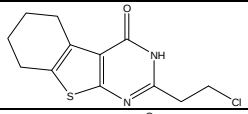
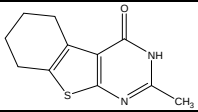
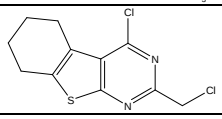
S. No.	Samples		µM	dye	FSC	SSC	FL-1	FAR	Peak Ch
1.	**PAR**		-	R123	458,30	172,15	941,45	-	865
2.	**PAR**		-	R123	459,37	173,16	970,00	-	1036
3.	**MDR**		-	R123	514,50	220,60	9,56	-	8
MDR mean					484,91	213,55	7,79	-	-
4.	**Verapamil**		21,99	R123	510,85	222,13	27,43	3,52	16
5.	**V*i***		4	R123	522,12	212,17	9,18	1,24	9
6.	**V*xviii***		4	R123	515,55	210,80	8,68	1,10	7
7.	**V*xxv***		4	R123	522,05	215,01	7,58	1,02	6
8.	**VI*i***		4	R123	511,71	215,04	7,70	1,12	7
9.	**VI*xiv***		*4*	*R123*	*485,09*	*217,45*	*20,75*	*2,66*	*14*
10.	**VI*xviii***		4	R123	491,21	216,08	11,29	1,44	7

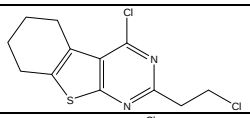
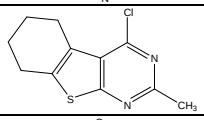
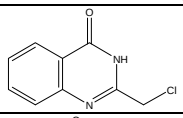
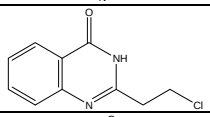
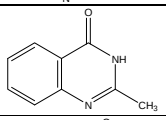
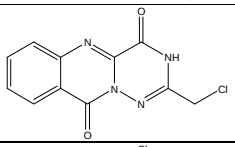
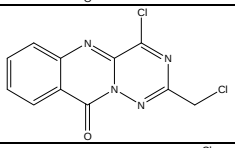
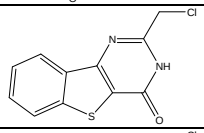
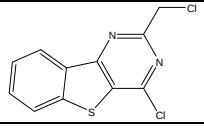
11.	**V*ii***		4	R123	465,20	207,98	15,19	1,94	10
12.	**V*xix***		4	R123	467,70	223,54	10,96	1,40	7
13.	**V*xxvi***		*4*	*R123*	*490,30*	*201,94*	*38,41*	*4,93*	*24*
14.	**VI*ii***		4	R123	446,37	199,20	19,99	2,56	12
15.	**VI*xv***		*4*	*R123*	*476,95*	*213,90*	*28,83*	*3,79*	*18*
16.	**VI*xix***		4	R123	436,05	211,16	12,40	1,59	10
17.	**V*iii***		4	R123	452,91	212,10	10,28	1,31	10
18.	**V*xx***		4	R123	428,75	201,21	10,30	1,32	10
19.	**V*xxvii***		4	R123	454,70	202,83	8,90	1,14	7
20.	**VI*iii***		4	R123	433,05	188,24	8,44	1,08	6
21.	**VI*xvi***		4	R123	466,70	201,41	8,62	1,10	7
22.	**VI*xx***		4	R123	444,29	204,16	7,35	0,94	5
23.	**DMSO control 20 µL**			R123	472,83	224,22	8,44	1,11	7

24.	**MDR**	-	R123	425,32	256,50	8,05	-	7

FAR: Fluorescence Activity Ratio

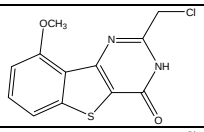
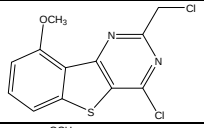
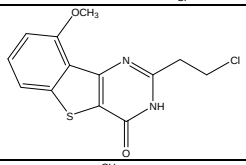
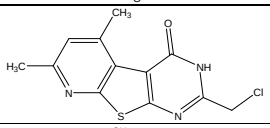
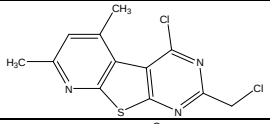
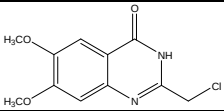
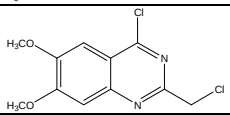
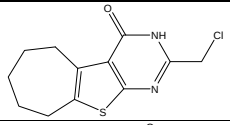
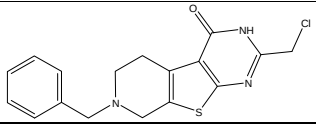
Table-41. MDR reversal effects of newly synthesized compounds on *mdr1*-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry

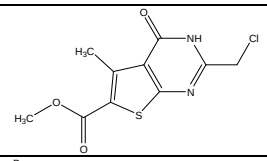
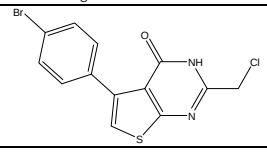
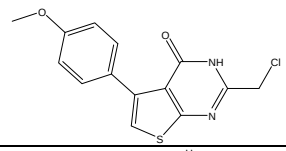
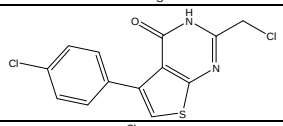
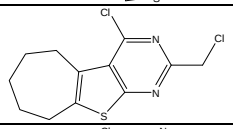
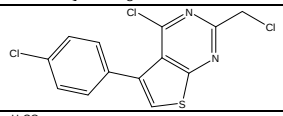
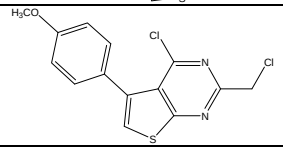
S. No.	Samples		µM	dye	FSC	SSC	FL-1	FAR	Peak Ch
1.	**PAR**		-	R123	509,50	196,92	953,39	-	956
2.	**PAR**		-	R123	517,57	201,82	979,59	-	1074
3.	**MDR**		-	R123	568,14	234,98	6,93	-	7
MDR mean					571,32	227,55	8,42	-	-
4.	**Verapamil**		21,99	R123	568,16	234,96	27,99	3,32	14
5.	**V*iv***		4	R123	533,26	215,30	6,38	0,75	5
6.	**V*xxi***		4	R123	563,43	225,41	6,09	0,75	5
7.	**V*xxviii***		4	R123	559,72	226,45	6,79	0,80	6
8.	**VI*iv***		4	R123	564,25	231,19	7,25	0,86	7
9.	**VI*xxi***		4	R123	561,81	216,36	7,67	0,91	8
10.	**V*v***		4	R123	559,66	222,12	8,44	1,00	8
11.	**V*xxii***		4	R123	561,10	220,10	5,79	0,68	5
12.	**V*xxix***		4	R123	550,09	219,91	8,40	0,99	8
13.	**VI*v***		4	R123	526,11	222,50	7,05	0,94	8

14.	**VI*xvii***		*4*	*R123*	*567,04*	*221,23*	*16,17*	*1,95*	*10*
15.	**VI*xxii***		4	R123	562,70	212,60	6,01	0,72	6
16.	**V*vi***		4	R123	568,09	221,80	6,78	0,80	6
17.	**V*xxiii***		4	R123	571,58	220,31	6,39	0,75	6
18.	**V*xxx***		*4*	*R123*	*580,66*	*222,19*	*25,47*	*5,40*	*26*
19.	**V*xiii***		4	R123	560,39	228,32	10,07	1,31	8
20.	**VI*ix***		4	R123	572,31	235,70	14,25	1,69	9
21.	**V*xiv***		*4*	*R123*	*569,64*	*218,59*	*38,68*	*4,50*	*22*
22.	**VI*x***		4	R123	560,60	239,91	21,67	2,57	12
23.	**DMSO control 20 µL**			R123	586,10	216,24	6,29	0,77	4
24.	**MDR**		-	R123	570,51	229,73	6,19	-	5

FAR: Fluorescence Activity Ratio

Table-42. MDR reversal effects of newly synthesized compounds on *mdr1*-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry

S. No.	Samples		µM	dye	FSC	SSC	FL-1	FAR	Peak Ch
1.	**PAR**		-	R123	506,87	202,17	983,79	-	858
2.	**PAR**		-	R123	499,44	206,08	1232,14	-	1336
3.	**MDR**		-	R123	503,98	237,33	13,42	-	11
MDR mean					481,13	221,05	11,75	-	-
4.	**Verapamil**		21,99	R123	512,22	253,01	120,78	10,21	50
5.	**V*xv***		*4*	*R123*	*515,23*	*239,45*	*95,88*	*8,16*	*41*
6.	**VI*xi***		4	R123	504,37	223,27	83,29	7,11	35
7.	**V*xxiv***		4	R123	543,46	220,40	74,58	6, 34	31
8.	**V*xvi***		4	R123	510,49	223,91	14,87	1,26	7
9.	**VI*xii***		4	R123	501,91	237,41	93,15	7,92	37
10.	**V*xvii***		4	R123	498,39	220,06	80,55	6,80	34
11.	**VI*xiii***		4	R123	489,47	227,03	85,44	7,27	35
12.	**V*vii***		*4*	*R123*	*491,04*	*213,91*	*139,86*	*11,90*	*60*
13.	**V*x***		*4*	*R123*	*486,19*	*222,54*	*142,10*	*12, 09*	*61*

14.	**V*xi***		4	R123	481,70	216,11	32,58	2,77	13
15.	**V*xii***		4	R123	465,70	215,09	30,18	3,24	16
16.	**V*viii***		4	R123	428,23	212,63	87,98	87,98	37
17.	**V*ix***		4	R123	472,45	211,30	56,84	4,83	24
18.	**VI*vi***		*4*	*R123*	*468,92*	*223,64*	*129,43*	*11,01*	*55*
19.	**VI*viii***		4	R123	483,27	210,21	80,18	6,82	34
20.	**VI*vii***		*4*	*R123*	*478,21*	*220,54*	*119,88*	*10,20*	*51*
23.	**DMSO control 20 µL**			R123	463,44	215,29	10,00	0,85	5
24.	**MDR**		-	R123	454,28	204,77	12,00	-	10

FAR: Fluorescence Activity Ratio

4.5.2 Discussion:

MDR reversal assay has gained importance in view of many cancerous cells developing multiple drug resistance (MDR) due to incorporation of MDR-1 gene coding of P-gp, a glycoprotein involved in MDR. The glycoprotein P-gp is driven by ATP and is responsible for efflux of drug from the cancerous cells leading to MDR. Therefore, MDR reversal agents are being exploited as potential anticancer agents.[7,8]

Condensed pyrimidines synthesized in this part of the thesis were tested for MDR reversal activity on MDR -1 gene transfected cell line 15178 by flow cytometry. Total of 52 compounds were tested for the MDR reversal activity. Details of the test protocol are given earlier. The tests were carried out in three sets with veerapamil as positive control.

The great majority of the compounds were ineffective on the MDR reversal efflux pump activity. The majority of the compounds were identified and characterized in this group as ineffective compounds when tested in 4 micro mol conc. No direct cytotoxic effect was found at the above concentration.

However, compounds 4-chloro-2-(2-chloroethyl)-5-*p*-tolylthieno[2,3-*d*]pyrimidine **VI*xiv***, 2,5,6-trimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **V*xxvi***, 4-chloro-2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidine **VI*xv***, 4-chloro-2-(2-chloroethyl)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine **VI*v***, 2-methylquinazolin-4(3*H*)-one **V*xxx***, 2-chloro-methyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **V*xiv***, 9-methoxy-2-chloromethyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **V*xv***, 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **V*vii***, 7-benzyl-2-chloromethyl-5,6,7,8-tetra-hydro-3*H*-9-thia-1,3,7-triaza-fluoren-4-one **V*x***, 4-chloro-2-chloromethyl-6,7,8,9-tetra-hydro-5*H*-10-thia-1,3-diaza-benzo[*a*]azulene **VI*vi*** and 4-chloro-2-(chloromethyl)-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidine **VI*vii*** showed moderate activity which is exhibited by fluorescence activity ratio. None of the compounds showed cytotoxic effect in the above said concentration which is desirable. This means that the above stated compounds were moderately effective in reversal of MDR efflux pump activity.

4.6 References:

1. Brown, D. J. In *The Pyrimidines*; The Chemistry of Heterocyclic Compounds; An Interscience Publication; John Wiley and Sons; New York: 1962, pp 162-167.
2. Brown, D. J. In *The Quinazolines*; The Chemistry of Heterocyclic Compounds; An Interscience Publication; John Wiley and Sons; New York: Vol.1; Supplement 1; 1962, pp 162-167.
3. Brown, D. J. In *Fused Pyrimidines*; *The Quinazolines*; The Chemistry of Heterocyclic Compounds; An Interscience Publication; John Wiley and Sons; New York: Vol.24; pp 219-227.
4. Kidwai, M.; Kahli, S.; Kumar, P. *J. Chem. Res.* **1998**, 586.
5. Phoujdar, M. S.; Kathiravan, M. K.; Bariwal, J. B.; Shah A. K.; Jain, K. S. *Tetrahedron Lett.* **2007,** *49,* 1269.
6. Alexandre, F. R.; Bercibar, A.; Wrigglesworth, R.; Besson, T. *Tetrahedron* **2003**, *59*, 1413.
7. Szabo, D.; Molnar, J. *Anticancer Res.* **1998,** *18,* 3039.
8. Molnar, J.; Gyemant, N.; Tanaka, M.; Hohmann, J.; Bergmann-Leitner, E.; Molnar, P.; Deli, J.; Diziapetris, R.; Ferreira, M. J. U. *Curr. Pharm. Design* **2006,** *12,* 287.

5. Experimental

5. Experimental

All the chemicals used in the synthesis were of laboratory grade. The melting points were determined in open capillary on Veego (VMP-D) electronic apparatus and are uncorrected.

The IR spectra of synthesized compounds were recorded on Perkin Elmer BX_2 FT-IR spectrophotometer in potassium bromide discs.

^{1}H NMR spectra were recorded on Varian Mercury YH-300 FT-NMR spectrometer using TMS (tetramethyl silane) as an internal standard $CDCl_3$ and DMSO-d_6 as a solvent at University of Pune, Pune.

Mass spectra were obtained on an Electron Impact Mass (GCMS-QP2010 spectrometer) 70 eV ionizing beam and using direct insertion probe at Department of Chemistry, Saurashtra University, Rajkot.

To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated silica plates (Merck Silicagel F_{254}) using hexane-ethyl acetate-glacial acetic acid, chloroform-methanol as the solvent systems and the spots were visualized by exposure to iodine vapors or under Ultra Violet (UV) light at 254 nm and 360 nm.

Microwave synthesizer (Questron Technologies Corp., Canada; model: Q-Pro M) having monomode open-vessel was used for the synthesis.

5.1 Synthesis of chloroacetonitrile

Choloroacetonitrile was synthesized as describe in the experimental procedure in the Part-1.

5.2 Synthesis of thiophene *o*-aminoesters and other cyclic *o*-aminoesters

These starting materials were synthesized as per procedure described in the experimental part in the Part-1.

5.3 Synthesis of condensed 2-chloromethylpyrimidin-4(3*H*)-ones under Microwave Irradiation (MWI) (V*i-xvii*)

1. Synthesis of 2-chloromethyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*i*)

A mixture of the ethyl 2-amino-4-**(**4-methylphenyl)thiophene-3-carboxylate (**I*vii*,** 5.2 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) was irradiated at 350W for 25 min in a microwave synthesizer. The progress of reaction was monitored using TLC at each 5 min intervals. The reaction mixture was allowed to cool to RT and poured on to ice-water mixture (50 ml). The resulting precipitated solid was filtered, washed with chilled water and dried. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*i*).**

M.P. : 258-260^{o}C (260-262^{o}C)[1]; Yield: 85%

Mol. Formula : $C_{14}H_{11}ClN_2OS$; Mol. Wt. 290.77

IR (KBr) cm^{-1} : 3438, (γ_{NH}), 2919(γ_{C-H}), 1648($\gamma_{C=O}$), 747(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 2.39 (3H, s, C*H*$_3$), 4.53 (2H, s, CH$_2$), 7.13 (1H, s, C*H*), 7.19-7.46 (4H, m, Ar-*H*), 10.43 (1H,s, N*H*)

2. Synthesis of 2-chloromethyl-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*ii*)

A mixture of the ethyl 2-amino-4,5-dimethylthiophene 3-carboxylate (**I*iv***, 3.98 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (30 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*ii*)**.

M.P. : 252-254^{o}C (253-255^{o}C)[2]; Yield: 92%

Mol. Formula : $C_9H_9ClN_2OS$; Mol. Wt. 228.7

IR (KBr) cm^{-1} : 2917 (γ_{C-H}), 1662($\gamma_{C=O}$), 769(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 4.51 (2H, s, C*H*$_2$), 10.03 (1H, s, br, N*H*)

3. Synthesis of 2-chloromethyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*iii*)

A mixture of ethyl 2-amino-4-phenylthiophene 3-carboxylate (**I*v***, 4.94 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (20 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*iii*)**.

M.P. : 220-222^{o}C (221-223^{o}C)[2]; Yield: 75%

Mol. Formula : $C_{13}H_9ClN_2OS$; Mol. Wt. 276.74

IR (KBr) cm^{-1} : 2855(γ_{C-H}), 1660($\gamma_{C=O}$), 748(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 4.58 (2H, s, C*H*$_2$), 7.31-7.52 (5H, m, Ar-*H* and 1*H* at 6 position), 12.69 (1H, s, br, N*H*);

4. Synthesis of ethyl 2-chloromethyl-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]-pyrimidine 6-carboxylate (V*iv*)

A mixture of diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**I*iii***, 5.14 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (27 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the ethyl 2-chloromethyl-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(V*iv*)**.

M.P. : 241-243^{o}C (243-246^{o}C)[2]; Yield: 90%

Mol. Formula : $C_{11}H_{11}ClN_2O_3S$; Mol. Wt. 286.73

IR (KBr) cm^{-1} : 2864(γ_{C-H}), 1725($\gamma_{C=O}$), 1670(γ_{CONH}), 763(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 1.41 (3H, t, *J* = 7, C*H*$_3$), 2.95 (3H, s, C*H*$_3$), 4.38 (2H, quartlet, *J* = 7, C*H*$_2$), 4.57 (2H, s, C*H*$_2$), 10.62 (1H, s, N*H*)

5. Synthesis of 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*v*)

A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene 3-carboxylate (**I*i***, 4.5 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (10 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform

mixture yielded the 2-chloromethyl-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*v*)**.

M.P. : 273-276°C (273-276°C)[3]; Yield: 86%
Mol. Formula : $C_{11}H_{11}ClN_2OS$; Mol. Wt. 254.74
IR (KBr) cm^{-1} : 2931(γ_{C-H}), 1663(γ_{CONH}), 754(γ_{C-Cl})
^{1}H NMR ($CDCl_3$)δppm : 1.86 (4H, s, C*H*$_2$ at 6 and 7), 2.79 (2H, s, C*H*$_2$ at 5), 3.02 (2H, s, C*H*$_2$ at 8), 4.55 (2H, s, C*H*$_2$), 10.65 (1H, s, br, N*H*)
MS m/e : 255(M^+), 221, 149.

6. Synthesis of 2-chloromethylquinazolin-4(3*H*)-one (V*vi*)

A mixture of methyl anthranilate (3.0 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (20 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethylquinazolin-4(3*H*)-one **(V*vi*)**.

M.P. : 257-258°C (257-258°C)[4]; Yield: 84%
Mol. Formula : $C_9H_7ClN_2O$; Mol. Wt. 194.62
IR (KBr) cm^{-1} : 2981(γ_{C-H}), 1697(γ_{CONH}), 776(γ_{C-Cl})
^{1}H NMR($CDCl_3$)δppm : 4.53 (2H, s, C*H*$_2$), 7.49-7.82 (4H, m, Ar-*H*), 12.56 (1H, s, br, N*H*)

7. Synthesis of 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diazabenzo[*a*]-azulen 4-one (V*vii*)

A mixture of ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene 3-carboxylate (**I*xi***, 4.78 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (12 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **(V*vii*)**.

M.P. : 188-190°C; Yield: 74%
Mol. Formula : $C_{12}H_{13}ClN_2OS$; Mol. Wt. 268.76

IR (KBr) cm^{-1} : 2925(γ_{C-H}), 1660(γ_{CONH}), 755(γ_{C-Cl})

8. Synthesis of 2-chloromethyl-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*viii*)

A mixture of ethyl 2-amino-4-(4-methoxyphenyl)thiophene-3-carboxylate (**I*vi***, 5.5 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (25 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-chloromethyl-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*viii*)**.

M.P. : 205-207^oC (208-210^oC)[2]; Yield: 84%

Mol. Formula : $C_{14}H_{11}ClN_2O_2S$; Mol. Wt. 306.77

IR (KBr) cm^{-1} : 2990(γ_{C-H}), 1680($\gamma_{C=O}$), 746(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 3.85 (3H, s, OC*H*$_3$), 4.48 (2H, s, *CH*$_2$Cl), 6.94-7.54 (5H, m, 4H, Ar-*H* and 1H at 6), 12.02 (1H, s, N*H*).

9. Synthesis of 2-chloromethyl-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*ix*)

A mixture of ethyl 2-amino-4-(4-chlorophenyl)thiophene-3-carboxylate (**I*ix***, 5.6 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (20 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-chloromethyl-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*ix*).**

M.P. : 233-234^oC (229-231^oC)[5]; Yield: 71%

Mol. Formula : $C_{13}H_8Cl_2N_2OS$; Mol. Wt. 311.19

IR (KBr) cm^{-1} : 3107(γ_{NH}), 1649(γ_{CONH}), 756(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 4.54 (2H, s, *CH*$_2$Cl), 7.23-7.57 (5H, m, 4H, Ar-*H* and 1*H* at 6), 11.5 (1H, s, N*H*).

10. Synthesis of 7-benzyl-2-chloroethyl-5,6,7,8-tetrahydro-3*H*-pyrido-[4',3':4,5]-thieno[2,3-*d*]pyrimidin-4-one (V*x*)

A mixture of ethyl 2-amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxylate (**I*xii*,** 6.32 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (15 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 7-benzyl-2-chloroethyl-5,6,7,8-tetrahydro-3*H*-pyrido-[4',3':4,5]thieno[2,3-*d*]pyrimidine-4-one **(V*x*).**

M.P. : 232-234°C (232-234°C)[3]; Yield: 78%
Mol. Formula : $C_{17}H_{16}ClN_3OS$; Mol. Wt. 345.85
IR (KBr) cm^{-1} : 3016(γ_{C-H}), 1669(γ_{CONH}), 743(γ_{C-Cl})

11. Synthesis of methyl 2-chloromethyl-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*] pyrimidine 6-carboxylate (V*xi*)

A mixture of 4-ethyl 2-methyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**I*ii***, 4.8 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (30 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded methyl 2-chloromethyl-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(V*xi*)**.

M.P. : 250-254°C; Yield: 86%
Mol. Formula : $C_{10}H_9ClN_2O_3S$; Mol. Wt. 272.71
IR (KBr) cm^{-1} : 2863(γ_{C-H}), 1724($\gamma_{C=O}$), 1664(γ_{CONH}), 763(γ_{C-Cl})

12. Synthesis of 5-(4-bromophenyl)-2-chloromethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xii*)

A mixture of ethyl 2-amino-4-(4-bromophenyl)thiophene-3-carboxylate (**I*viii***, 6.5 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (20 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 5-(4-bromophenyl)-2-chloromethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xii*).**

M.P. : 247-249^{o}C; Yield: 68%

Mol. Formula : $C_{13}H_8BrClN_2OS$; Mol. Wt. 355.64

IR (KBr) cm^{-1} : 2980(γ_{C-H}), 1655($\gamma_{C=O}$), 775(γ_{C-Cl})

13. Synthesis of 2-chloromethyl-4*H*-[1,2,4]triazino[6,1-*b*]quinazolin-4,10-dione (V*xiii*)

A mixture of 3-amino-2-carbethoxyquinazolin-4-one (**I*xiv***, 4.66 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (30 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-4*H*-[1,2,4]triazino[6,1-*b*]quinazolin-4,10-dione **(V*xiii*).**

M.P. : 240-243^{o}C (242-244^{o}C)[6]; Yield: 60%

Mol. Formula : $C_{11}H_7Cl\,N_4O_2$; Mol. Wt. 262.2

IR (KBr) cm^{-1} : 2896(γ_{C-H}), 1686(γ_{CONH}), 778(γ_{C-Cl}).

14. Synthesis of 2-chloromethyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (V*xiv*)

A mixture of ethyl 3-aminobenzo[*b*]thiophene 2-carboxylate (**I*xviii***, 4.42 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (45 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **(V*xiv*).**

M.P. : 150-152^{o}C; Yield: 62%

Mol. Formula : $C_{11}H_7ClN_2OS$; Mol. Wt. 250.7

IR (KBr) cm^{-1} : 2980(γ_{C-H}), 1680(γ_{CONH}), 740(γ_{C-Cl}).

15. Synthesis of 2-chloromethyl-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (V*xv*)

A mixture of ethyl 3-amino-4-methoxybenzo[*b*]thiophene 2-carboxylate (**I*xvi***, 5.02 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (40 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture

yielded the 2-chloromethyl-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **(V*xv*).**

M.P. : 265-267^{o}C; Yield: 83%
Mol. Formula : $C_{12}H_9Cl\,N_2O_2S$; Mol. Wt. 280.7
IR(KBr)cm^{-1} : 2978(γ_{C-H}), 1676(γ_{CONH}), 736(γ_{C-Cl})

16. Synthesis of 2-chloromethyl-7,9-dimethylpyrido[3', 2':4, 5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (V*xvi*)

A mixture of 3-amino-2-carbethoxy-4,6-dimethylthieno[2,3-*b*]pyridine (**I*xiii***, 5.02 gm; 0.02 mol), chloroacetonitrile (1.65 gm; 0.022 mol) and catalytic amount of conc. HCl (0.5 ml) were reacted under microwave irradiation (50 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidine-4(3*H*)-one **(V*xvi*).**

M.P. : 275-277^{o}C (273-275^{o}C)[7]; Yield: 76%
Mol. Formula : $C_{12}H_{10}Cl\,N_3OS$; Mol. Wt. 279.7
IR (KBr) cm^{-1} : 3013(γ_{C-H}), 1675(γ_{CONH}), 746(γ_{C-Cl})
MS m/e : 281(M+1), 279(M^+), 244, 216.

17. Synthesis of 2-chloromethyl-6,7-dimethoxyquinazolin-4(3*H*)-one (V*xvii*)

A mixture of methyl-2-amino-4,5-dimethoxybenzoate (**I*xv***, 4.22 gm; 0.02 mole), chloroacetonitrile (1.65 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (40 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded the 2-chloromethyl-6, 7-dimethoxyquinazolin-4(3*H*)-one **(V*xvii*).**

M.P. : 240-245^{o}C (240-245^{o}C)[8]; Yield: 70%
Mol. Formula : $C_{11}H_{11}Cl\,N_2O_3$; Mol Wt. 254.6
IR (KBr) cm^{-1} : 3012(γ_{ArH}), 2888(γ_{CH2}), 1666(γ_{CONH}), 754(γ_{C-Cl})
MS m/e : 254(M^+), 239, 219

5.4 Synthesis of condensed 2-chloroethylpyrimidin-4(3*H*)-ones under Microwave Irradiations (MWI) (V*xviii-xxiv*)

18. Synthesis of 2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xviii*)

A mixture of ethyl 2-amino-4-(4-methylphenyl)thiophene-3-carboxylate (**I*vii***, 5.2 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (35 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xviii*)**.

M.P. : 166-168^{o}C (168-170^{o}C)[9]; Yield: 81%

Mol. Formula : $C_{15}H_{13}ClN_2OS$; Mol. Wt. 304.79

IR (KBr) cm^{-1} : 2837(γ_{C-H}), 1672(γ_{CONH}), 762(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 2.40 (3H, s, C*H*$_3$), 3.06 (2H, t, *J* = 7, C*H*$_2$), 3.87 (2H, t, *J* = 7, C*H*$_2$), 7.06 (1H, s, C*H*), 7.15-7.45 (4H, m, Ar-*H*), 12.99 (1H, s, N*H*).

19. Synthesis of 2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xix*)

A mixture of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (**I*iv***, 3.9 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (60 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloro-ethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xix*).**

M.P. : 200-202^{o}C (200-202^{o}C)[9]; Yield: 86%

Mol. Formula : $C_{10}H_{11}ClN_2OS$; Mol. Wt. 242.73

IR (KBr) cm^{-1} : 2922(γ_{C-H}), 1666(γ_{CONH}), 758(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 2.38 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 3.19 (2H, t, C*H*$_2$, *J* = 7.5), 3.97 (2H, t, C*H*$_2$, *J* = 7.2), 12.34 (1H, s, br, N*H*).

20. Synthesis of 2-(2-chloroethyl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xx*)

A mixture of ethyl 2-amino-4-phenylthiophene-3-carboxylate (**I*v***, 4.9 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (45 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloroethyl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xx*).**

M.P. : 268-270^{o}C (268-270^{o}C)[9]; Yield: 90%

Mol. Formula : $C_{14}H_{11}ClN_2OS$; Mol. Wt. 290.77

IR (KBr) cm^{-1} : 2848(γ_{C-H}), 1670($\gamma_{C=O}$), 748(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 3.14 (2H, t, C*H*$_2$, *J* = 7.2), 4.02 (2H, t, C*H*$_2$, *J* = 7.5), 7.30-7.50 (6H, m, 5*H* Ar-*H* and 1*H* at 6 position), 12.40 (1H, s, br, N*H*).

21. Synthesis of ethyl 2-(2-chloroethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]-pyrimidine 6-carboxylate (V*xxi*)

A mixture of diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**I*iii***, 5.1 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (70 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded ethyl 2-(2-chloroethyl)-3,4-dihydro-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(V*xxi*).**

M.P. : 250-252^{o}C (250-252^{o}C)[9]; Yield: 72%

Mol. Formula : $C_{12}H_{13}ClN_2O_3S$; Mol. Wt. 300.76

IR (KBr) cm^{-1} : 2865(γ_{C-H}), 1719($\gamma_{C=O}$), 1670(γ_{CONH}), 762(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 1.41 (3H, t, *J* = 7.3, C*H*$_3$), 2.9 (3H, s, C*H*$_3$), 3.24 (2H, t, *J* = 6.7, C*H*$_2$), 4.29 (2H, t, *J* = 7.3, C*H*$_2$) , 4.3(2H, q, *J* = 7.1, C*H*$_2$), 12.30 (1H, s, N*H*).

22. Synthesis of 2-(2-chloroethyl)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]-pyrimidin-4-one (V*xxii*)

A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**I*i***, 5.1 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml)

were reacted under microwave irradiation (55 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloroethyl)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **(V*xxii*).**

M.P. : 156-158^{o}C (156-158^{o}C)[9]; Yield: 83%

Mol. Formula : $C_{12}H_{13}ClN_2OS$; Mol. Wt. 268.76

IR (KBr) cm^{-1} : 2935(γ_{C-H}), 1665(γ_{CONH}), 665(γ_{C-Cl}).

^{1}H NMR ($CDCl_3$)δppm : 1.90 (4H, s, C*H*$_2$ at 6 and 7), 2.81 (2H, s, C*H*$_2$ at 5), 3.02 (2H, s, C*H*$_2$ at 8), 3.23 (2H, t, C*H*$_2$, *J* = 7.0), 4.02 (2H, t, C*H*$_2$, *J* = 7.2), 11.90 (1H, s, br, N*H*)

23. Synthesis of 2-(2-chloroethyl)quinazolin-4(3*H*)-one (V*xxiii*)

A mixture of methylanthranilate (3.2 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (40 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloroethyl)quinazolin-4(3*H*)-one **(V*xxiii*).**

M.P. : 200-202^{o}C; Yield: 91%

Mol. Formula : $C_{10}H_9ClN_2O$; Mol. Wt. 208.64

IR (KBr) cm^{-1} : 2979(γ_{C-H}), 1665(γ_{CONH}), 771(γ_{C-Cl}).

^{1}H NMR ($CDCl_3$)δppm : 3.18 (2H, t, C*H*$_2$, *J* = 6.3, 7.2), 4.06 (2H, t, C*H*$_2$ at 2, *J* = 6.3, 7.2), 7.44-8.07 (4H, m, Ar-*H*).

24. Synthesis of 2-(2-chloroethyl)-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (V*xxiv*)

A mixture of methyl 3-amino-4-methoxybenzo[*b*]thiophene-2-carboxylate (**I*xvi***, 4.7 gm; 0.02 mole), acrylonitrile (1.21 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (75 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-(2-chloro-ethyl)-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **(V*xxiv*)**.

M.P. : 260-262^{o}C; Yield: 63%

Mol. Formula : $C_{12}H_9Cl\,N_2O_2S$; Mol Wt. 280.7

IR(KBr)cm^{-1} : 2978($\gamma_{C\text{-}H}$), 1676(γ_{CONH}), 736($\gamma_{C\text{-}Cl}$)

5.5 Synthesis of condensed 2-methylpyrimidin-4(3*H*)-ones under Microwave Irradiation (MWI) (V*xxv-xxx*)

25. Synthesis of 2-methyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xxv*)

A mixture of ethyl 2-amino-4-(4-methylphenyl)thiophene-3-carboxylate (**I*vii***, 5.22 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (40 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-methyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxv*).**

M.P. : 97-99^{o}C; Yield: 80%

Mol. Formula : $C_{14}H_{12}N_2OS$; Mol. Wt. 256.3

IR (KBr) cm^{-1} : 2898($\gamma_{C\text{-}H}$), 1667(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 2.39 (3H, s, C*H*$_3$), 2.47 (3H, s, C*H*$_3$), 7.04 (1H, s, *H*), 7.16-7.48 (4H, m, Ar-*H*), 11.90 (1H, s, N*H*).

26. Synthesis of 2,5,6-trimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xxvi*)

A mixture of ethyl 2-amino-4,5-dimethylthiophene 3-carboxylate (**I*iv***, 3.98 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (55 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2,5,6-trimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxvi*)**.

M.P. : 102-104^{o}C (102-104^{o}C)[10]; Yield: 70%

Mol. Formula : $C_9H_{10}N_2OS$; Mol. Wt. 194.25

IR (KBr) cm^{-1} : 2918($\gamma_{C\text{-}H}$), 1665(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 2.37 (3H, s, C*H*$_3$), 2.46 (3H, s, C*H*$_3$), 2.51 (3H, s, C*H*$_3$), 12.04 (br, s, 1H, N*H*).

27. Synthesis of 2-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (V*xxvii*)

A mixture of ethyl 2-amino-4-phenylthiophene-3-carboxylate (**I*v***, 4.9 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (45 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxvii*)**.

M.P. : 235-237^{o}C (235-237^{o}C)[10]; Yield: 77%

Mol. Formula : $C_{13}H_{10}N_2OS$; Mol. Wt. 242.3

IR (KBr) cm^{-1} : 2998(γ_{C-H}), 1667(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 3.36 (3H, s, C*H*$_3$), 7.31-7.50 (5H, m, Ar-*H* and 1H at 6), 12.28 (1H, s, N*H*).

28. Synthesis of ethyl 3,4-dihydro-2,5-dimethyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate (V*xxviii*)

A mixture of diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate (**I*iii***, 5.1 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (30 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded ethyl 3,4-dihydro-2,5-dimethyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate **(V*xxviii*).**

M.P. : 278-280^{o}C (280-283^{o}C)[11]; Yield: 99%

Mol. Formula : $C_{11}H_{12}N_2O_3S$; Mol. Wt. 252.29

IR (KBr) cm^{-1} : 2960(γ_{C-H}), 1718($\gamma_{C=O}$) 1667(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 1.40 (3H, t, C*H*$_3$, *J* = 7.3), 2.55 (3H, s, C*H*$_3$), 2.94 (3H, s, C*H*$_3$), 4.36 (2H, C*H*$_2$ quartlet, *J* = 7.1), 10.95 (1H, s, N*H*).

29. Synthesis of 2-methyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (V*xxix*)

A mixture of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**I*i***, 4.5 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (30 min) as per procedure described for the

compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-methyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **(V*xxix*)**.

M.P. : 155-158^{o}C (157-159^{o}C)[10]; Yield: 91%

Mol. Formula : $C_{11}H_{12}N_2OS$; Mol. Wt. 220.29

IR (KBr) cm^{-1} : 2920(γ_{C-H}), 1661(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 1.77 (4H, s, 2 X C*H*$_2$ at 6 and 7), 2.30 (3H, s, C*H*$_3$ at 2), 2.70 (2H, s, C*H*$_2$ at 5), 2.83 (2H, s, C*H*$_2$ at 8), 7.07 (br, s, 1H, N*H* at 3).

30. Synthesis of 2-methylquinazolin-4(3*H*)-one (V*xxx*)

A mixture of methylanthranilate (3.0 gm; 0.02 mole), acetonitrile (1.0 gm; 0.022 mole) and catalytic amount of HCl (0.5 ml) were reacted under microwave irradiation (40 min) as per procedure described for the compound **V*i***. The crude product on recrystallization from methanol-chloroform mixture yielded 2-methylquinazolin-4(3*H*)-one **(V*xxx*)**.

M.P. : 237-239^{o}C (240-240^{o}C)[12]; Yield: 71%

Mol. Formula : $C_9H_8N_2O$; Mol. Wt. 160.17

IR (KBr) cm^{-1} : 2918(γ_{C-H}), 1666(γ_{CONH}).

^{1}H NMR ($CDCl_3$)δppm : 2.50 (2H, s, C*H*$_3$), 7.38-7.74 (4H, m, Ar-*H*), 12.13 (1H, br, s, N*H*)

5.6 Synthesis of condensed 4-chloro-2-chlormethylpyrimidines under Microwave Irradiations (MWI) (VI*i-xiii*)

31. Synthesis of 4-chloro-2-chloromethyl-5-(4-methylphenyl)thieno[2,3-*d*]-pyrimidine (VI*i*)

A mixture of 2-chloromethyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*i*,** 5.22 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) was irradiated at 350W for 4 min in a microwave synthesizer. The progress of reaction was monitored using TLC after each 2 min intervals (chloroform: methanol::4.5: 0.5). After completion of the reaction, the reaction mixture was allowed to cool to room temperature and poured on to ice-water mixture (100 ml). The resulting precipitated solid was filtered, washed with chilled water and dried. The crude product on recrystallization from hexane (60-

80^{o}C) that yielded 4-chloro-2-chloromethyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidine (**VI*i***).

M.P. : 85-87^{o}C (85-87^{o}C)[3]; Yield: 90%
Mol. Formula : $C_{14}H_{10}Cl_2N_2S$; Mol. Wt. 309.1
IR (KBr) cm^{-1} : 2979(γ_{C-H}), 1458($\gamma_{C=C}$), 722(γ_{C-Cl}).
^{1}H NMR ($CDCl_3$)δppm : 2.42 (3H, s, C*H*$_3$), 4.82 (2H, s, C*H*$_2$Cl), 7.25-7.51 (5H, m, Ar-*H* and H at 6).

32. Synthesis of 4-chloro-2-chloromethyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (VI*ii*)

A mixture of 2-chloromethyl-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*ii*,** 4.5 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (5 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-5,6-dimethylthieno[2,3-*d*]pyrimidine (**V*ii*).**

M.P. : 116-118^{o}C (118-120^{o}C)[3]; Yield: 92%
Mol. Formula : $C_9H_8C_2N_2S$; Mol. Wt. 247.16
IR (KBr) cm^{-1} : 2980(γ_{C-H}), 677(γ_{C-Cl}).
^{1}H NMR ($CDCl_3$)δppm : 2.45 (3H, s, C*H*$_3$), 2.50 (3H, s, CH_3), 4.82 (2H, s, *CH*$_2$Cl).

33. Synthesis of 4-chloro-2-chloromethyl-5-phenylthieno[2,3-*d*]pyrimidine (VI*iii*)

A mixture of 2-chloromethyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**V*iii*,** 5.5 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-5-phenylthieno[2,3-*d*]pyrimidine **(VI*iii*).**

M.P. : 68-70^{o}C (70-72^{o}C)[3]; Yield: 77%
Mol. Formula : $C_{13}H_8Cl_2N_2S$; Mol. Wt. 295.2
IR (KBr) cm^{-1} : 2932(γ_{C-H}), 1510($\gamma_{C=C}$).
^{1}H NMR ($CDCl_3$)δppm : 4.82 (2H, s, *CH*$_2$Cl), 7.25-7.62 (4H, m, Ar-*H* and 1H at 6).

34. Synthesis of ethyl 4-chloro-2-chloromethyl-5-methylthieno[2,3-*d*]pyrimidine 6-carboxylate (VI*iv*)

A mixture of ethyl 2-chloromethyl-5-methyl-4-oxothieno[2,3-*d*]pyrimidine 6-carboxylate (**V*iv***, 5.7 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (6 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded ethyl 4-chloro-2-chloromethyl-5-methylthieno[2,3-*d*]pyrimidine 6-carboxylate **(VI*iv*)**.

M.P. : 135-137^{o}C (135-137^{o}C)[3]; Yield: 75%

Mol. Formula : $C_{11}H_{10}Cl_2N_2O_2S$; Mol. Wt. 305.2

IR (KBr) cm^{-1} : 2984(γ_{C-H}), 1718($\gamma_{C=O}$), 1534($\gamma_{C=C}$).

^{1}H NMR ($CDCl_3$)δppm : 1.43 (3H, t, CH_2CH_3, *J* = 7.2 & 6.9), 3.06 (3H, s, CH_3), 4.42 (2H, q, CH_2CH_3, *J* = 6.9 & 7.2), 4.78 (2H, s, CH_2Cl).

MS m/e : 304 (M^+), 289, 276.

35. Synthesis of 4-chloro-2-chloromethyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno-[2,3-*d*]pyrimidine (VI*v*)

A mixture of 2-chloromethyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**V*vii***, 5.0 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidine **(VI*v*)**.

M.P. : 80-82^{o}C (80-82^{o}C)[3]; Yield: 70%

Mol. Formula : $C_{11}H_{10}Cl_2N_2S$; Mol. Wt. 273.2

IR (KBr) cm^{-1} : 2941(γ_{C-H}), 1447($\gamma_{C=C}$), 736(γ_{C-Cl})

^{1}H NMR ($CDCl_3$)δppm : 1.99 (4H, s, CH_2 at 6 & 7), 2.57 (3H, s, CH_3), 2.95 (3H, s, CH_3 at 5), 3.12 (3H, s, CH_3 at 8), 4.80 (2H, s, CH_2Cl).

MS m/e : 275(M^+), 244, 237, 209.

36. Synthesis of 4-chloro-2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulene (VI*vi*)

A mixture of 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diazabenzo[*a*]azulen-4-one **(V*vii*,** 5.0 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (5 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diazabenzo[*a*]azulene **(VI*vi*)**.

M.P. : 75-77^{o}C; Yield: 85%
Mol. Formula : $C_{12}H_{12}Cl_2N_2S$; Mol. Wt. 287.21
IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1658($\gamma_{C=C}$), 755 (γ_{C-Cl}).

37. Synthesis of 4-chloro-2-chloromethyl-5-(4-methoxyphenyl)thieno[2,3-*d*]-pyrimidine (VI*vii*)

A mixture of 2-chloromethyl-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*viii*,** 6.1 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (6 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-5-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidine (**VI*vii*).**

M.P. : 122-124^{o}C (124-126^{o}C)[3]; Yield: 90%
Mol. Formula : $C_{14}H_{10}Cl_2N_2OS$; Mol. Wt. 325.21
IR (KBr) cm^{-1} : 3001(γ_{C-H}), 1608($\gamma_{C=C}$), 787(γ_{C-Cl}).
^{1}H NMR ($CDCl_3$)δppm : 3.90 (3H, s, OC*H*$_3$), 4.92 (2H, s, C*H*$_2$ at 2), 6.95-7.55 (5H, m, Ar-*H* & 1*H* at 6).
MS m/e : 325(M^+), 309, 289.

38. Synthesis of 4-chloro-2-chloromethyl-5-(4-chlorophenyl)thieno[2,3-*d*]-pyrimidines (VI*viii*)

A mixture of 2-chloromethyl-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*ix***, 6.2 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-5-(4-chlorophenyl)thieno[2,3-*d*]pyrimidine **(VI*viii*)**.

M.P. : 175-177°C (175-177°C)[3]; Yield: 70%

Mol. Formula : $C_{13}H_7Cl_3N_2S$; Mol. Wt. 329.63

IR (KBr) cm^{-1} : 3030(γ_{C-H}), 1543($\gamma_{C=C}$), 787 (γ_{C-Cl}).

^{1}H NMR ($CDCl_3$)δppm : 4.90 (2H, s, C*H*$_2$Cl), 2.71 (3H, s, C*H*$_3$), 7.20-7.57 (5H, m, Ar-*H* & 1*H* at 6)

MS m/e : 329(M^+), 263, 257.

39. Synthesis of 4-chloro-2-chloromethyl-10*H*-[1,2,4]triazino[6,1-*b*]quinazolin-10-one (VI*ix*)

A mixture of 2-chloromethyl-3*H*-[1,2,4]triazino[6,1-*b*]quinazoline-4,10-dione **(V*xiii*,** 5.2 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (10 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-10*H*-[1,2,4]triazino[6,1-*b*]quinazolin-10-one **(VI*ix*)**.

M.P. : 90-92°C; Yield: 88%

Mol. Formula : $C_{11}H_6Cl_2N_4O$; Mol. Wt. 281.1

IR (KBr) cm^{-1} : 3046(γ_{C-H}), 1608($\gamma_{C=C}$), 756(γ_{C-Cl}).

40. Synthesis of 4-chloro-2-chloromethylbenzo[4,5]thieno[3,2-*d*]pyrimidine (VI*x*)

A mixture of 2-chloromethyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (**V*xiv*,** 5.0 gm; 0.02 mole) and phosphorus oxychloride (6.0 gm; 0.04 mole) were reacted under microwave irradiation (12 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-benzo[4,5]thieno[3,2-*d*]pyrimidine **(VI*x*)**.

M.P. : 220-222°C; Yield: 76%

Mol. Formula : $C_{11}H_6Cl_2N_2S$; Mol. Wt. 269.15

IR (KBr) cm^{-1} : 3040(γ_{C-H}), 1547($\gamma_{C=C}$), 768(γ_{C-Cl}).

41. Synthesis of 4-chloro-2-chloromethyl-9-methoxybenzo[4,5]thieno[3,2-*d*]-pyrimidine (VI*xi*)

A mixture of 2-chloromethyl-9-methoxy-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one (**V*xv***, 5.0 gm; 0.02 mole) and phosphorus oxychloride (5.6 gm; 0.04 mole) were reacted

under microwave irradiation (18 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-9-methoxy-benzo[4,5]thieno[3,2-*d*]pyrimidine **(VI*xi*)**.

M.P. : 180-182^{o}C; Yield: 80%
Mol. Formula : $C_{12}H_8Cl_2N_2OS$; Mol. Wt. 299.18
IR (KBr) cm^{-1} : 2980(γ_{C-H}), 1534($\gamma_{C=C}$), 718(γ_{C-Cl})

42. Synthesis of 4-chloro-2-chloromethyl-7,9-dimethylpyrido[3',2': 4,5]thieno[3,2-*d*]pyrimidine (VI*xii*)

A mixture of 6-chloromethyl-2,4-dimethyl-7*H*-9-thia-1,5,7-triaza-fluoren-8-one (**V*xvi*,** 5.5 gm; 0.02 mole) and phosphorus oxychloride (5.6 gm; 0.04 mole) were reacted under microwave irradiation (10 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-7,9-dimethylpyrido[3',2': 4,5]thieno[3,2-*d*]pyrimidine **(VI*xii*)**.

M.P. : 116-118^{o}C; Yield: 86%
Mol. Formula : $C_{12}H_9Cl_2N_3S$; Mol. Wt. 298.19
IR (KBr) cm^{-1} : 2990(γ_{C-H}), 1629($\gamma_{C=C}$), 730(γ_{C-Cl})

43. Synthesis of 4-chloro-2-chloromethyl-6,7-dimethoxyquinazoline (VI*xiii*)

A mixture of 2-chloromethyl-6,7-dimethoxyquinazolin-4(3*H*)-one (**V*xvii*,** 5.0 gm; 0.02 mole) and phosphorus oxychloride (5.6 gm; 0.04 mole) were reacted under microwave irradiation (6 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-chloromethyl-6,7-dimethoxy-quinazoline **(VI*xiii*)**.

M.P. : 162-164^{o}C; Yield: 90%
Mol. Formula : $C_{11}H_{10}Cl_2N_2O_2$; Mol. Wt. 273.12
IR (KBr) cm^{-1} : 2963(γ_{C-H}), 1502($\gamma_{C=C}$), 743(γ_{C-Cl}).

5.7 Synthesis of condensed 4-chloro-2-chloroethylpyrimidines under Microwave Irradiation (MWI) (**VI*xiv-xvii***)

44. Synthesis of 4-chloro-2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]-pyrimidine (**VI*xiv***)

A mixture of 2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xviii*,** 6.0 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (5 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-(2-chloroethyl)-5-(4-methyl-phenyl)thieno[2,3-*d*]pyrimidine **(VI*xiv*)**.

M.P. : 160-162^{o}C (160-162^{o}C)[9]; Yield: 81%
Mol. Formula : $C_{15}H_{12}Cl_2N_2S$; Mol. Wt. 323.24
IR (KBr) cm^{-1} : 2923(γ_{C-H}), 1496($\gamma_{C=C}$), 794(γ_{C-Cl}).

45. Synthesis of 4-chloro-2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidine (VI*xv*)

A mixture of 2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xix*,** 4.8 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-(2-chloroethyl)-5,6-dimethylthieno[2,3-*d*]pyrimidine **(VI*xv*)**.

M.P. : 40-42^{o}C; Yield: 70%
Mol. Formula : $C_{10}H_{10}Cl_2N_2S$; Mol. Wt. 261.17
IR (KBr) cm^{-1} : 2930(γ_{C-H}), 1478($\gamma_{C=C}$), 841(γ_{C-Cl}).

46. Synthesis of 4-chloro-2-(2-chloroethyl)-5-phenylthieno[2,3-*d*]pyrimidine (VI*xvi*)

A mixture of 2-(2-chloroethyl)-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**V*xx*,** 5.8 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (6 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-(2-chloroethyl)-5-phenylthieno[2,3-*d*]pyrimidine **(VI*xvi*)**.

M.P. : >300°C (>300°C)[9]; Yield: 67%

Mol. Formula : $C_{14}H_{10}Cl_2N_2S$; Mol. Wt. 309.21

IR (KBr) cm^{-1} : 2940 (γ_{C-H}), 1553($\gamma_{C=C}$), 759(γ_{C-Cl}).

47. Synthesis of 4-chloro-2-(2-chloroethyl)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine (VI*xvii*)

A mixture of 2-(2-chloroethyl)-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one **(V*xxii*,** 5.3 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-(2-chloro-ethyl)-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine **(VI*xvii*)**.

M.P. : 62-64°C (68-70°C)[9]; Yield: 90%

Mol. Formula : $C_{12}H_{12}Cl_2N_2S$; Mol. Wt. 287.21

IR (KBr) cm^{-1} : 2939 (γ_{C-H}), 1528($\gamma_{C=C}$), 735(γ_{C-Cl}).

^{1}H NMR ($CDCl_3$)δppm : 1.94 (4H, s, CH_2 at 6 & 7), 2.92 (2H, s, CH_2 at 5), 3.05(2H, s, CH_2 at 8), 3.42 (2H, 7, CH_2CH_2Cl, J = 6.9 & 7.1), 4.05 (2H, 7, CH_2CH_2Cl, J = 6.9 & 7.1).

MS m/e : 287(M^+), 286(M-1), 253, 251, 225, 209.

5.8 Synthesis of condensed 4-chloro-2-methylpyrimidines under Microwave Irradiation (MWI) (VI*xviii*-*xxii*)

48. Synthesis of 4-chloro-2-methyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidine (VI*xviii*)

A mixture of 2-methyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxv*,** 5.1 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (3 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-methyl-5-(4-methylphenyl)thieno[2,3-*d*]pyrimidine **(VI*xviii*)**.

M.P. : 97-99°C; Yield: 65%

Mol. Formula : $C_{14}H_{11}ClN_2S$; Mol. Wt. 274.77

IR (KBr) cm^{-1} : 1553($\gamma_{C=C}$), 791(γ_{C-Cl}).

^{1}H NMR ($CDCl_3$)δppm : 2.47 (3H, s, C*H*$_3$), 4.82 (2H, s, C*H*$_2$ at 2), 7.18-7.50 (5H, m, 4-Ar-*H* and 1*H* at 6)

49. Synthesis of 4-chloro-2,5,6-trimethylthieno[2,3-*d*]pyrimidine (VI*xix*)

A mixture of 2,5,6-trimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxvi*,** 3.8 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2,5,6-trimethylthieno[2,3-*d*]-pyrimidine **(VI*xix*)**.

M.P. : 102-104^{o}C; Yield: 90%

Mol. Formula : $C_9H_9ClN_2S$; Mol. Wt. 212.7

IR (KBr) cm^{-1} : 1560($\gamma_{C=C}$), 841(γ_{C-Cl}).

50. Synthesis of 4-chloro-2-methyl-5-phenylthieno[2,3-*d*]pyrimidine (VI*xx*)

A mixture of 2-methyl-5-phenylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **(V*xxvii***, 4.8 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (6 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-methyl-5-phenylthieno[2,3-*d*]pyrimidine **(VI*xx*).**

M.P. : 235-237^{o}C; Yield: 90%

Mol. Formula : $C_{13}H_9ClN_2S$; Mol. Wt. 260.74

IR (KBr) cm^{-1} : 2932(γ_{C-H}), 1510($\gamma_{C=C}$), 818(γ_{C-Cl}).

51. Synthesis of ethyl 4-chloro-2,5-dimethylthieno[2,3-*d*]pyrimidine 6-carboxylate (VI*xxi*)

A mixture of ethyl 3,4-dihydro-2,5-dimethyl-4-oxo-thieno[2,3-*d*]pyrimidine 6-carboxylate **(V*xxviii*,** 5.0 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (4 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded ethyl 4-chloro-2,5-dimethylthieno[2,3-*d*]pyrimidine 6-carboxylate **(VI*xxi*)**.

M.P. : 280-282^{o}C; Yield: 90%

Mol. Formula : $C_{11}H_{11}ClN_2O_2S$; Mol. Wt. 270.74

IR (KBr) cm^{-1} : 1718($\gamma_{C=O}$), 1534($\gamma_{C=C}$).

^{1}H NMR ($CDCl_3$)δppm : 2.43 (3H, t, C*H*$_3$CH$_2$COO, *J* = 6.9), 3.05 (3H, s, C*H*$_3$ at 5), 4.35 (2H, q, CH$_3$C*H*$_2$COO, *J* = 6.9, 7.2), 4.82 (2H, s, C*H*$_2$ at 2).

52. Synthesis of 4-chloro-2-methyl-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]-pyrimidine (VI*xxii*)

A mixture of 2-methyl-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**V*xxix***, 4.4 gm; 0.02 mole) and phosphorus oxychloride (5.3 gm; 0.04 mole) were reacted under microwave irradiation (5 min) as per procedure described for the compound **VI*i***. The crude product on recrystallization from hexane that yielded 4-chloro-2-methyl-5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidine **(VI*xxii*)**.

M.P. : 157-159^{o}C; Yield: 90%

Mol. Formula : $C_{11}H_{11}ClN_2S$; Mol. Wt. 238.74

IR (KBr) cm^{-1} : 2939(γ_{C-H}), 1413($\gamma_{C=C}$).

5.9 Protocol for MDR Reversal Activity of Condensed Pyrimidines

MDR reversal effects of this series on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) was carried out by Prof. Joseph Molnár at Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Hungary.

MDR reversal effects of V and VI series on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry.

Assay for reversal of MDR in tumour cells[13,14]:

The cells were adjusted to a density of 2×10^6/ml, resuspended in serum-free McCoy's 5A medium and distributed in 0.5-ml aliquots into Eppendorf centrifuge tubes. The tested compounds were added at various concentrations in different volumes (2.0-20.0 μl) of the 1.0-10.0 mg/ml stock solutions, and the samples were incubated for 10 min at room temperature. Next, 10 μl (5.2 μM final concentration) of the indicator rhodamine 123 was added to the samples and the cells were incubated for a further 20 min at 37°C, washed twice and resuspended in 0.5 ml PBS for analysis. The fluorescence of the cell population was measured with a Beckton Dickinson FACScan flow cytometer. Verapamil was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared with the untreated cells. An activity ratio R was calculated via the following equation, on the basis of the measured fluorescence values:

$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\ treated \,/\ parental\,control}$$

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PART-III

Synthesis, Characterization and Anticancer Activity of 3-Aza-Analogues of DP-7

1. Advanced Dihydropyridines and Dihydropyrimidines as Novel Multidrug Resistance Modifiers and Reversing Agents in Cancer Chemotherapy

1. Advanced Dihydropyridines and Dihydropyrimidines as Novel Multidrug Resistance Modifiers and Reversing Agents in Cancer Chemotherapy

1.1 Cancer and the Current Status in World

With more than 10 million new cases every year, cancer has become one of the most devastating diseases worldwide. The disease burden is immense, not only for affected individuals but also for their relatives and friends. At the community level, cancer has posed considerable challenges for the health care systems in poor and rich countries alike. World Cancer Report (WCR) of 2007 provides a unique global view of cancer. It documents the frequency of cancer in different countries and trends in cancer incidence and mortality as well as describing the known causes of human cancer[1] (Table 43).

Table 43: The estimated numbers of new cases and deaths for each common cancer type[2,3]:

Cancer Type	Estimated New Cases	Estimated Deaths
Bladder	67,160	13,750
Breast (Female -- Male)	178,480 -- 2,030	40,460 -- 450
Colon and Rectal (Combined)	153,760	52,180
Endometrial	39,080	7,400
Kidney (Renal Cell) Cancer	43,512	10,957
Leukemia (All)	44,240	21,790
Lung (Including Bronchus)	213,380	160,390
Melanoma	59,940	8,110
Non-Hodgkin's Lymphoma	63,190	18,660
Pancreatic	37,170	33,370
Prostate	218,890	27,050
Skin (Non-melanoma)	>1,000,000	<2,000
Thyroid	33,550	1,530

1.2 Role of ABC Transporters

Cancer chemotherapy is the treatment of choice in many malignant diseases. A major form of resistance against a variety of the antineoplastic agents currently used involves the function of a group of membrane proteins that extrude cytotoxic molecules, thus keeping intracellular drug concentration below a cell-killing threshold. Multidrug transporters belong to the superfamily of ATP Binding Cassette (ABC) proteins, present in organisms from bacteria to humans. The medical significance of ABC proteins exceeds

their role in cancer chemotherapy resistance; the transport function of several members was found to hinder the effective therapy of anticancer agents for many other widespread diseases (*e.g.* malaria, AIDS), and inherited diseases were also linked to mutations in these genes. The transport activity of ABC proteins has an important effect in general pharmacology, that is, in modulating the absorption, distribution and excretion of numerous pharmacological cancer agents.[4]

These substrate molecules exhibit a wide variety of chemical structures. Some ABC proteins facilitate the transport of inorganic ions, whereas others pump various organic compounds, including lipids, bile acids, glutathione and glucuronide conjugates, or even short peptides. Most ABC family proteins utilize the energy of ATP hydrolysis for this transport activity (active transporters), but some ABC transporters form specific membrane channels.[4]

1.3 Structure of ABC Proteins

The typical structure of an ABC protein consists of membrane-embedded transmembrane domains (TMD) and ATP binding domains. Typically, the transmembrane regions anchor the protein to the membrane and form a pore through which the transport of a surprisingly large variety of substrates occurs. The cytoplasmic nucleotide binding domains provide the molecular compartment, where the energy of ATP is released. It is not known how the energy is conveyed from the ABC domains to the site of the transport and the precise mechanism of transport also remains elusive.[4]

1.4 Role of Resistance in Cancer-The Players

Numerous clinical data revealed that the multidrug resistance (MDR) phenotype in tumors is associated with the overexpression of certain ABC transporters, termed as MDR proteins. The P-glycoprotein (Pgp, MDR1, ABCB1)-mediated MDR was the first discovered[5-7] and probably still is the most widely observed mechanism in clinical MDR.[8-11] Soon after the cloning and characterization of MDR1, it became evident that other efflux-pumps may also play a significant role in the transport-associated drug resistance. There are two other ABC transporters, which have been definitively demonstrated to participate in the MDR of tumors: the MDR protein 1 (MRP1, ABCC1), and the mitoxantrone resistance protein[11-15] (MXR/BCRP, ABCG2). Furthermore, other human ABC proteins capable of actively transporting various compounds out of cells may

also be their players in selected cases of MDR. These include ABCB4 (MDR3) and ABCB11 (sister Pgp or BSEP), two proteins residing predominantly in the liver with a function involved in the secretion of phosphatidyl choline and bile acids, respectively.[16-18] MDR3 has been already shown to transport certain drugs as well.[19] In addition to MRP1, five homologues (MRP2-MRP6) have been cloned. Overexpression of MRP2 (an organic anion transporter which can also extrude hydrophobic compounds) was definitively shown to confer cancer MDR.[13,20] MRP3, an organic conjugate transporter, and MRP5, a nucleoside transporter, are also candidate proteins for causing certain forms of drug resistance.[13]

1.4.1 Basic Mechanism of MDR in Cancer

The generally accepted mechanism of MDR is that the MDR proteins actively expel the cytotoxic drugs from tumor cells, maintaining the anticancer drug level below a cell-killing concentration. Drug extrusion mediated by these primary active transporters is driven by the energy of ATP hydrolysis. The most intriguing characteristic distinguishing the MDR proteins from other mammalian transporters is their wide substrate specificity. Unlike other selective (classical) transport proteins, multidrug transporters have been recognized and handled as a wide range of substrates. This wide substrate specificity explains the cross-resistance to several chemically unrelated compounds, the characteristic feature found in the MDR phenotype.[8-11]

Different tumors with MDR protein overexpression (*e.g.* hepatomas, lung or colon carcinomas) often show primary (or intrinsic) resistance to cancer chemotherapy. In addition, cancer chemotherapy itself might induce the overexpression of these proteins, so that the MDR clones become less sensitive to chemotherapy (secondary drug resistance). Treatment failure due to MDR is also found in connection with conditions other than cancer, including some autoimmune disorders and infectious diseases.[21-23]

1.4.2 Nomenclature, Basic Structure and Membrane Topology of MDR Proteins

The ABC superfamily is one of the largest families in proteins. The most recent annotation of the human genome sequence revealed 48 genes for ABC proteins. The ABC proteins were grouped into seven sub-classes, ranging from ABCA to ABCG[24-28] based on genomic organization, order of domains and sequence homology. The phylogenetic tree of the ABC transporters involved in cancer MDR is presented in Figure 1. A thick

line and a circle label the three definite players, while the close relatives, which may have a role in drug resistance, are also indicated on this evolutionary diagram.

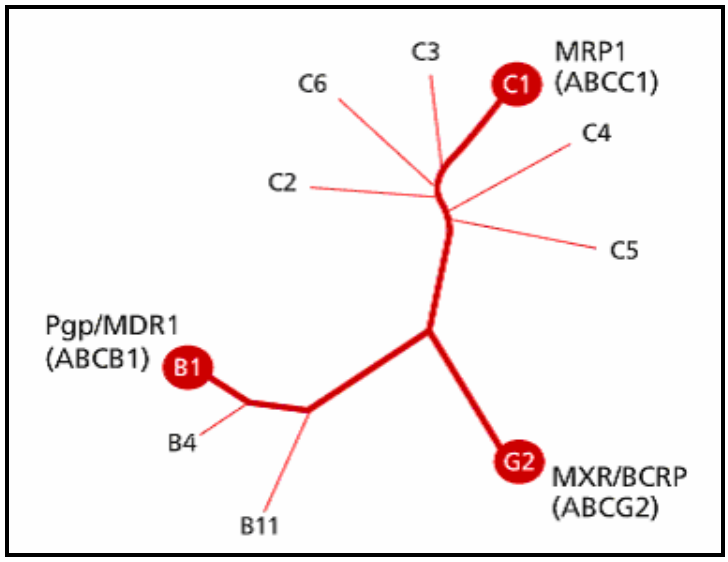


Figure-1. Phylogenic tree of the MDR- related ABC transporters. The thick lines represent the proteins definitively involved in multi-drug resistance. (Reproduced from reference-4)

All ABC proteins contain at least three characteristic peptide sequences: the Walker A and B motifs and the so-called ABC-signature sequence. Whereas the Walker motifs are present in several classes of ATP binding proteins, the presence of the signature region is diagnostic for the ABC proteins. It is generally accepted that the minimum functional unit requirement for an ABC transporter is the presence of two transmembrane domains (TMDs) and two ATP Binding Cassette (ABC) units. These may be present within one polypeptide chain ("full transporters"), or within a membrane-bound homo- or heterodimer of "half transporters".[8-11,27,28] There are no high-resolution structural data presently available for any mammalian ABC transporter; therefore computer modeling and laborious biochemical experiments are necessary to elucidate the position and orientation of membrane spanning segments and other domains within the polypeptide chain. Figure 2 presents the most plausible membrane topology models for the key MDR-ABC transporters. As shown in Figure 2, Pgp-MDR1 (ABCB1) is a "full transporter" with six TM helices in both TMDs of the protein, each followed by an ABC domain. A similar membrane topology has been predicted for ABCB4 (MDR3), and ABCB11 (sister Pgp) as well.[24-28]

MRPs belong to the ABCC-subfamily, comprising eleven members in the human genome. Most of these proteins (ABCC1-6) have been identified as active, ATP-dependent membrane transporters for various anticancer agents and organic anions.[12-14,17] In contrast to these active transporters, the cystic fibrosis transmembrane conductance regulator, ABCC7 (CFTR) is a regulated chloride channel, while ABCC8 (SUR1) and ABCC9 (SUR2) are called sulfonylurea receptors and best described as intracellular ATP sensors, regulating the permeability of specific K^+ channels. Nothing is currently known about the function of ABCC10 and ABCC11.[11,13,27,28]

The predicted membrane topology of MRP1 is shown in Figure 2. According to current notion, in addition to an MDR1-like core, MRP1 contains an additional *N*-terminal segment of about 280 amino acids. A major part of this region is membrane-embedded with five transmembrane helices (TMD0), while a small cytoplasmic loop of about 80 amino acids (L0) connects this area to the core region.[29-32] Recent studies revealed that the TMD0 domain of ABCC1 does not play a crucial role in either the transport activity or the proper routing of the protein. However, the presence of the membrane-associated cytoplasmic L0 region (together with the core region) is necessary for both the transport activity and the proper intracellular routing of the protein. These studies indicate that the L0 region forms a distinct structural and functional domain, which interacts with the membrane and the core region of the MRP1 transporter.[33]

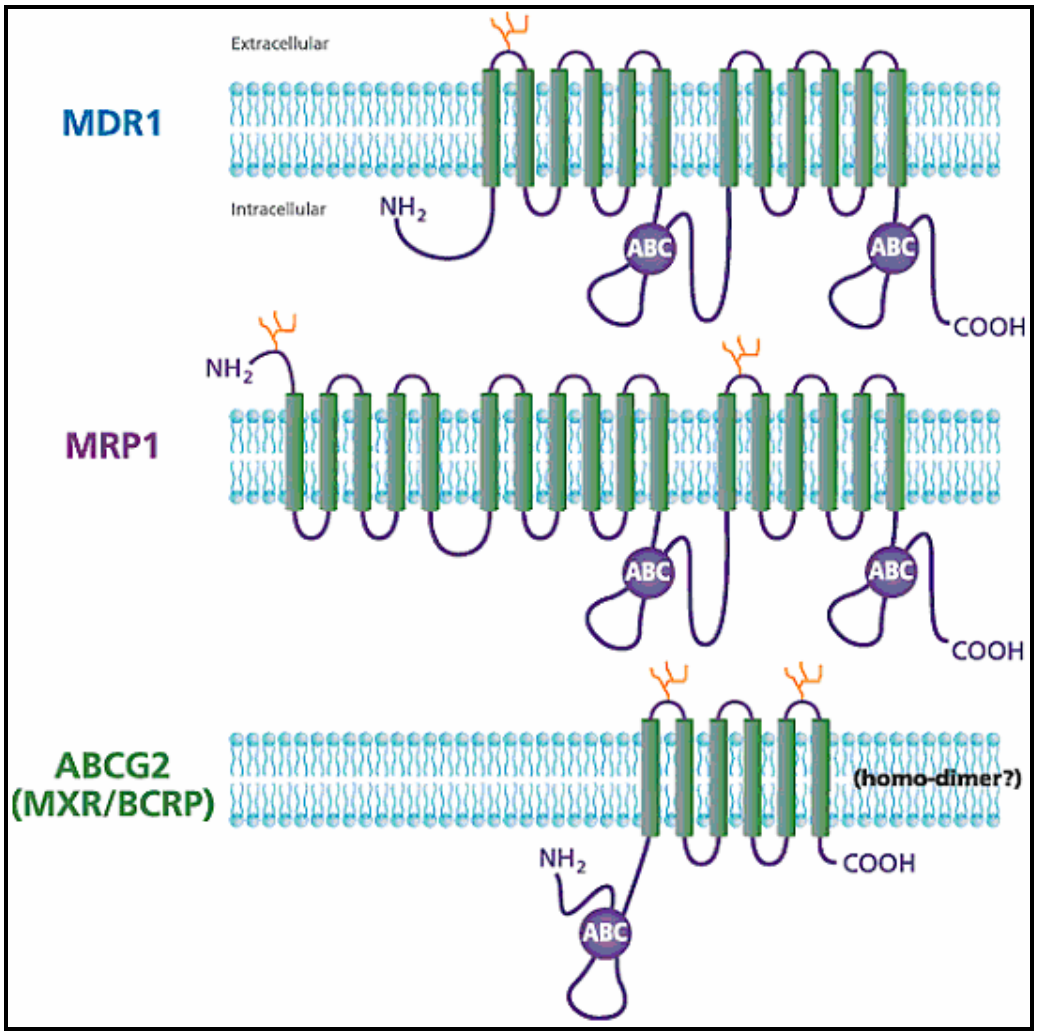


Figure-2. Membrane topology models for the MDR-related ABC transporters. Green Bars represents predicted transmembrane helices, the purple circles represents the ABC domains, the gold tree are glycosylation sites at the extra cellular surface. (Reproduced from reference-4)

The third ABC protein believed to play a role in clinical MDR, ABCG2 (MXR/BCRP) is a half transporter[15,34], with a unique domain arrangement, where the ABC is located at the *N*-terminus (Figure-2). This protein performs an active extrusion of hydrophobic, positively charged molecules from the cells in an *N*-glycosylated mature form, and in contrast to many other ABC half-transporters is probably localized in the plasma membrane. Recently, it has been shown that the human ABCG2 MDR protein forms an active homodimer for its transport function.[35,36]

There is no high-resolution three-dimensional structure available for any of the mammalian ABC transporters, thus the structural background of the MDR molecular mechanism is currently unresolved. A low-resolution structure of the MDR1[37] indicates

that the protein is embedded into the membrane as a cylinder with a large central pore, which is closed at the inner (cytoplasmic) face of the membrane. This structure also included an opening of this cylinder to the lipid phase.

The structure of a bacterial ABC transporter, MsbA of *E. coli*, has recently been determined by X-ray crystallography.[38] MsbA is a half-transporter with a TMD-ABC domain arrangement, organized as a homodimer. The structure reveals that each MsbA subunit contains a transmembrane domain with six transmembrane helices, an ABC-domain, and an "intracellular domain" which is composed of the three intracellular loops connecting the transmembrane segments to the ABC-domain. One of the most important conclusions of the MsbA structure is that the membrane-spanning segments of the polypeptide are indeed α-helices. The organization and interactions of these peptide domains will probably be a valuable foundation towards elucidating the structures of mammalian multidrug transporter ABC proteins.

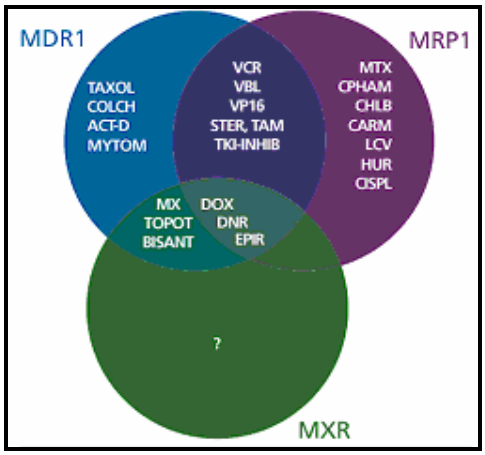
1.4.3 Substrate Specificity of MDR-ABC Transporter

The three major MDR proteins are highly promiscuous transporters; they share the ability of recognizing and translocating a large number of structurally diverse, mainly hydrophobic compounds. In addition to their overlapping substrate specificity, each transporter can handle unique compounds.

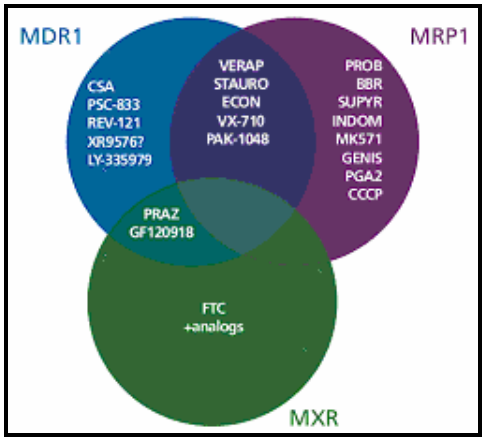
Pgp is a transporter for large hydrophobic, either uncharged or slightly positively charged compounds, while the MRP family primarily transports hydrophobic anionic conjugates and extrudes hydrophobic uncharged anticancer drugs. The MRP1-related uncharged drug transport is quite an enigma, and is somehow linked to the transport or allosteric effect of cellular free reduced glutathione[13]. The exact spectrum of the MXR (ABCG2) transported substrates has not yet been explored in detail, and these studies are complicated by the variable substrate-mutants of MXR observed in the most recent studies.[39]

In order to put the MDR substrates in their medical and pharmacological context, we present some of the key molecules in separate figures. Figure 3A shows anticancer drugs, which are, unfortunately for the patients, also MDR substrates. Figure 3B shows the chemical MDR modulators used experimentally or in clinical trials, while Figure 3C

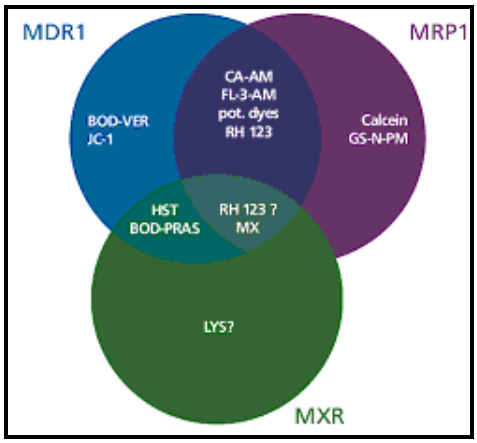
compiles the best-known MDR substrates used for functional diagnosis of the proteins.[8-11,40-43]



3A: MDR-substrate anticancer agents. Abbreviations: VCR: vincristine, VP-16: etoposide, STER: steroids, TAM: tamoxiphen, TKI-INHIB: tyrosin kinase inhibitors e.g. STI-571, DOX: doxorubicine or adriamycin, DNR: daunorubicin, , EPIR: epirubicin, MX: mitoxantrone, TOPOT: topotecan, iridotecan, BISANT: bisanthrone, COLCH: colchicin, ACT-D: actinomycin D, MYTOM: mytomycin, TX: methotrexate, CPHAM: cyclophosphamide, CHLB: chlorambucil, CARM: carmustine, LCV: leucovorin, HUR: hydroxy urea, CISPL: cisplatin, TAXOL: paclitaxel. (Reproduced from reference-4)



3B: MDR-Modulating agents. Abbreviations: CSA: cyclosporin A, VERAP: verapamil STAURO: staurosporine, ECON: econazole, PRAZ: prazosine, FTC: fumitremorgin C, PROB: probenecide, BBR: benzbromarone, SUPYR: sulfinpyrazone, INDOM: indomethacin, GENIS: genistein, PGA2: prostaglandin A2, CCCP: chlorocarbonyl cyanide phenylhydrazine. (Reproduced from reference- 4)



3C: Fluorescent Compounds for the functional detection of multi drug resistance. Abbreviations: CA-AM: calcein AM, FL-3-AM: fluo-3AM, Pot. Dyes: potentiomeric dyes, RH123: rhodamine123, HST: Hoechst dye No. 33342, GS-N-PM: N-Pyrenemaleimide glutathione conjugate, BOD-VER: BODIPY verapamil, BOD-PRAS: BODIPY prazosin, MX: mitoxantrone, LYS: LysoTracker dye. (Reproduced from reference-4)

Figure-3. Venn-diagram for selected compounds interacting with the key MDR-related ABC transporters.

1.4.4 Cellular and Tissue Distribution of MDR-ABC Transporter

The tissue distribution of the MDR-ABC proteins is as varied as their substrate specificity. MRP1 is almost ubiquitously expressed, while the expression of Pgp is more restricted to tissues involved in absorption and secretion.[8-11] High level MDR1 expression has also been shown in certain pharmacological barriers of the body, such as the blood-brain barrier (BBB) and the choroid plexus.[44,45] It has been reported that MXR is highly expressed in the placenta, liver, and most interestingly, in various stem cells.[34-46] All multidrug transporters are localized predominantly in the plasma membrane. In polarized cells, Pgp-MDR1 is localized in the apical (luminal) membrane surface (*e.g.* in the epithelial cells of the intestine and the proximal tubules of kidney, or in the biliary canalicular membrane of hepatocytes).[47-49] In contrast, MRP1 expression in polarized cells is restricted to the basolateral membrane. The expression of MRP2, MDR3, and of Sister Pgp (BSEP) is predominant in the canalicular membrane of hepatocytes, while MRP3 and MRP5 are expressed in the basolateral membranes of these cells (Figure 4). MRP2 is also highly expressed in the apical membranes of kidney proximal tubules. In polarized cells, the MXR expression was reported to be mostly apical.[50]

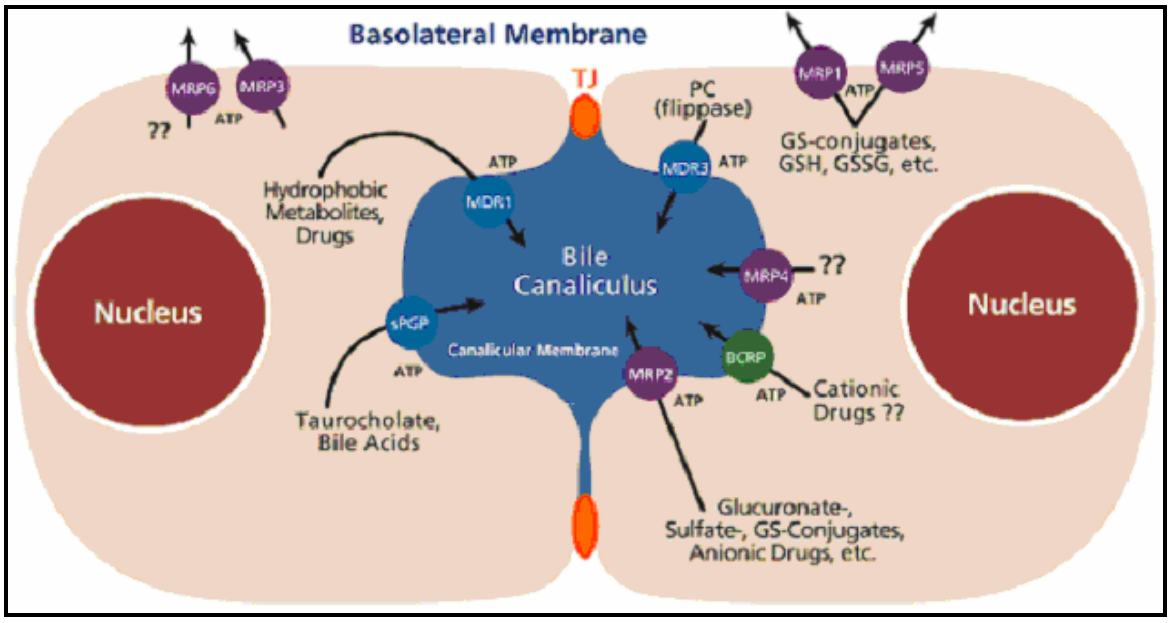
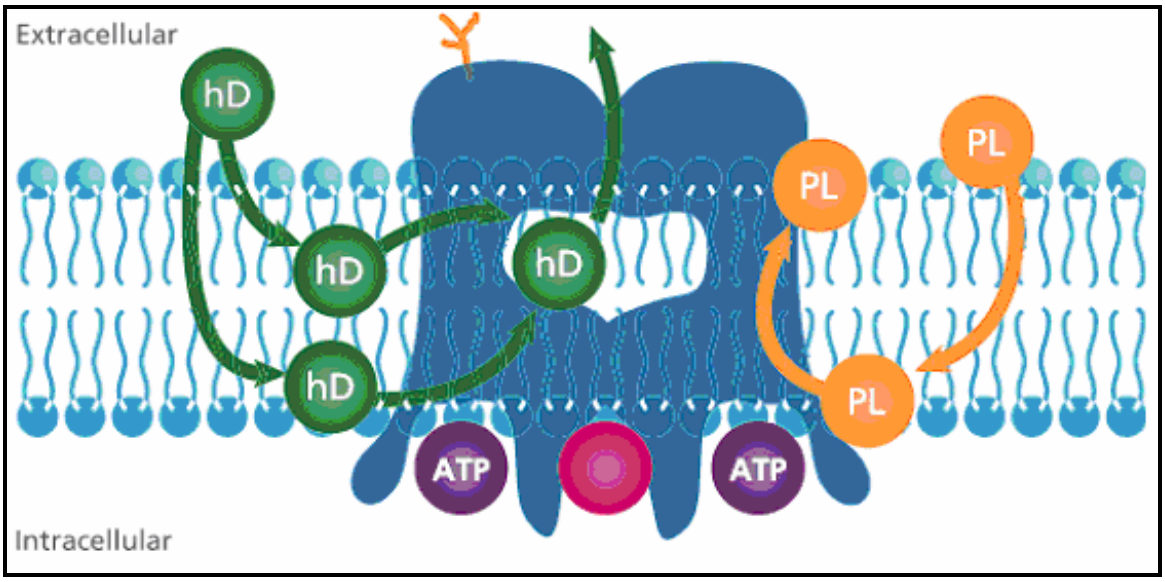


Figure-4. Multi-drug transporters in the human liver hepatocytes. Abbreviations: TJ, tight junction. (Reproduced from reference- 4)

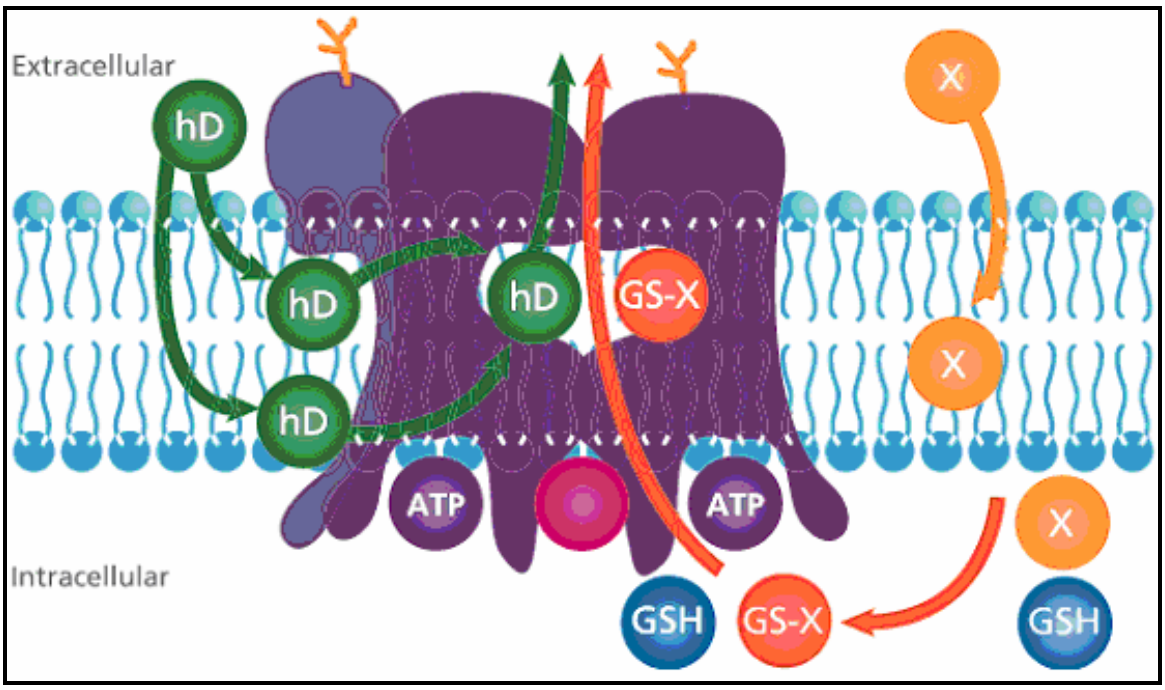
1.4.5 Molecular Mechanism of the Multidrug Pumps

Drug transport by MDR proteins requires the energy of ATP-hydrolysis, controlled by drug interaction, and closely coupled to the actual drug translocation. Interaction with the drug-substrate significantly enhances the basal ATPase activity of the multidrug transporters, that is, the transported drug-substrates increase the rate of ATP cleavage.[51-53] The schematic pictures of the proposed molecular mechanisms of the MDR1 and MRP1 proteins, as depicted in Figure-5.

The site(s) in multidrug transporters interacting with the drug-substrates are probably encoded in the transmembrane domains. Detailed mutagenesis studies of MDR1 and photochemical labeling with the reactive drug-derivatives revealed that transmembrane helices 5 and 6 (in the *N*-proximal transmembrane domain), helices 11 and 12 (in the C-proximal transmembrane domain), as well as the short cytoplasmic loops connecting these helices, are involved in the formation of an extended drug-binding site(s).[54] There are strong indications that the hydrophobic substrates of MDR1 are recognized within the membrane bilayer or in its vicinity, and this type of recognition makes the MDR1 protein a highly effective pump, preventing the cellular entry of toxic compounds.[55] In the case of MRP1 a similar picture has emerged. Recent studies have explored some parts of the transmembrane domains involved in drug interactions.[56]



5A: MDR1-P-glycoprotein (substrates are recognized in, or near to the membrane lipid phase). Abbreviations: hD: hydrophobic drugs, PL: Phospholipids. (Reproduced from reference- 4)



5B: MRP1. Both hydrophobic drugs and anionic conjugates, such as glutathione, are transported. The transport of some hydrophobic drugs may be coupled to reduced gluthatione (GSH) as GS-X molecules. (Reproduced from reference- 4)

Figure-5. Possible model for the molecular mechanism of multidrug transporters.

Based on the three-dimensional structures of bacterial ABC-units, the nucleotide binding sites appear as shallow, more or less open grooves, forming atypical active sites. The close interaction of the two ABC units' likely results in the formation of a fully competent catalytic site. The regions connecting the ABC units to the transmembrane domains have

an active key role in the transfer of conformational information within the protein, and the ABC signature region may have a special function in this regard.[57]

The transport and ATPase cycle of the MDR proteins is blocked by vanadate, a phosphate-mimicking inhibitory anion, which stabilizes a transition state intermediate of the ATPase cycle. An occluded nucleotide in the catalytic sites is locked within the ABC protiens in this interaction. Similar to their ATPase activity, the rate of the vanadate-dependent nucleotide occlusion in MDR-ABC proteins is greatly accelerated by the transported drug-substrates.[58] It has recently been shown, that in the case of MDR1 the MDR1*MgADP*Vi complex exhibits a dramatically reduced binding affinity for the transported drug substrate, as compared to the MDR1*MgATP complex.[59] This observation suggests that the hydrolytic step triggers conformational changes, which reduce drug binding to the binding site (and presumably makes drug binding to another site favorable, from which the drug can be released to the extracellular space).

1.5 MDR Modulators

Considerable interest exists in circumventing MDR by a variety of strategies. The pharmacological approach began with the report by Tsuruo that the calcium channel blocker verapamil and a phenothiazine derivative trifluperazine potentiate the activity of vincristine.[60] MDR modulators (MDR reversal agents, MDR inhibitors, chemosensitizers) can be defined as compounds that permit the anticancer drug to reenter the cell by occupying the protein active or allosteric site(s), or by altering the physicochemical properties of the biomembranes.

The very heterogeneous chemical structure of the compounds with MDR reversal activity has prevented structure-activity studies, although most MDR inhibiting molecules share a basic structural pattern comprising a cationic protonable site linked to an aromatic lipophilic part by a spacer of variable length.[61] Structure-activity relationship (SAR) studies yielded only qualitative indications[62-64] unless very homogeneous series of molecules are studied.[65]

Most modulators identified interfere with Pgp by competitive or noncompetitive inhibition[66] of its drug effluxing activity. The modulators are normally Pgp substrates, but some of them can only bind to the protein but are not effluxed from the cells, and can thus

be considered as pure antagonists. At least two other types of binding sites have been identified in the Pgp in addition to the ATP site, one for transport and other for modulation. It is, therefore, unknown whether one or more pharmacophores exist in the Pgp. The problem is complicated by the possible existence of mutant forms of the Pgp in different tumors with modified responses to modulators.

Furthermore, the expression and function of the Pgp can be modulated by indirect mechanisms, such as interactions with membrane lipids[67] or inhibition of protein kinase C. The reversal of MDR is established using tumor cells lines that are made resistant by the exposure to an anticancer agent or by transfection of the *mdr1* or *mrp1* genes. The parameter most widely used to show the activity of MDR reversal agents is reversal factor (RF). This type of assay assumes that the reversal agent does not show inherent cytotoxicity at the concentrations tested.

The function and structure of ABC transporters along with their role and also in acquired immunodeficiency syndrome (AIDS) related lymphoma has been reviewed recently.[68-72] The MDR modulators according to their chemical structures includes the arylalkylamines including verapamil and its analogs (verapamil **1**, devapamil **2** *etc.*), tiapamil and its analogs (tiapamil **3** and DMDP **4**) and miscellaneous arylalkylamines (SR33557) **5**, aryloxypropanolamines (propafenone-related compound **6**, quinolyloxypropanolamine derivatives (MS-073) **7**, anthranylamides (XR9576) **8**, salicylamides **9** and related derivatives, nitrogen heterocycles including pyrrole derivatives (A-30312 **10**, HWL-12 **11**), staurosporine and analogs (NA-381 **12**, NA-382 **13** and SF-2370 **14**), indole derivatives (yohimbine **15** and reserpine **16**), quinoline and isoquinoline derivatives (chloroquine **17**, mefloquine **18** and quinine **19**), acridin-9-ones and related compounds (GF-120918 (GG-918)) **20**, quinazolines (AV-200) **21**, phenothiazines and related heterocycles[73] (flupentixol **22** and trifluoperazine **23**, pteridines and related condensed heterocycles (BIBW22BS) **24**, 1,3,5-triazines and related compounds (S-9788) **25**, oxygen heterocycles includes pyran derivatives **26**, flavonoids (kaempferol **27** and quercetin **28** and coumarin derivatives (novobiocin **29**), glutathione-related compounds as MRP reverters (MK-571) **30**, cyclic peptides (cyclosporin A **31**, SDZ PSC 833 **32**), depsipeptides (SDZ 280-446) **33** and *macrolactones and macrolactams* (FK506 **34** and VX-710 **35**, steroids and related derivatives (megestrol acetate **36**, medroxyprogesterone acetate **37**), terpenes and miscellaneous lipophilic compounds (taxuspine **38** and taxinine

derivatives **39**) and most important dihydropyridines.[74] Structures of representing molecules from each class are given in Figure 6.

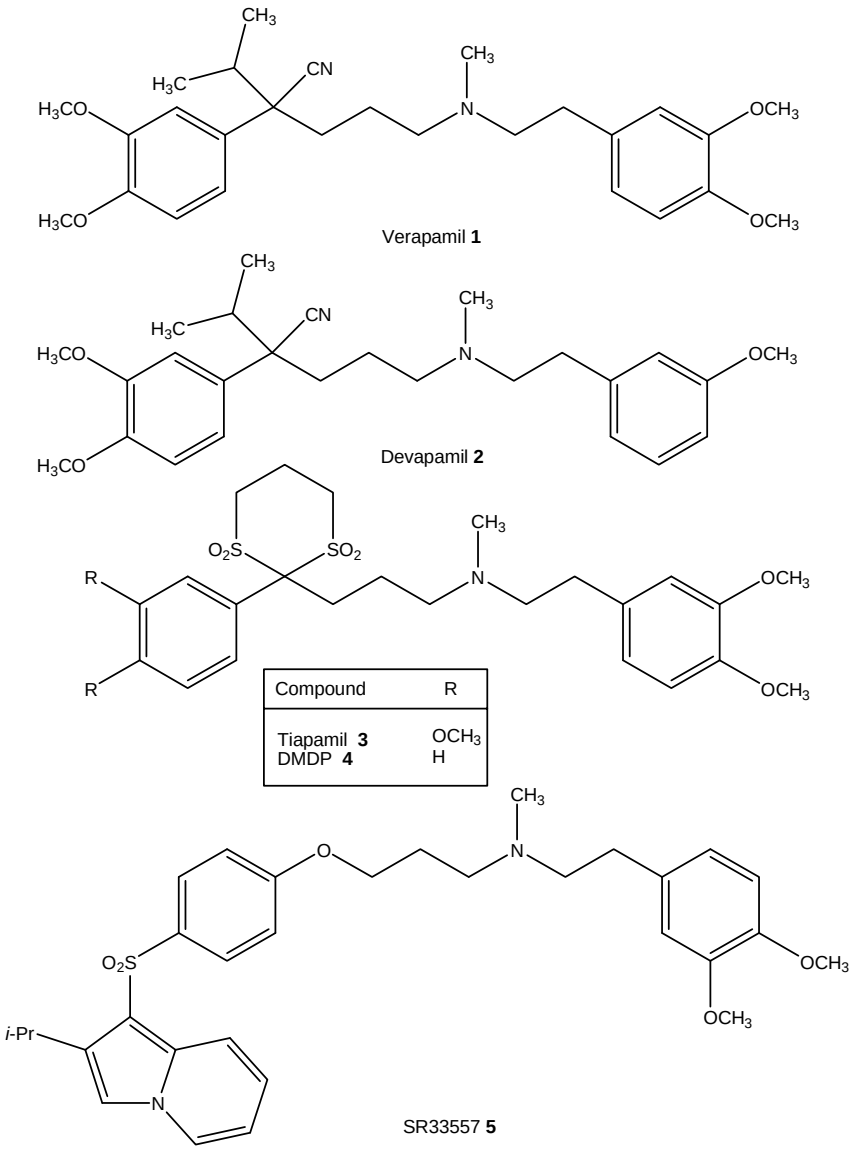


Figure-6. Structures of various classes of drugs used as MDR reversal agents (contd.1)

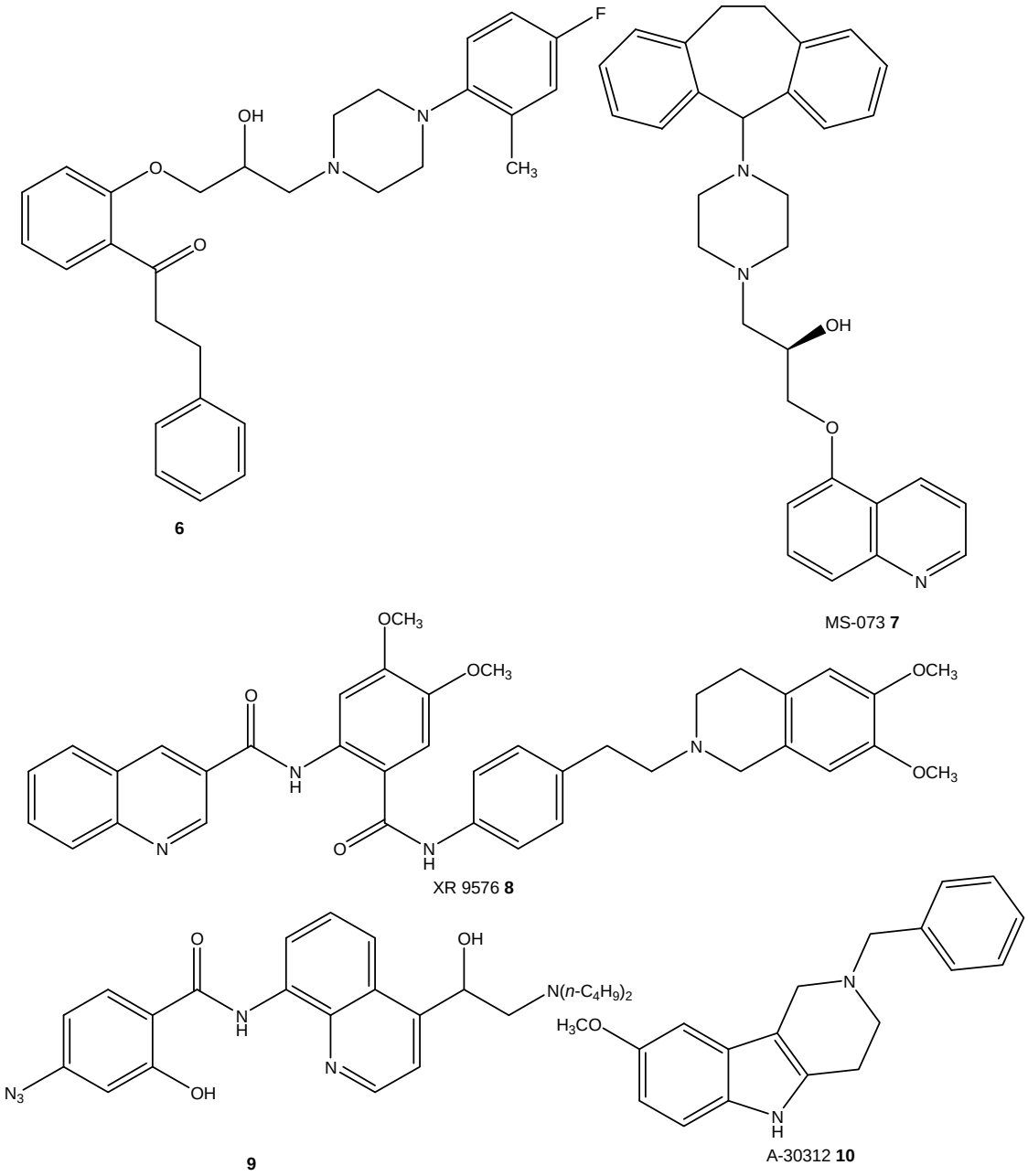
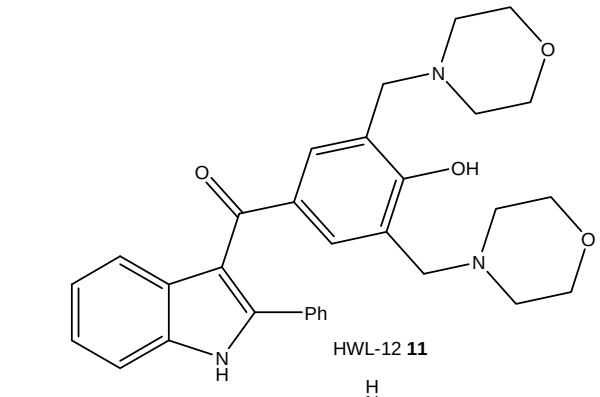
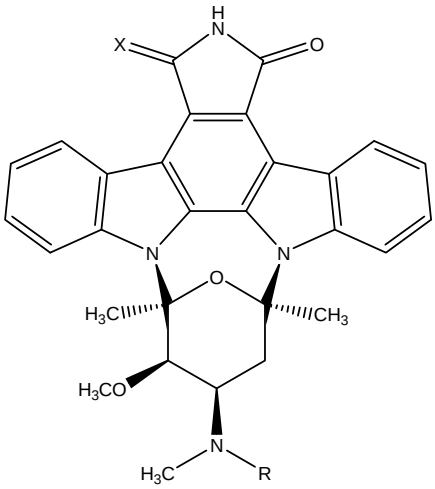


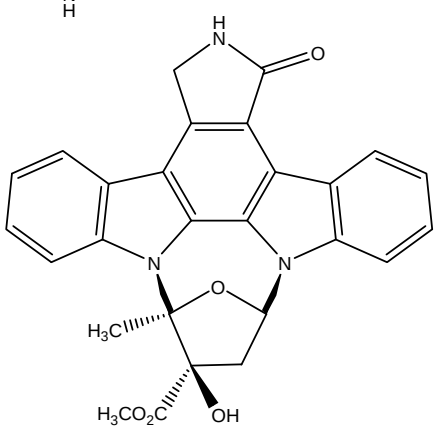
Figure-6. Structures of various classes of drugs used as MDR reversal agents (contd.1)



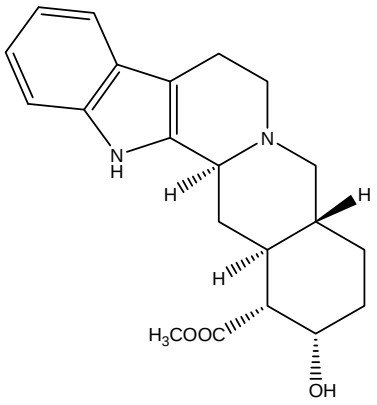
HWL-12 **11**



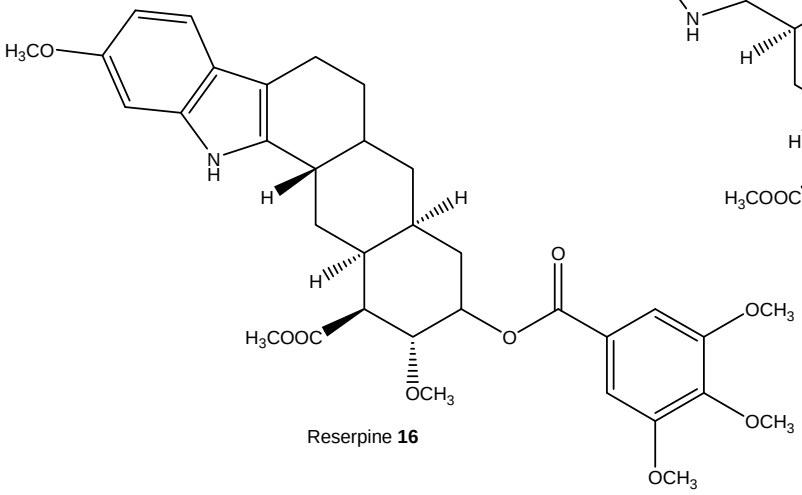
Compound	R	X
NA-381 **12**	CO_2Et	H
NA-382 **13**	CO_2Et	O



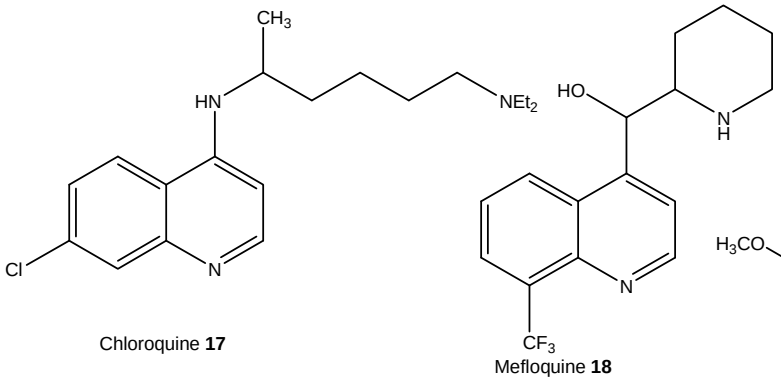
SF-2370 (K-252a) **14**



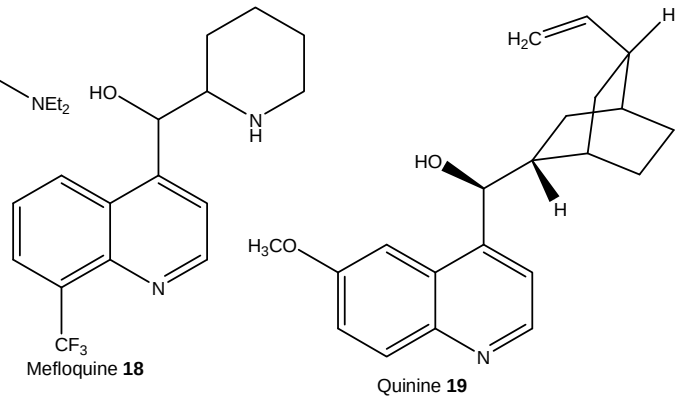
Yohimbine **15**



Reserpine **16**



Chloroquine **17**



Mefloquine **18**

Quinine **19**

Figure-6. Structures of various classes of drugs used as MDR reversal agents (contd.)

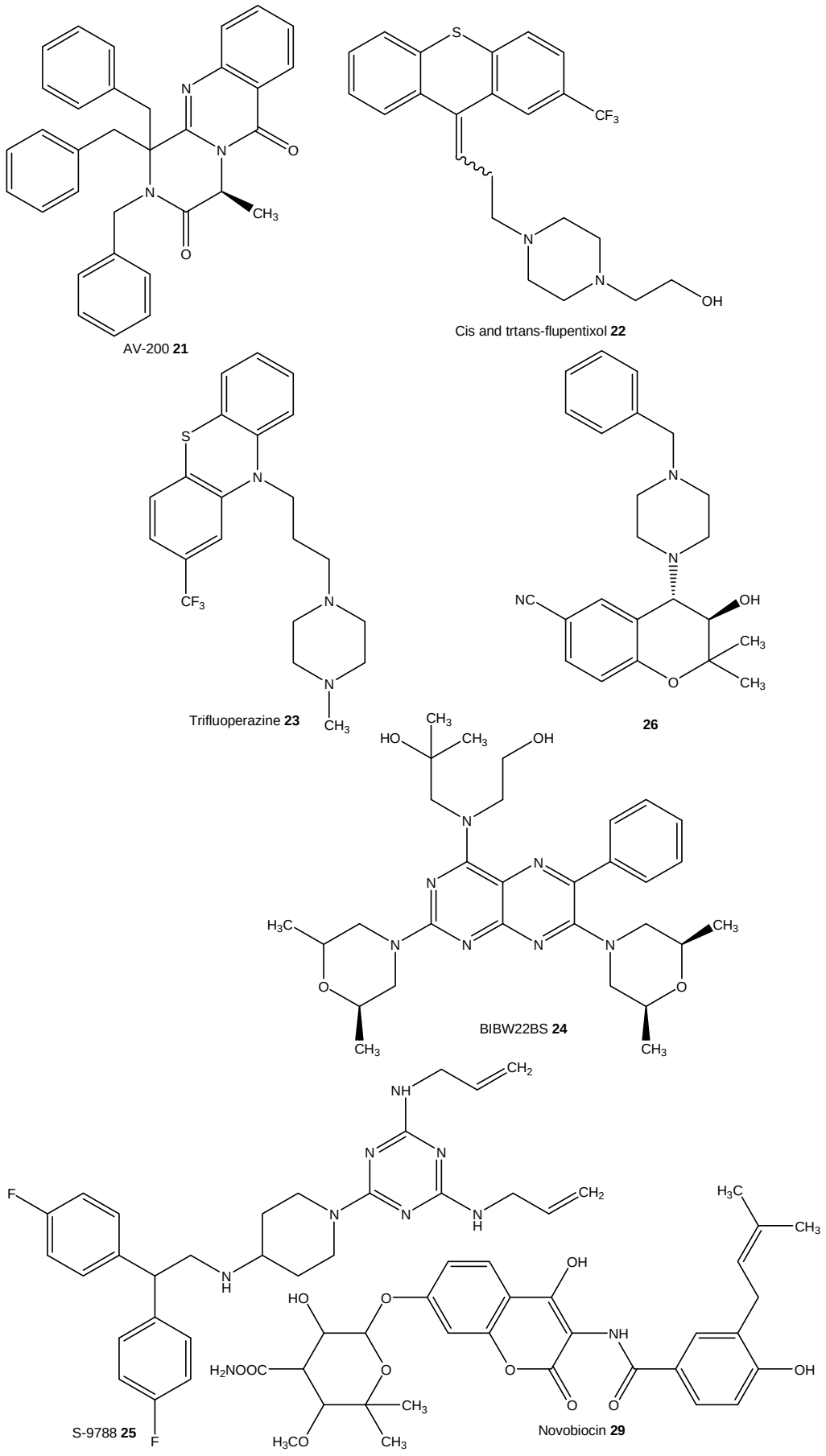


Figure-6. Structures of various classes of drugs used as MDR reversal agents (contd.)

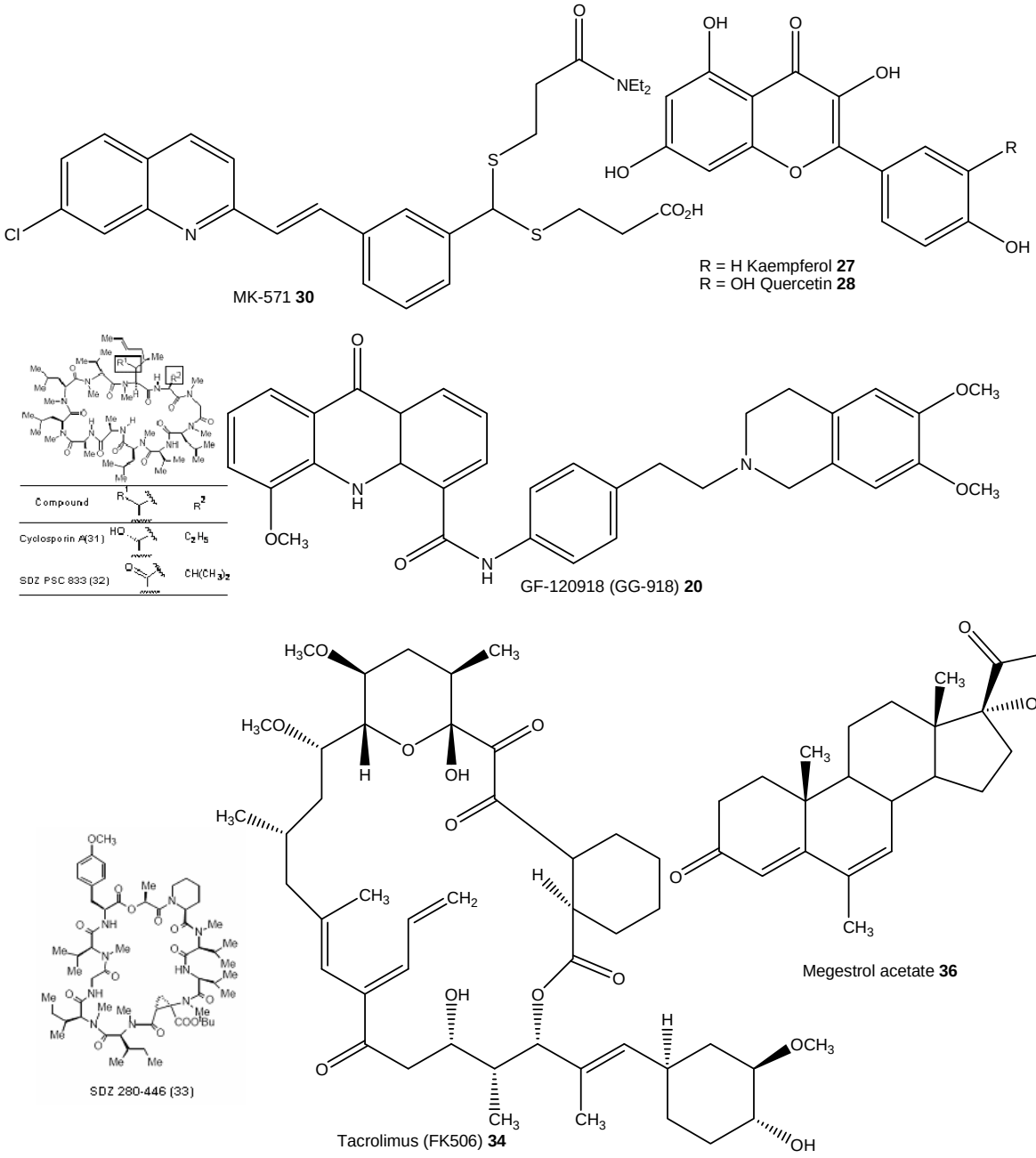


Figure-6. Structures of various classes of drugs used as MDR reversal agents (contd.)

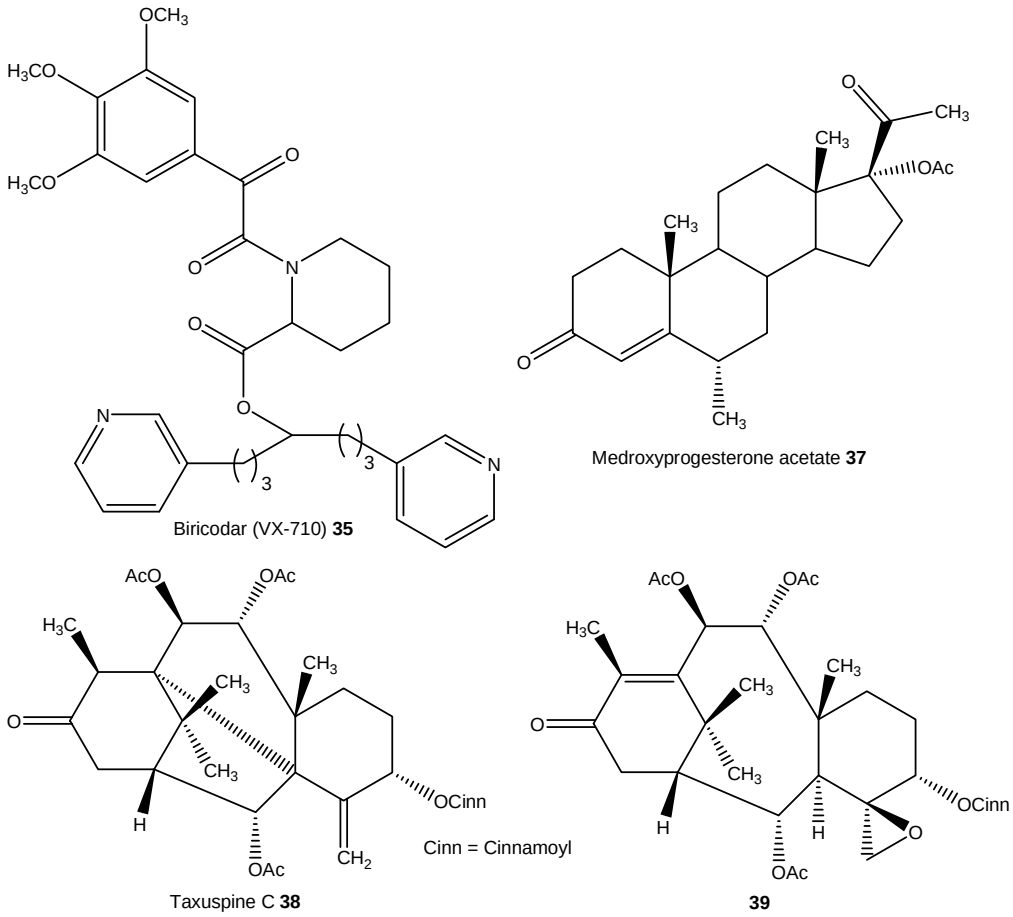


Figure-6. Structures of various classes of drugs used as MDR reversal agents

1.6 DHPs as Potential MDR Reversal Agents

Historical Background:

Some members of calcium channel blockers, such as nicardipine **40** and nimodipine **41**, were identified as potent MDR antagonists. This early work stressed the lack of correlation between the calcium channel blocking and anti-MDR potencies.[75] It has been reported that DHPs bind to a site which is allosterically coupled to the receptor site which binds anticancer agents and other MDR reversal agents.[76,77] DHPs are well recognized as "privileged structure" for their multi receptor affinity.[78,79]

In the derivatives bearing a stereogenic center at C-4, such as nicardipine, nimodipine, nitrendipine **42**, felodipine **43**, isradipine **44** and niguldipine **45**, both stereoisomers differ markedly in their potencies as calcium channel blockers but they are about equally

effective as MDR reversal modulators.[80,81] This has led to use of the *R* isomers as MDR modulators, as in the case of dexniguldipine **45**.

The ability to overcome MDR in many 1,4-DHPs varies considerably with the nature of the 3,5-substituents. The pyridylalkyl esters are specially suitable, as in the case of NIK-250 **46**[82,83] and related derivatives bearing dihydro-1,4-dioxene, dihydro-1,4-dithiane or dihydropyran substituents at C-4.[84] Other representatives of this group that contain an alkyl group at C-4 **47** have also shown potent and selective anti-MDR activity.[85] Compounds PAK-200 **48**[86,87] and PAK 104P **49**[88] exemplify the absence of correlation between calcium channel and MDR antagonism, since neither *N*-alkyl-1,4- DHPs nor pyridines have significant calcium channel blocking activity.

A systematic study of *N*-alkylated DHPs as MDR modulators has shown that the derivatives with an arylalkyl substituent on the nitrogen atom were more active than verapamil in potentiating the anticancer activity of vincristine in *in vitro*, but not in *in vivo*. However, the additional introduction of basic substituents in the C-3 ester group led to DHPs with *in vivo* activity[89] (*e.g.* compound **50**).

The most widely studied anti-MDR DHPs is dexniguldipine hydrochloride **45** (DNIG).[90,91] In preclinical studies, it was particularly effective in taxane resistances of ovariam carcinoma MDR cell lines, where other chemosensitizers were rather ineffective.[92] Besides its ability to reverse the MDR, dexniguldipine is a potent anticancer agent with well-documented anti-protein kinase C activity[93,94] and it inhibits cleavage and relegation reactions of eukaryotic DNA topoisomerase I in a similar fashion to campothecin.[95]

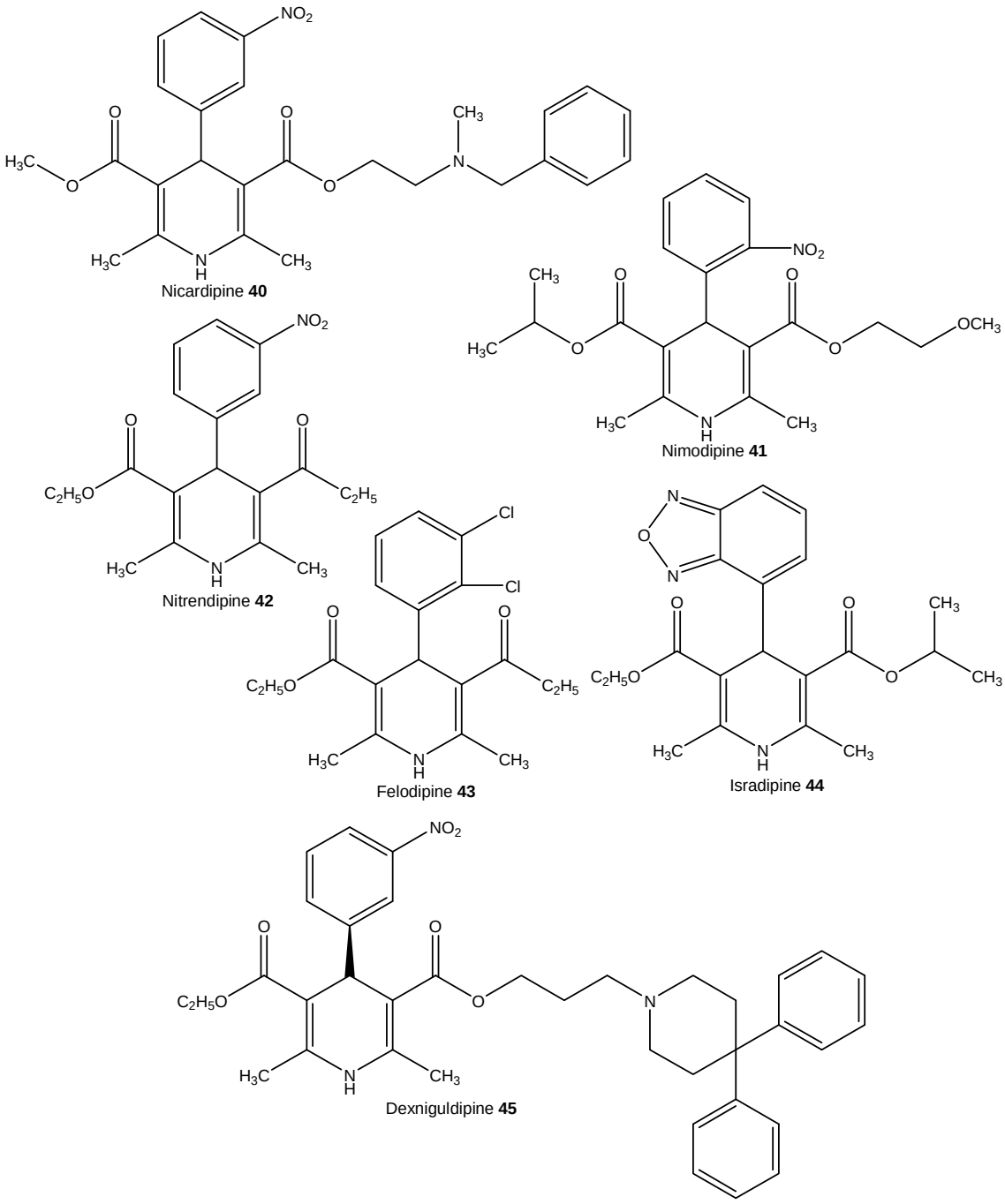


Figure-7. Structures of various DHPs known as MDR reversal agents (contd.)

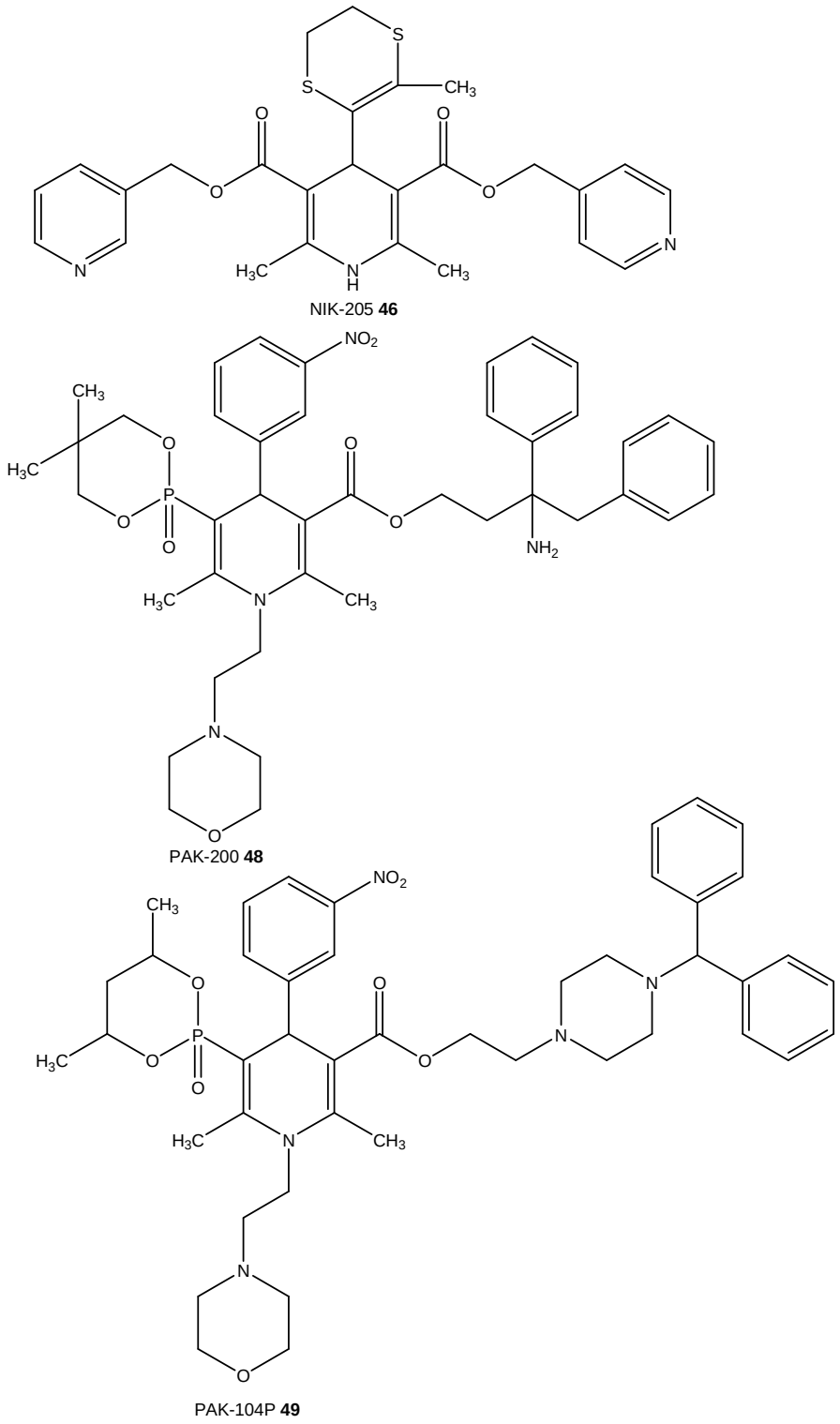


Figure-7. Structures of various DHPs known as MDR reversal agents (contd.)

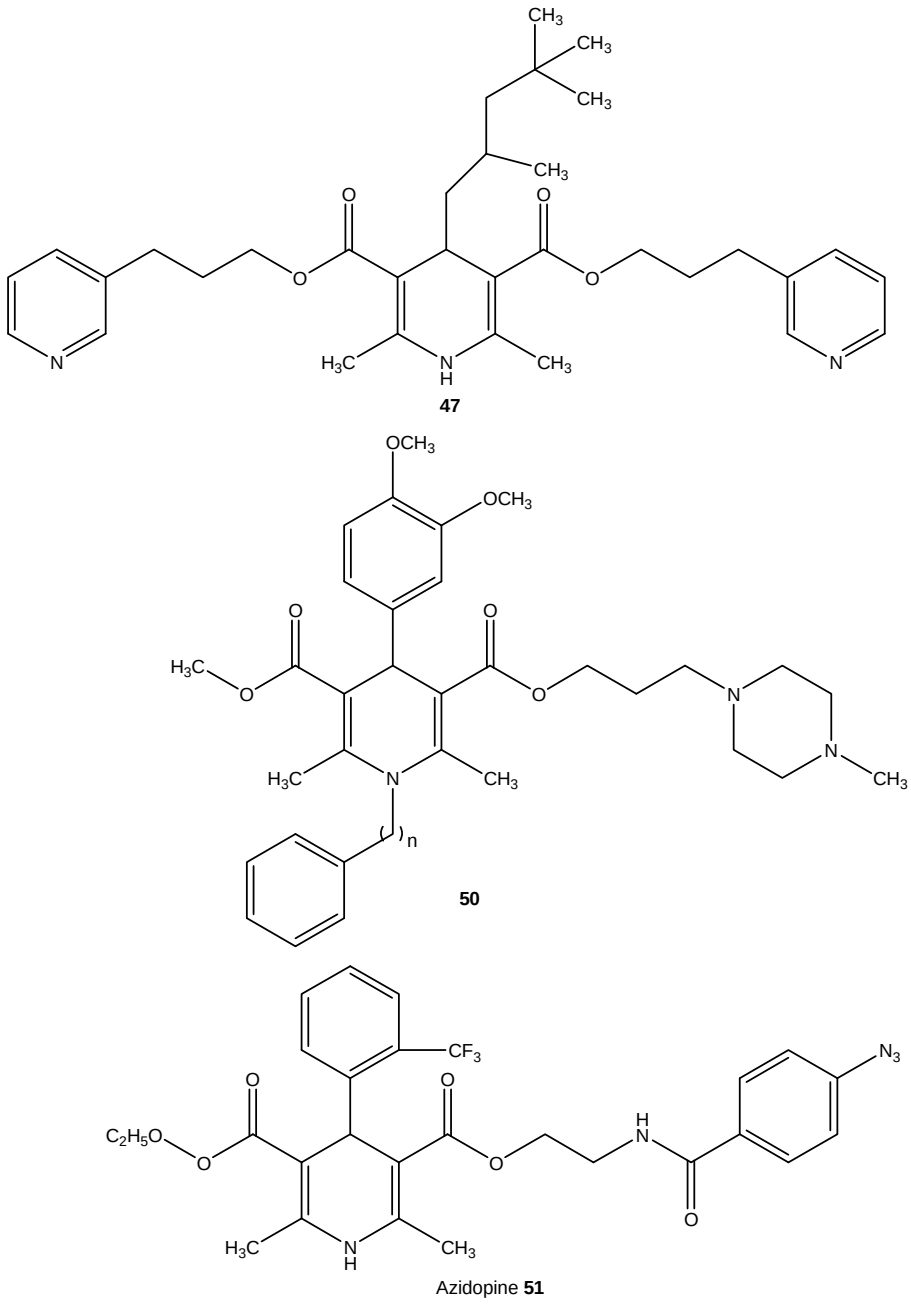


Figure-7. Structures of various DHPs known as MDR reversal agents

Over and above, these clinically established molecules, some other DHPs could also highlight themselves for their potent MDR reversal property.

[3*H*]azidopine **51**, a radioactive photoactive DHPs calcium channel blocker, photolabels Pgp in membrane vesicles from KBCl cells. This photolabeling was almost completely inhibited by the excess DHPs analogues that reversed or lowered drug resistance. In contrast, the labeling was not significantly inhibited by analogues that do not reverse resistance. Inferencing from this Kamiwatari *et al.,* screened a series of DHPs analogues

for their MDR reverting ability in human KB cells. PAK-1 **52** was found to be a weaker calcium channel-blocking activity, when compared with other members of the series including the standard nifedipine but completely reverses the drug resistance. Though, nifedipine **53** and other analogs are better at blocking calcium channels than PAK-1, but they only partially reverse the resistance.[96]

Two isomers of teludipine **54**, *R*-enantiomer (GR66234A) and *L*-enantiomer (GR66235 A) which were originally developed as a new lipophilic calcium channel blocker by Glaxo were evaluated for daunorubicin resistance reversal activity and found to be more effective than verapamil. Additionally, the difference in activity was also found on different cells. Verapamil and the enantiomers of teludipine are more active in ARNII cells than in MCF 7/R cells. There were no apparent differences in cellular daunorubicin accumulation between ARNII and MCF 7/R following exposure to teludipine, no differences in intracellular daunorubicin distribution in the presence of either MDR reversing agent was observed.[97]

In an attempt to characterize chemosensitizer domains on Pgp, Boer *et al.,* found that DHPs label multiple chemosensitizer domains on Pgp, distinct from the vinblastine interaction site. (-)-[3*H*]BZDC-DHPs **55** represents a valuable tool to characterize the molecular organization of chemosensitizer binding domains on Pgp by both reversible binding and photo-induced covalent modification. It provides a novel simple screening assay for Pgp active drugs. Photoreactive DHPs, BZDC-DHPs (2,6-dimethyl-4-(2-(trifluoromethyl)-phenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid {2-[3-(4-benzoyl-phenyl)propionylamino]ethyl} ester ethyl ester), and its tritiated derivative were synthesized as novel probes for human Pgp. (-)-[3*H*]BZDC-DHPs specifically photolabeled Pgp in membranes of multidrug-resistant CCRF-ADR5000 cells. In reversible labeling experiments a saturable, vinblastine-sensitive and high-affinity binding component was present in CCRF-ADR5000 membranes but absent in the sensitive parent cell line. Binding was inhibited by cytotoxics and known chemosensitizers with a Pgp. The DHPs such as niguldipine and a structurally related pyrimidine stereoselectively stimulated reversible (-)-[3*H*]BZDC-DHPs binding, suggesting that more than one DHPs molecule can bind to Pgp at the same time.[98-99]

B859-35, a DHP, which was previously shown *in vitro* to be highly effective in reversing MDR of Pgp positive tumor cell lines, such as the adriamycin (ADR) resistant erythroleukemia F4-6RADR cells. In *in vivo* studies, B859-35 was highly active in reducing the number of viable cells in the resistant tumor nodule by 67±9%. This model provides evidence that even *in vivo*, MDR modulators can be effective in reversing drug resistance. In addition, it presents a potentially useful and rapid preclinical system for *in vivo* studies on the modification of drug resistance.[100]

The modulatory activity of the novel pyridine analogue PAK-104P on MRP-mediated resistance to doxorubicin and paclitaxel was investigated in two doxorubicin-selected human tumor cells [HT1080/DR4 (sarcoma) and HL60/ADR (leukemia)]. The experiment demonstrated that PAK-104P was effective in restoring cellular doxorubicin concentrations in resistant cells to levels comparable to those obtained in parental cells. In addition to reversing Pgp-mediated MDR, the pyridine analogue provides an example of an effective *in vivo* modulator of MRP-mediated MDR.[101]

From QSAR studies of several hundreds of DHPs, seven DHPs were found to be very active. From these predictions, manidipine (CV-4093) **56**, a newly synthesized DHPs calcium channel blocker, were predicted to be an extremely active MDRR agent. The probability for the DHP to show MDRR activity is very high (99%), owing to the presence of several biophores.[102]

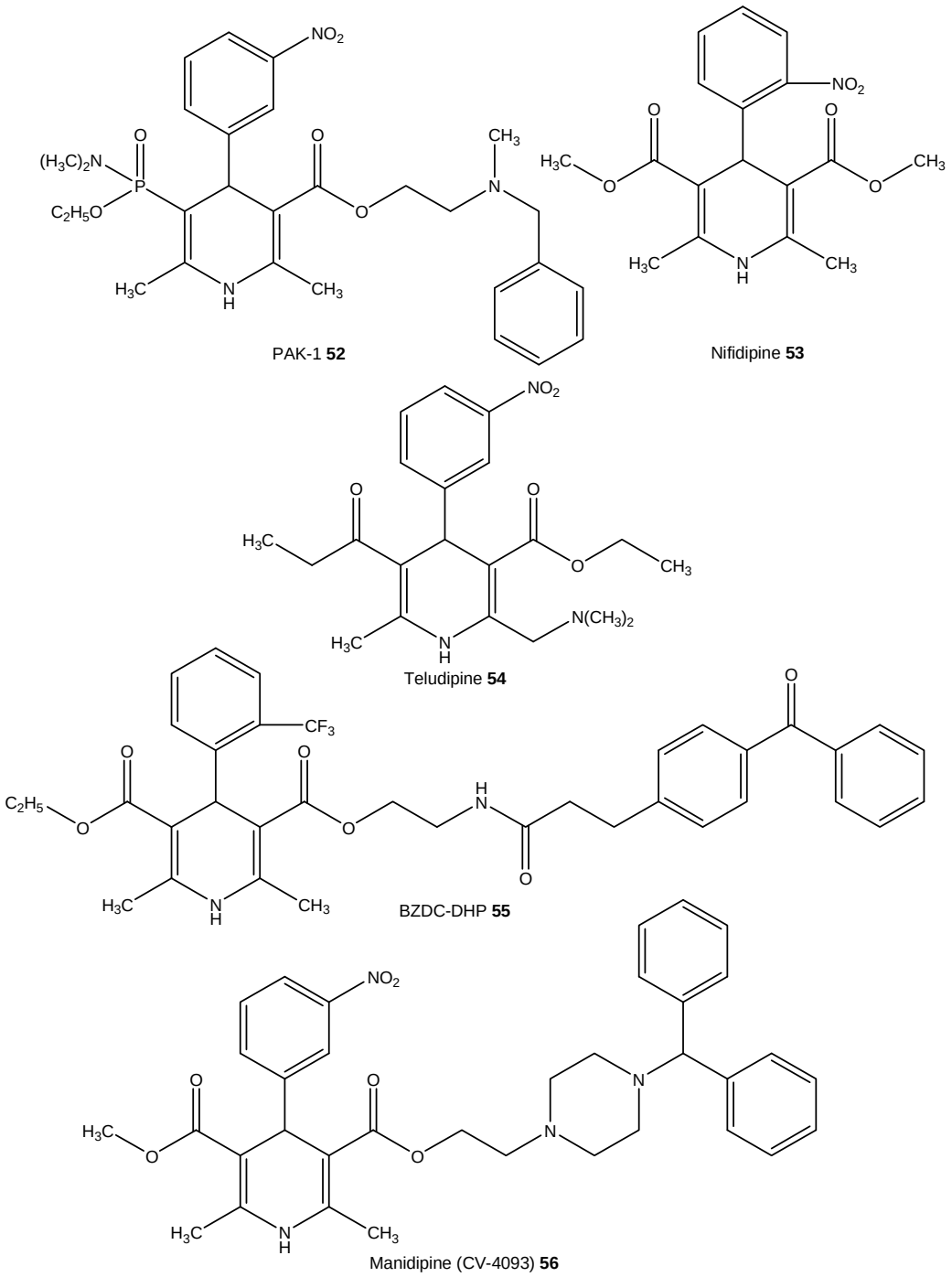


Figure-8. Structures of new DHPs

Earlier, *N*-alkylated 1,4-DHPs of general formula **57** & **58** were synthesized which were found to possess a remarkable activity for overcoming resistance to anticancer agents. The DHPs were used in combination with anticancer agents. The DHPs were also found very potent in enhancing the therapeutic activity of anticancer agents.[103]

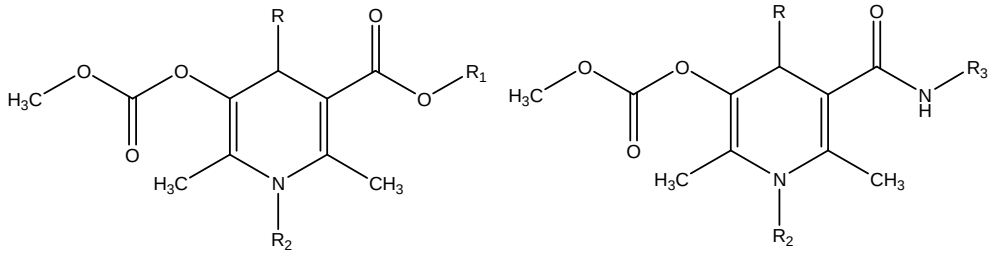


Figure-9. Structures of 57 and 58 DHPs.

In continuation to this, new *N*-alkylated 1,4- DHPs derivatives were synthesized and their ability to overcome MDR was examined in vincristine-resistant P388 cells (P388/VCR cells). DHPs that possessed an arylalkyl substituent on the DHPs ring nitrogen **59, 60, 61** were more potent than verapamil in potentiating the cytotoxicity of vincristine against P388/VCR cells. However, neither drug effectively enhanced the antitumor activity of vincristine in tumor-bearing mice. Introduction of basic nitrogen-containing substituents on the side chain of 1,4- DHPs gave improved activity *in vitro* and *in vivo*. The piperazine derivative **62** and **63** were more than 10 times as potent as verapamil *in vitro*. Four compounds **64, 65, 66** and **67** selected for *in vivo* testing showed superior antitumor activity in P388/VCR-bearing mice in combination with vincristine. The SARs of the compounds are discussed.[89]

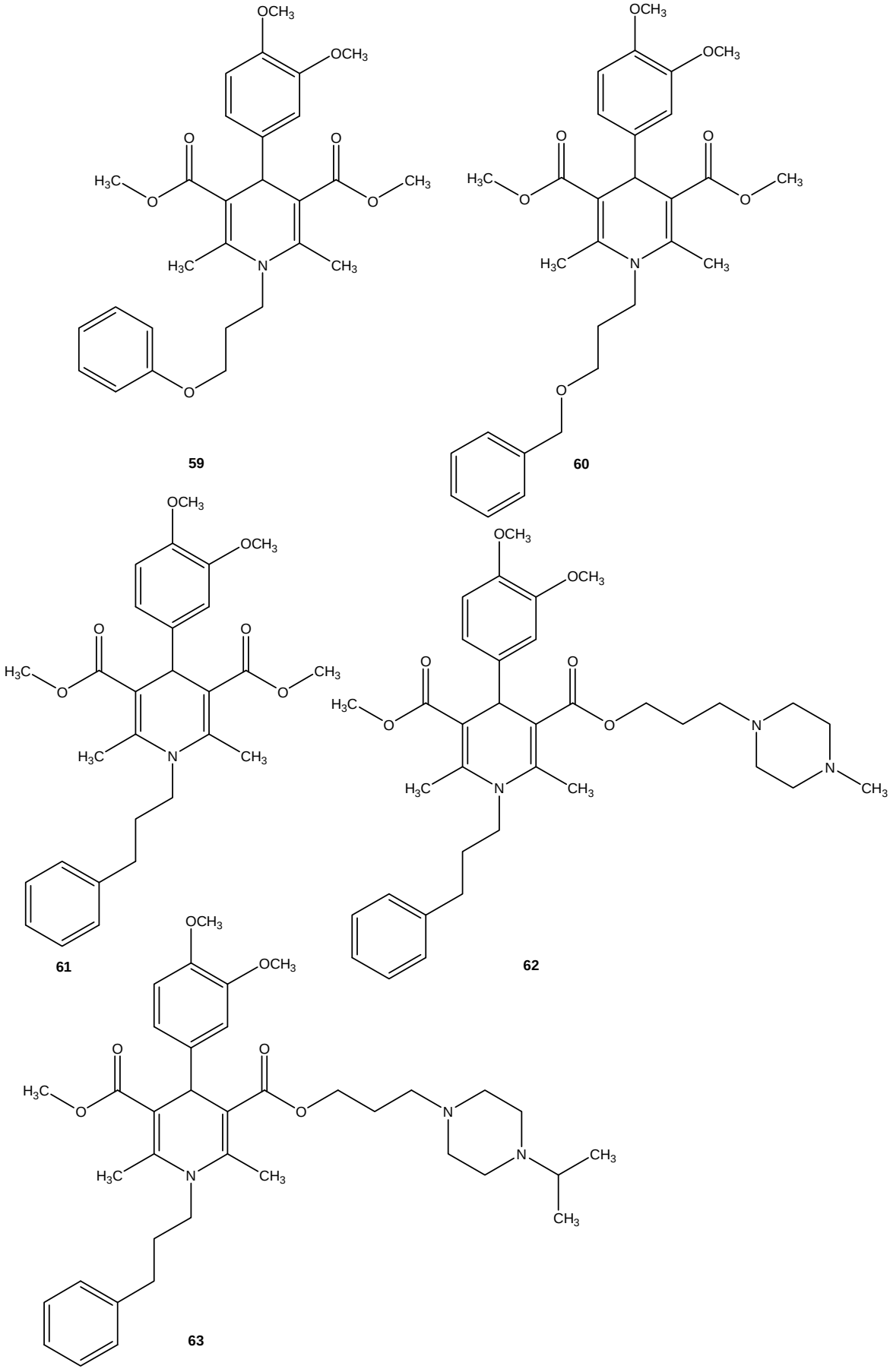
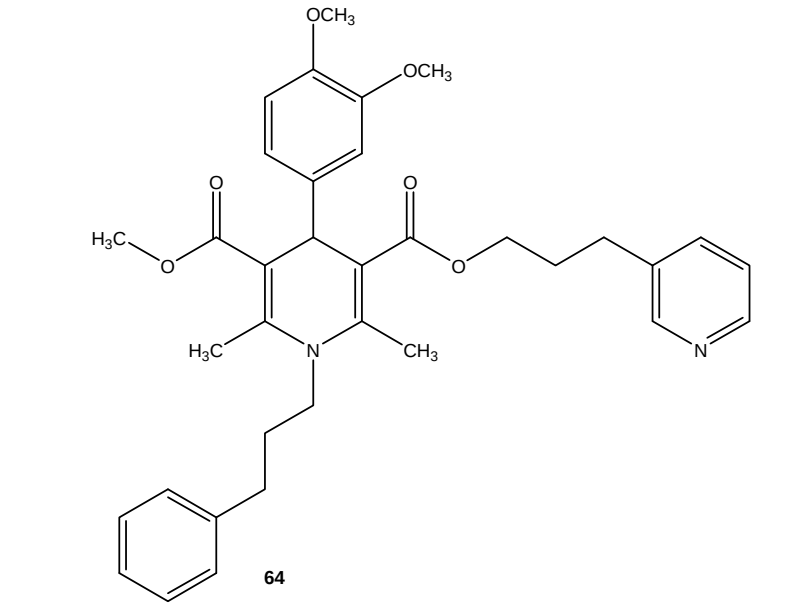


Figure-10. Structures of various *N*-substituted DHPs (contd.2)



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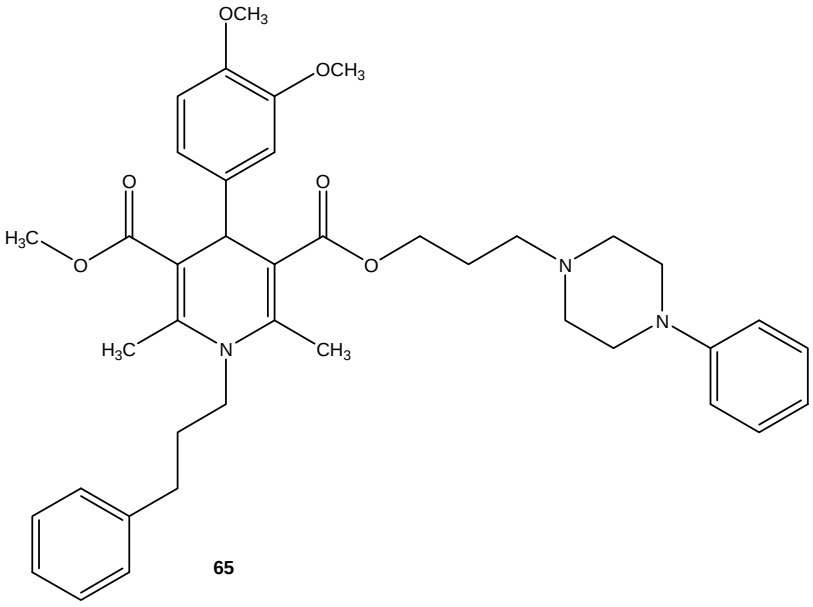

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Figure-10. Structures of various *N*-substituted DHPs (contd.3)

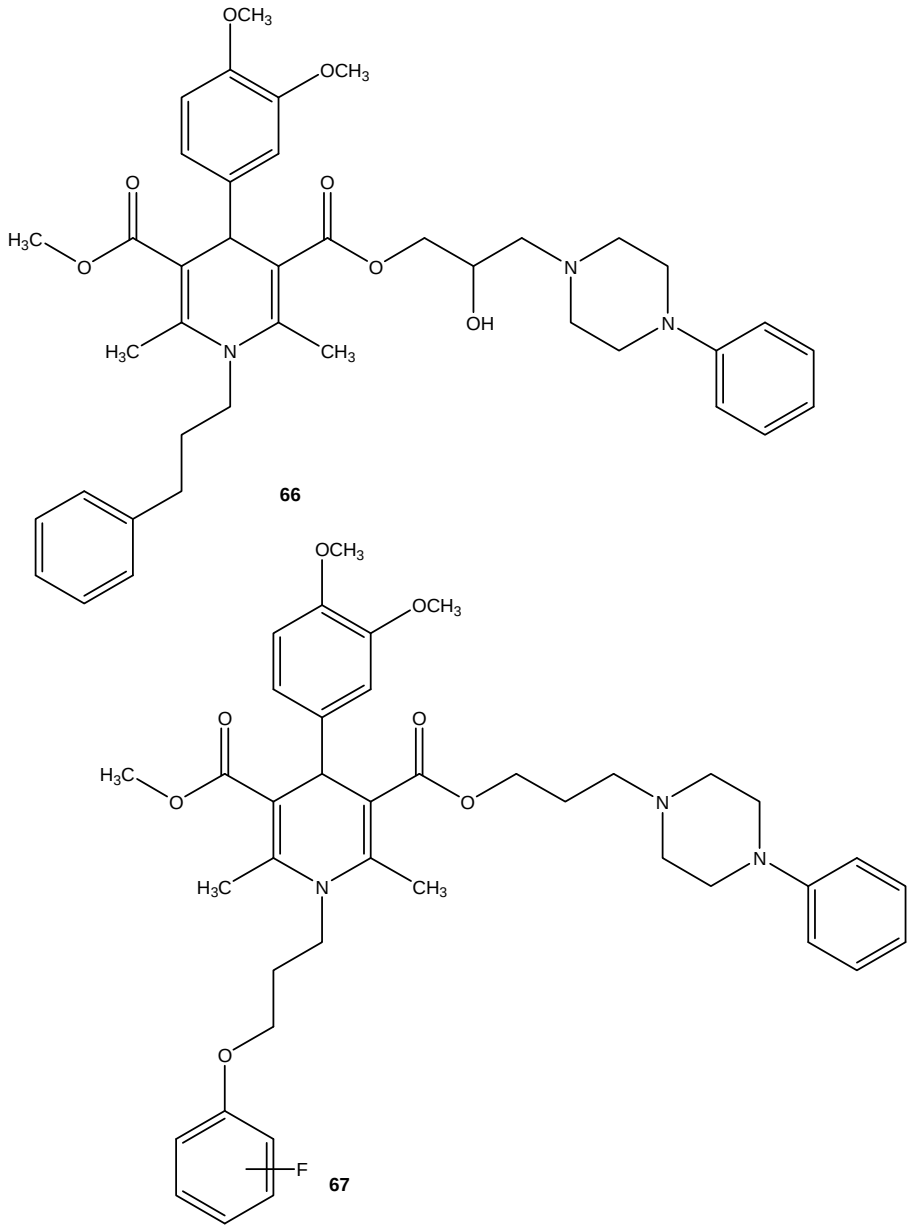


Figure-10. Structures of various *N*-substituted DHPs

Further, eleven 4-phenyl-3,5-diacetyl- 1,4- DHPs substituted at the C-4 phenyl ring (G series) were synthesized and compared for their cytotoxic activity and MDR reversing activity in *in vitro* assay systems. Among them, compound **68** showed the highest cytotoxic activity against human promyelocytic leukemia HL-60 and human squamous cell carcinoma HSC-2 cells. However, no compounds tested produced radicals at pH 7.4-12.5. The activity of Pgp responsible for MDR in tumor cells was reduced by compounds **69, 70, 71, 72, 73, 74** and **75**. However, compounds **76, 68** and **77** were hardly active, while **78** did not show a MDR reversing effect at 2.0-20 μg/mL[104]. These DHPs also showed synergistic interaction with ampicillin and erythromycin on *E. coli*

K12LE140/F'lac. The antibacterial effect of ampicillin was enhanced by most analogues. But none of the DHPs had any effect on a MDR clinical isolate of *E.coli* Gy-1/$A_{res}Er_{res.}$[105]

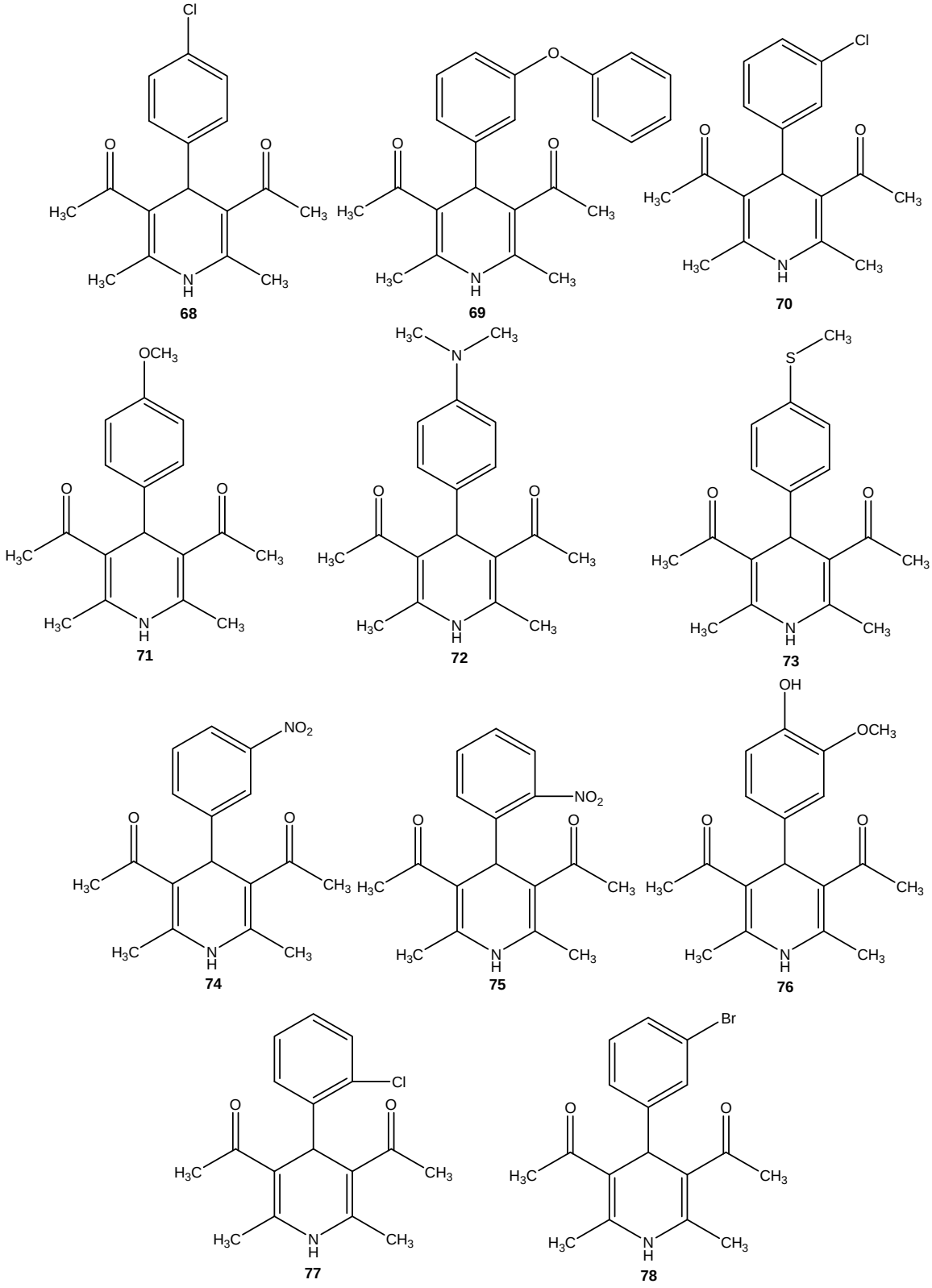


Figure-11. Structure of G series DHPs

When the acetyl group of G series was replaced with the benzoyl group (3, 5-dibenzoyl-1,4-DHPs) for GB series and test for their antibacterial effect along with Erythromycin, the MIC values are reduced against clinical isolates of *E.coli* Gy-1/$A_{res}Er_{res.}$ Compound **79** was the most effective in enhancing the activity of erythromycin.[106] Fifteen 4-phenyl-3,5-dibenzoyl-1,4-dihydropyridines substituted at the 4-phenyl ring were synthesized and compared for their cytotoxic activity and MDR reversing activity in *in vitro* assay systems. Among them, 2-CF_3, 2-Cl and 3-Cl derivatives showed the highest cytotoxic activity against human oral squamous carcinoma (HSC-2) cells. The activity of Pgp response for MDR in tumor cells was reduced by some of new derivatives, verapamil and nifedipine. These data suggest that 3,5-dibenzoyl-4-(3-chlorophenyl)-1,4-dihydro-2,6-dimethylpyridine **79** can be recommended as a new drug candidate for MDR cancer treatment.[107]

Further, it was found that 4-(2'trifluoromethylphenyl)-**80** and 4-(3'chlorophenyl)-3,5-dibenzoyl-2,6-dimethyl-1,4-dihydropyridine **79** showed not only MDR reversal activity, but also markedly higher cytotoxicity against two human oral tumor cell lines than one normal cell (human gingival fibroblast). In this report, tumor-specificity of **80** and **79** was first confirmed using a total of seven human cells, including four tumor cell lines (squamous cell carcinoma HSC-2, HSC-3, submandibular carcinoma HSG, promyelocytic leukemia HL-60) and three normal cells (gingival fibroblast HGF, pulp cells HPC and periodontal ligament fibroblast HPLF). Compound **80** and **79** were also capable to induce apoptotic cell death in HL-60 and HSC-2 cells, monitored by using several apoptosis associated markers, such as internucleosomal DNA fragmentation, activation of caspases -3, -8 and -9 and expression of pro-apoptotic proteins and an anti-apoptotic protein (Bcl-2). It was proposed that cell death was induced by **80** and **79** *via* radical-mediated reaction.[108]

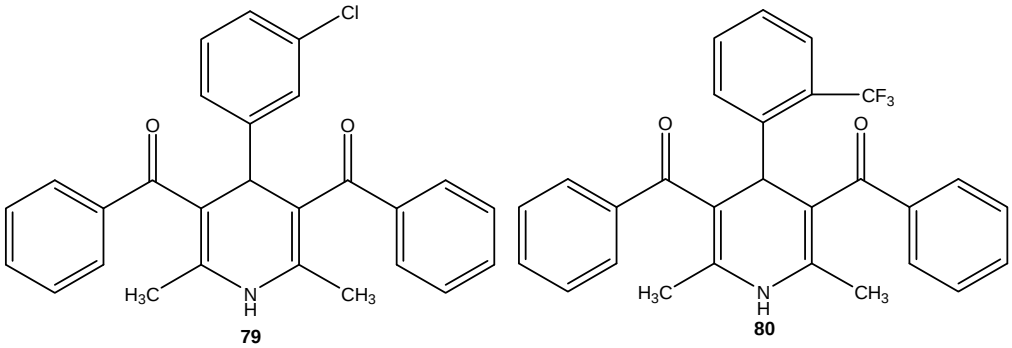


Figure-12. Structures of active GB DHPs

When the effects of DP series (selective molecules from G and GB series) namely 3,5-diacetyl and 3,5-dibenzoyl-1,4- DHPs were investigated on vascular functions *in vitro*, by comparing their mechanical and electrophysiological actions in rat aorta rings and single rat tail artery myocytes, respectively, along with their MDR reversing activity in L5178 Y mouse T-lymphoma cells transfected with MDR1 gene, DP7 **81** was found to inhibit L-type Ca^{2+} current recorded in artery myocytes in a concentration-dependent manner, with IC_{50} (M) values ranging between 1.12×10^{-6} and 6.90×10^{-5}. Other derivatives which are tested for MDR reveritng activity tested in L5178 MDR cell line, compound **75, 69** and DP7, exhibited an MDR reversal activity, with IC_{50} values ranging between 3.02×10^{-7} and 4.27×10^{-5}, DP7 being the most potent. From this study, DP7 represent a lead compound for the development of potent DHPs MDR chemosensitizers devoid of vascular effects.[109] DP7 has been shown to be a powerful Pgp inhibitor, almost devoid of cardiovascular effects, but capable of inhibiting liver CYP3A. DP7 is now considered a lead compound for the development of novel DHPs which do not affect CYP enzyme system but still retain the activity towards ABC-efflux transporters.[110] Cardiac effects of DP7 using Langendorff-perfused rat heart have been investigated and compared to that of nifedipine. Nifedipine decreased concentration-dependent ($IC_{50} = 8.89 \pm 1.09 \times 10^{-8}$ M) left ventricular pressure leaving unaltered coronary perfusion pressure, whereas DP7 did not affect these parameters. Nifedipine did not modify QRS and QT intervals of ECG.[111] It has also been investigated that neither pyruvate kinase nor lactate dehydrogenase was inhibited by DP7 which, however, inhibited concentration-dependently both Pgp ATPase activities, with IC_{50} value of 1μM.[112]

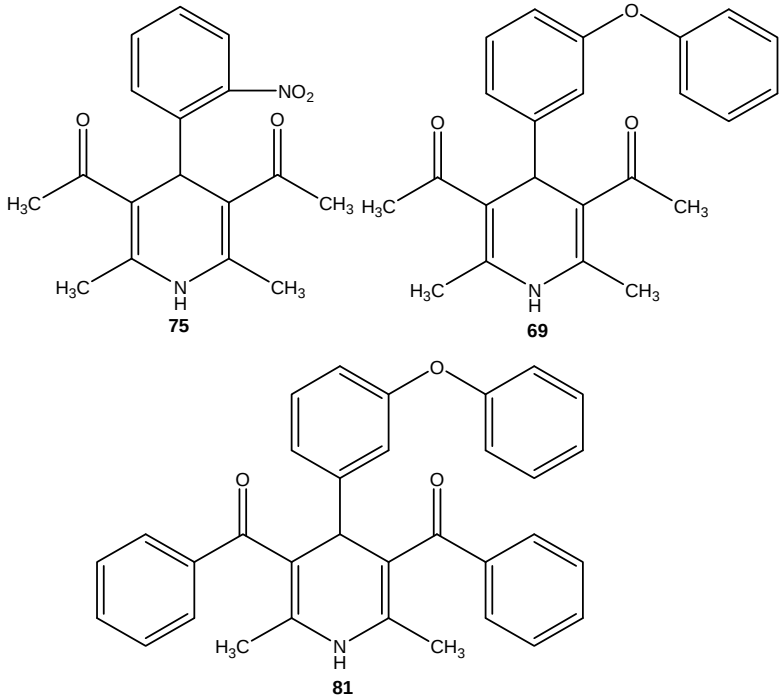
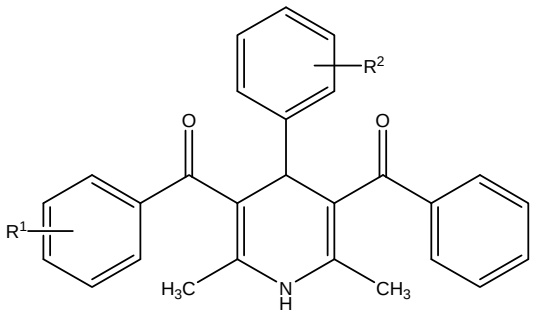


Figure-13. Structures of active DP DHPs

In an attempt to further modify the DP7, various modifications were done, particularly on C-5 benzoyl group and C-4 phenyl ring as in HK series **82.**[113]



HK series **32**

R^1 - Cl, CH_3, OCH_3
R^2 = 2-Cl, 3-Cl, 4-Cl, 4-OH, 4-CH_3, 4-OCH_3, phenoxy, 2,5-dimethoxy, 2-NO_2, 3-NO_2, 3-Br *etc.*

Figure-14. Common structure of HK series DHPs

In a recent study by Azizi E *et al.,* the work apparently initiated on basis of results from DP series, new series of DHPs modified at C-4 position carrying 1,3-thiazole substituted at C-2 has been investigated for inhibitory effects on cell proliferation of parental and moderately resistant T47D breast cancer cells. New DHPs were also studied for their effects on MDR1 reversal agent in these breast cancer cells and compared to verapamil as

standard. Two DHPs of **83** and **84** showed noticeable potentiation of doxorubicin cytotoxicity compared to doxorubicin alone, particularly in resistant cells. This effect was similar to that of verapamil. Compound **83** showed the highest effect on resistant cells. Two newly synthesized DHPs derivatives, **83** and **83**, are promising potential new MDR1 reversal agents.[114]

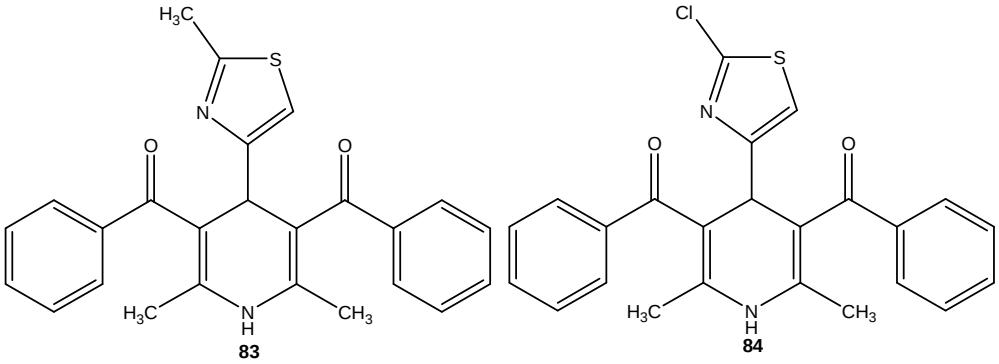


Figure-15. Structure of **83** and **84**

Some new hybridized derivatives of 1,4-DHPs (DL series) having *m*-nitrophenyl group at C-4 and changing variable substitutions at C-3 and C-4 were investigated for their inhibitory activity for Pgp by flow cytometry in the MDR human colon cancer cell lines (COLO320) and in human MDR1 gene-transfected mouse lymphoma cells (L 5178 Y). The cytotoxicities of the DHPs were also examined against human normal and cancer cell lines. The majority of the tested DHPs proved to be effective inhibitors of rhodamine 123 outward transports. Some DHPs displayed higher cytotoxic activity against four human oral tumour cell lines against three normal human oral cell lines. New ring substituents could well prevent the oxidation of the ring of the aromatic compound. Some DHPs at the higher concentration was found to be toxic as indicated by deformation in the cell size and the intracellular structures of the cells were changed during the short-term experiments. The majority of the DHPs tested were shown to enhance the drug retention in the cells by inhibiting the efflux-pump activity. Among the DHPs, **85, 86, 87, 88, 89** and **90** were found to be the most effective MDR modulators. These DHPs caused a dose-dependent inhibition of the MDR Pgp.[115]

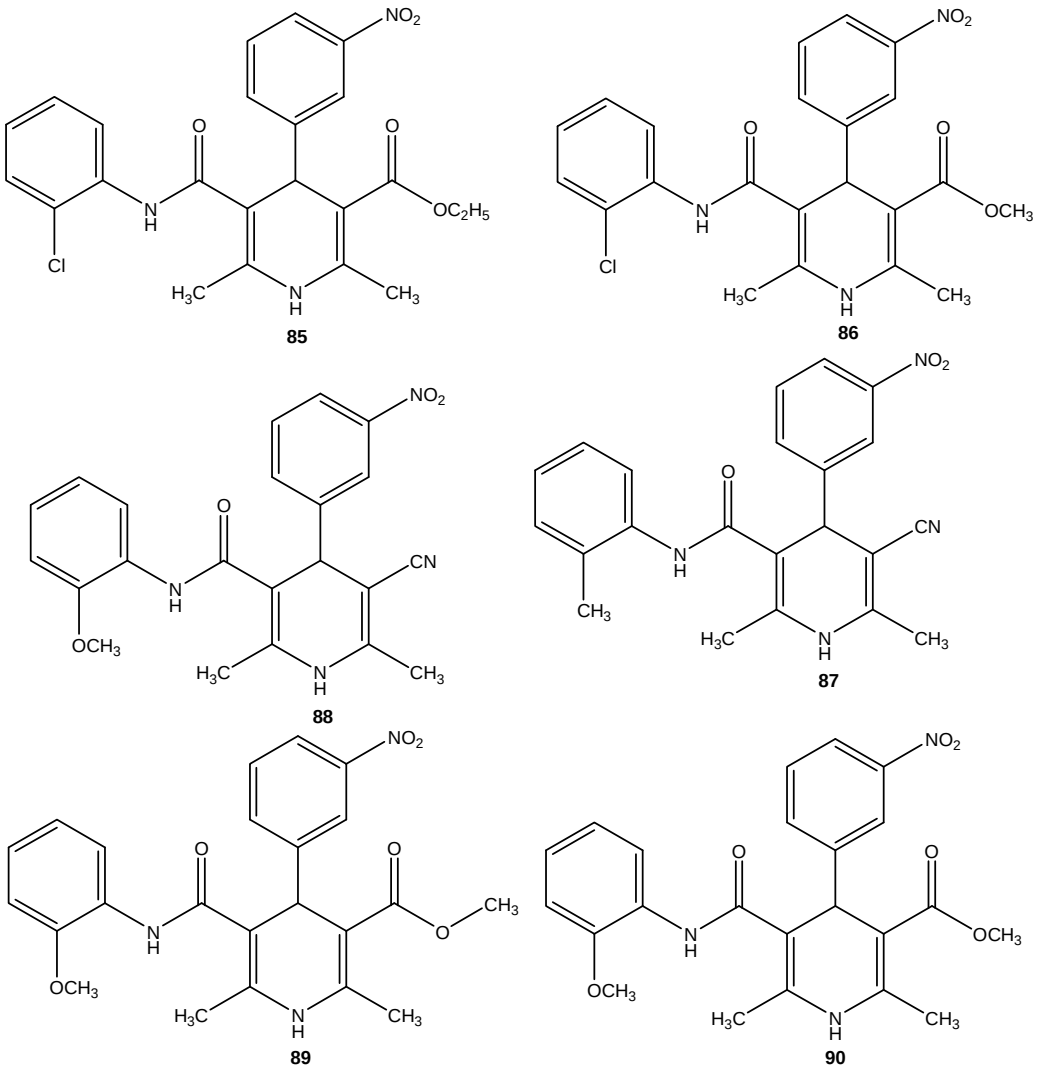


Figure-16. Structure of active DL series of DHPs

Further, symmetrical di-carbamoyl and di-carboxamide derivatives **91**, **92** & **93** series were synthesized and studied for their anti Pgp activity, most of the studied compounds were moderately active against L-5178 cells.[116]

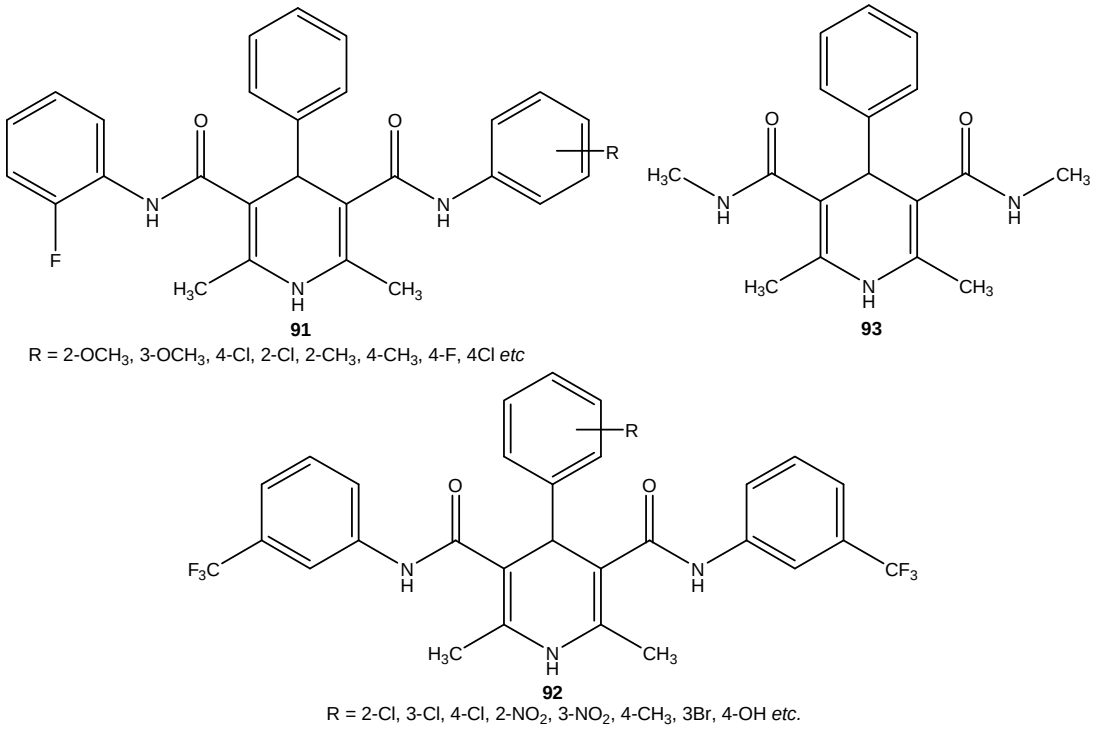


Figure-17. Structure of **91, 92** and **93** series DHPs

The structures of the DHPs reported from our research group were proved by X-ray crystallographic studies.[117-119] Some of similar DHPs were also found very potent against *M. tuberculosis.*[120,121]

New C-4 fused heterocyclic systems in DHPs, AHC-52 **94** (methyl 2-(*N*-benzyl-*N*-methylamino)ethyl-2,6-dimethyl-4-(2-isopropyl-pyrazolo[1,5-*a*]pyridine-3-yl)-1,4-dihydropyridine-3,5-dicarboxylate) and its pyridine analog AHC-93 **95** has also been reported to reverse MDR by inhibiting Pgp.[122-124]

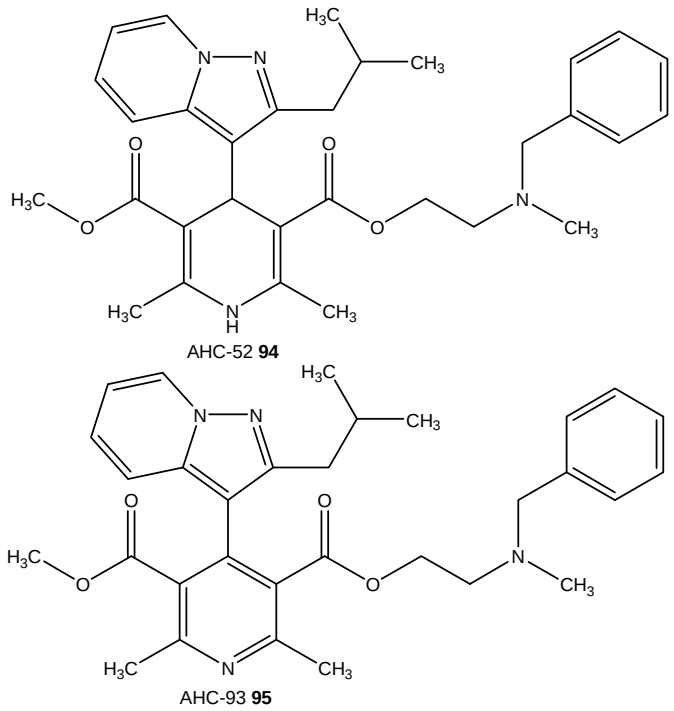


Figure-18. Structure of AHC-52 and AHC-93

A series of 4-aryl-1,4-DHPs and corresponding aromatized 4-arylpyridines has been synthesized aimed to enhance MDR activity, while minimizing Ca^{2+} channel binding. Synthesized DHPs were evaluated for [^{3}H]vinblastine accumulation studies. 4-Aryl-1,4-DHPs and all 4-arylpyridines can successfully restore intracellular accumulation of vinblastine in a resistant human breast adenocarcinoma cell line, MCF-7/adr, which over expresses Pgp. The most potent DHPs **96, 97** and **98** led to an approximately 15-fold increase of vinblastine accumulation. All of the DHPs tested were also able to substantially reduce IC_{50} values of daunomycin and increase its cytotoxicity in MCF-7/adr-resistant cells, confirming the results of the vinblastine accumulation studies. Out of these DHPs, eight DHPs have negligible effect on calcium channel binding over the concentration range from 15 to 2500 nM.[125]

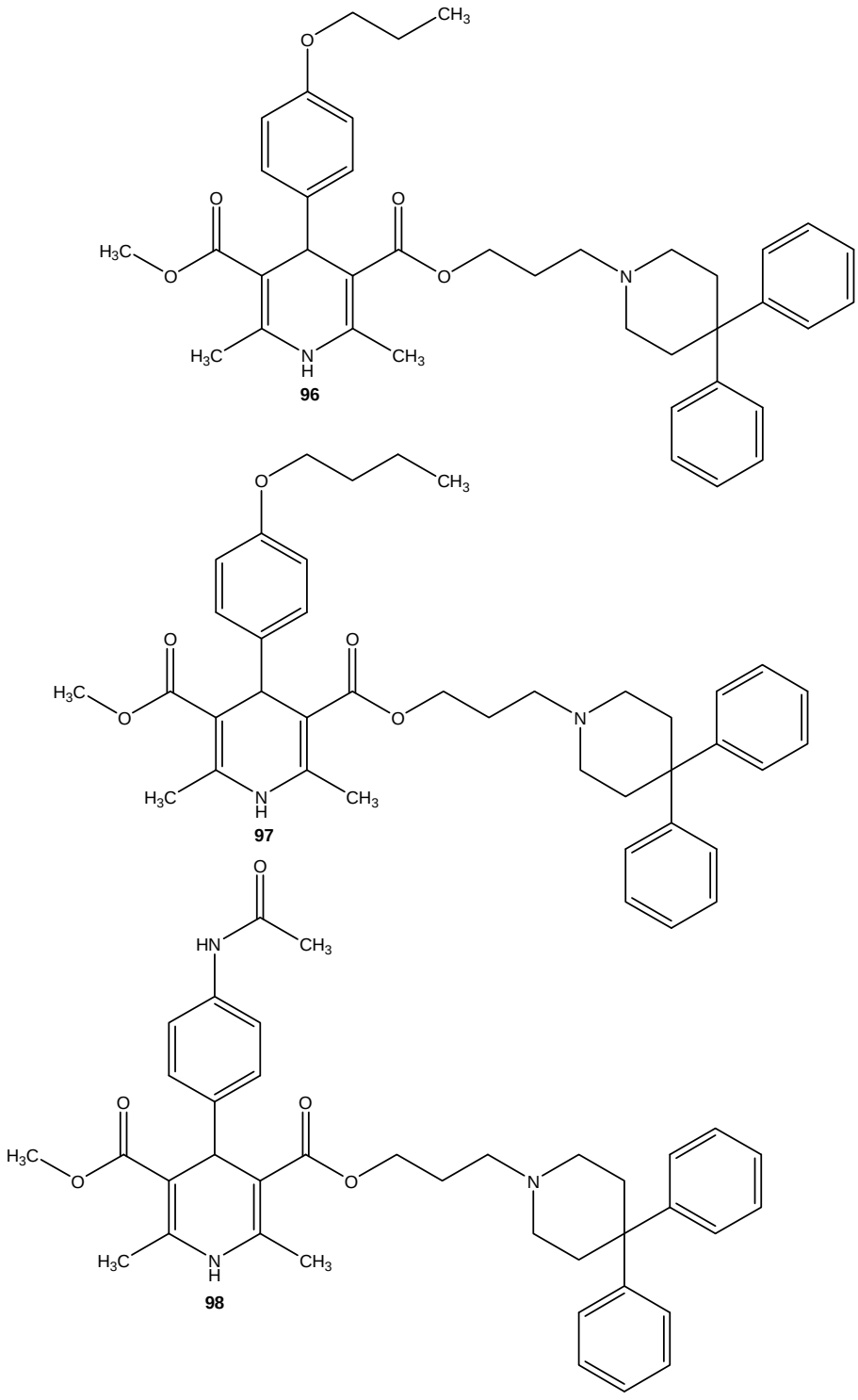
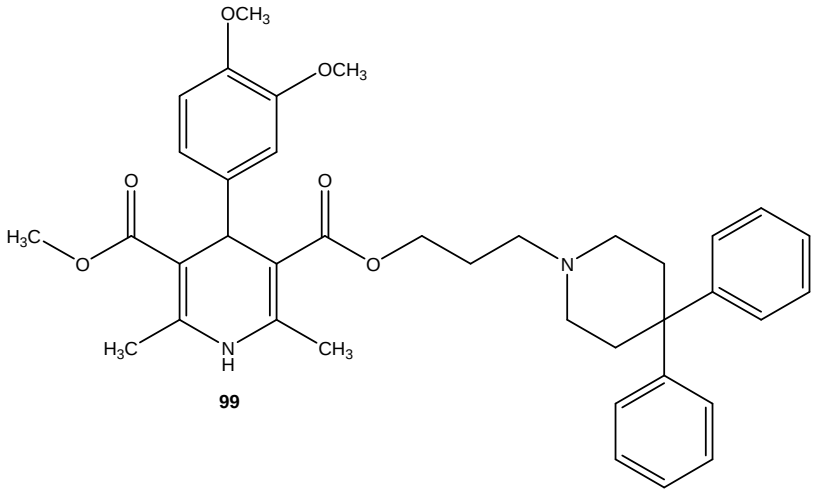


Figure-19. Structures of active DHPs **96, 97** and **98**

(±)3-(3-(4,4-Diphenylpiperidin-1-yl)propyl) 5-methyl 4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ((±)-DHPs-014) **99**, is a new 4-aryl-1,4-DHPs that can reverse MDR mediated by the ATP-binding cassette (ABC) transport proteins, Pgp, MDR1 and breast cancer resistance protein; This DHP exhibits negligible

calcium channel blocking activity. Three intravenous (1, 2 and 5 mg/kg) and two oral (25 and 50 mg/kg) doses were administered to female Sprague-Dawley rats. A two-compartment model with nonlinear elimination best characterized the pharmacokinetic profiles after intravenous and oral administration in rats. The terminal half-life of **99** increased and the systemic clearance significantly decreased at higher doses, indicating nonlinear elimination. The dose-dependent clearance is likely due to saturation of metabolism. The apparent volume of distribution of this DHP was 2.0 L/kg in rats and was unchanged with increasing intravenous doses. The estimated oral bioavailability was 8.2%. The poor bioavailability is likely due to the poor solubility of the compound, as well as to substantial first-pass elimination.[126]

While comparing **99** with niguldipine, nicardipine, nifedipine, and nitrendipine for their effects on breast cancer resistance protein (BCRP) mediated efflux and on the cytotoxicity of the BCRP substrate and chemotherapeutic agent mitoxantrone, **99** was found to be a potent BCRP and Pgp inhibitor *in vitro*. This DHP may be promising agents for clinical application due to their potent inhibition of both BCRP and Pgp. This study represents the first report that DHPs and pyridines as potent inhibitors of BCRP.[127]



99

Figure-20. Structure of DHPs-014

Quantitative structure–activity/pharmacokinetic relationships (QSAR/QSPKR) for a series of synthesized DHPs and pyridines as Pgp **100** & **101** inhibitors was generated by 3D molecular modeling using SYBYL and KowWin programs. A multivariate statistical technique, partial least square (PLS) regression, was applied to derive a QSAR model for

Pgp inhibition and QSPKR models. Cross-validation using the "leave-one-out" method was performed to evaluate the predictive performance of models. For Pgp reversal, the model obtained by PLS could account for most of the variation in Pgp inhibition (R^2 = 0.76) with fair predictive performance (Q^2 = 0.62). Nine structurally related 1,4-DHPs drugs were used for QSPKR analysis. The models could explain the majority of the variation in clearance (R^2 = 0.90), and cross-validation confirmed the prediction ability (Q^2 = 0.69)[128].

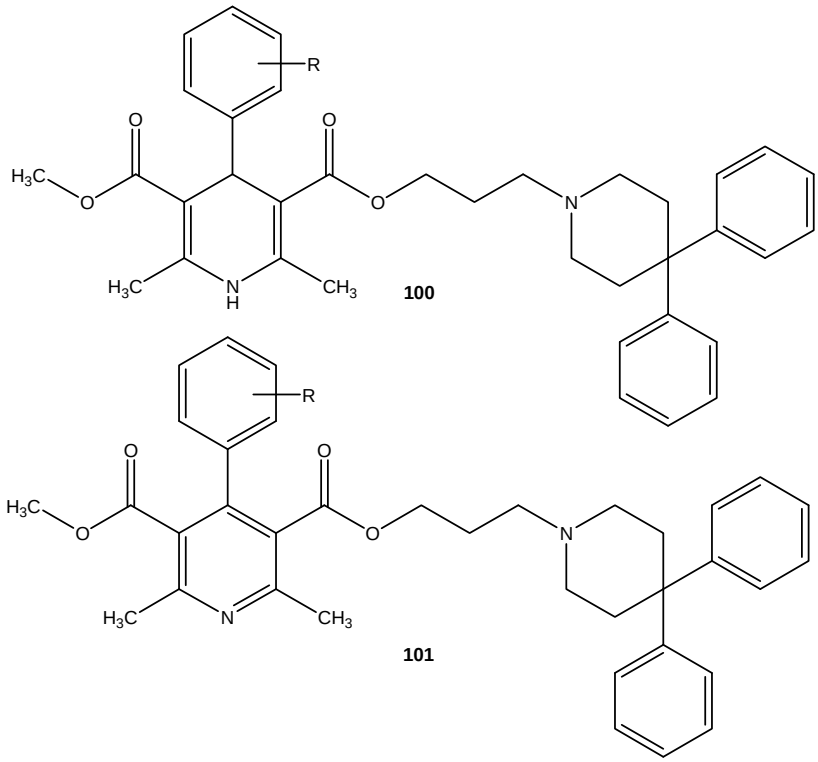
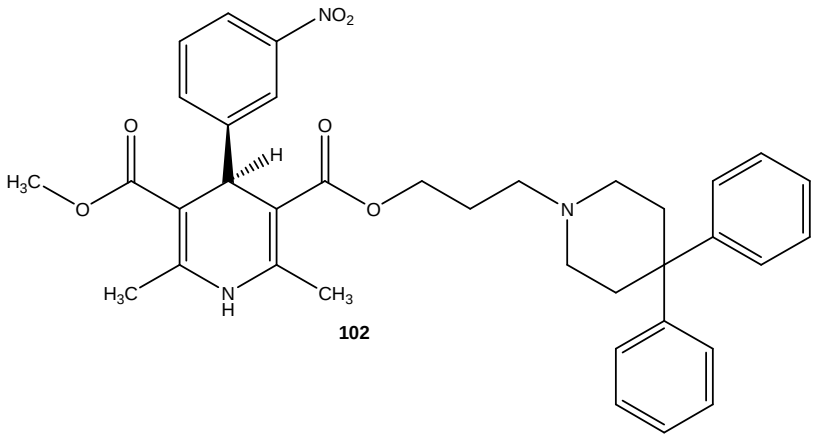


Figure-21. Structure of Type-I **100** and Type-II **101** DHPs

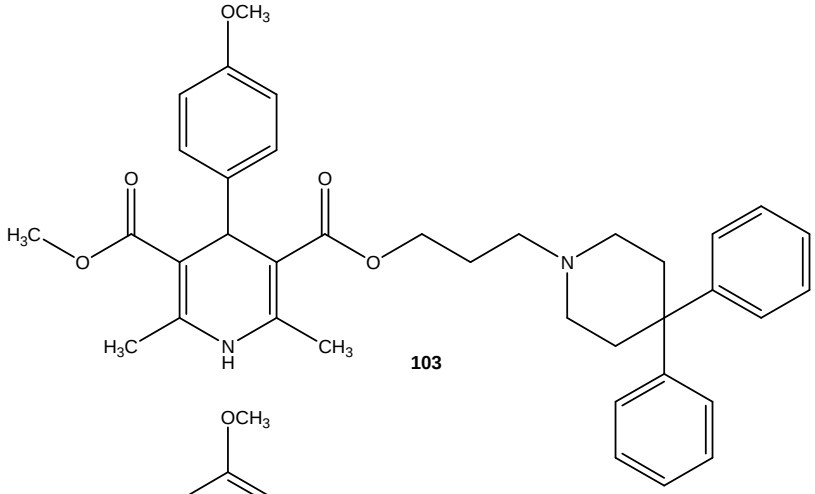
Optically pure DHPs substituted at C-4 with 3-nitro phenyl as shown in structure **102** are capable of potentiating the activity of anticancer agents in tumor cell (synergism). This overcoming of resistance is not only limited to resistance to cytostatics but also to other therapeutics such as for the treatment of malaria.[129]



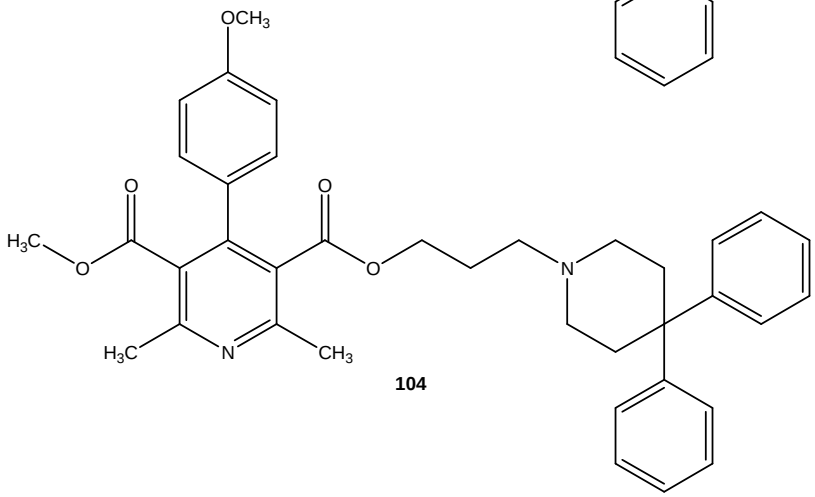
102

Figure-22. Structure of **102**

In a similar vein, several newly synthesized 4-aryl-1,4-DHPs and respective aromatized pyridines on drug efflux mediated by MDR associated protein 1 (MRP1, ABCC1) in human small cell lung cancer H69AR (overexpressing MRP1) and wild type H69 cells, five out of sixteen DHPs and six out of nine pyridines were found to significantly increase the intracellular accumulation of vinblastine in resistant H69AR cells (p<0.01) at a concentration of 2.5 µM. Four DHPs, which significantly increased vinblastine accumulation, were tested for their effect on daunomycin cytotoxicity in H69AR cells and found to significantly decrease the IC_{50} of daunomycin, confirming the accumulation study results. The DHPs were also tested for their effect on intracellular glutathione (GSH) concentrations, a co-substrate for MRP1-mediated efflux in H69AR and Panc-1 cells. After 2-hr and 24-hr incubation with a DHP compound, **103** and its pyridine derivative **104** there was a small (~20%) but statistically significant decrease in intracellular GSH in Panc-1 cells.[130]



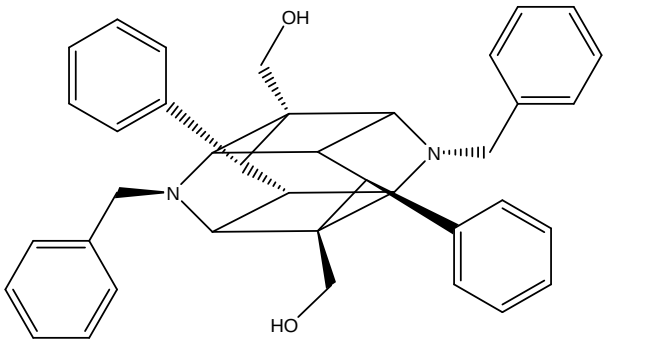
103



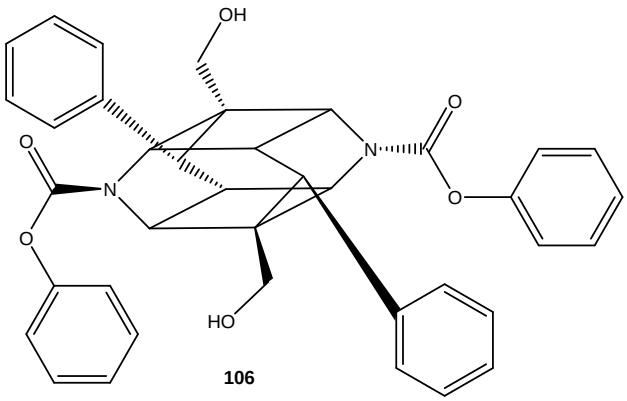
104

Figure-23. Structures of **103** and **103** DHPs

A series of *N*-substituted cage dimeric 1,4-DHPs was also evaluated as inhibitors of membrane efflux pump Pgp in MDR cancer cells. Some of the reported 1,4-DHPs have MDR modulating effect on Pgp, significantly superior to that of verapamil. The most active 1,4-DHPs are lipophilic substituted *N*-benzyl and -phenyloxycarbonyl derivatives **105** and **106**. Some P-gp substrate properties have been suggested only for the *N*-phenyloxycarbonyl compound **106**. Competitive studies with cytotoxic Pgp substrate epirubicin indicated the overcoming of MDR in comparison of the cell line at concentrations below cytotoxic ranges of the most effective MDR-modulating concentrations of the compounds themselves. The *N*-benzyl DHPs exhibited the highest activity and practically no Pgp substrate properties. It could be a promising lead candidate for further clinical studies and structural improvement for the overcoming of MDR[131].



105



106

Figure-24. Structure of **105** and **106** DHPs

Additional study on quantitative structure activity relationships of newly synthesized 1,4 DHPs possessing a 1-pentyl group at the 4-position was carried out. 3-Pyridylpropylester was found to be one of the effective fragments for overcoming Pgp mediated MDR in cultured human cancer cells, *in vitro*. It was found to increase the life span of mice having Pgp over expressing MDR P388 leukemia. All 1,4-DHPs had weak calcium antagonistic activities, but there appeared no relationship between MDR reversing effect and calcium antagonistic activity. Some 1,4-DHPs such as **107** and **108** with weak calcium antagonistic activities showed effective MDR reducing activities both *in vitro* and *in vivo*. In particular, compound **108** was expected to be the most suitable compound to overcome MDR.[132]

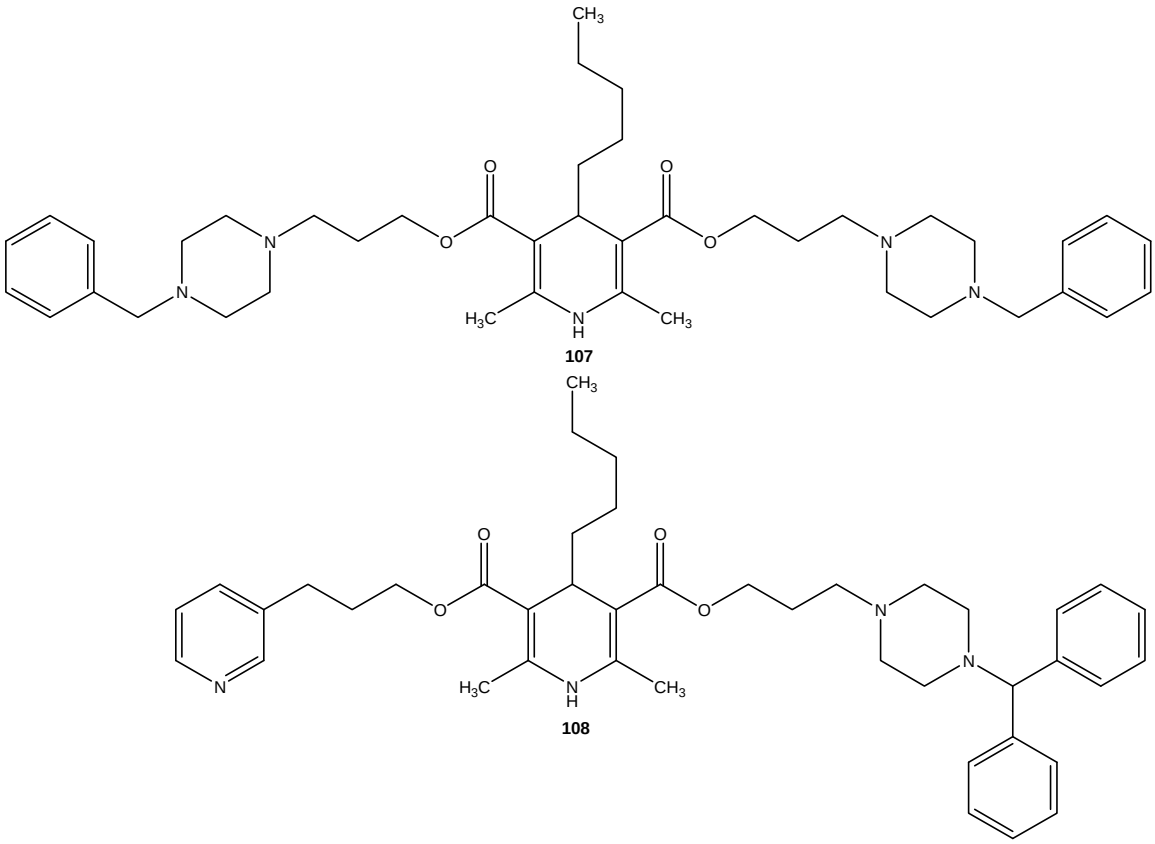


Figure-25. Structures of **107** and **108**

Two other DHPs, **109** and **110** were also found to be potent antagonist against cancer cells with potentiation of anticancer agents.[133,134]

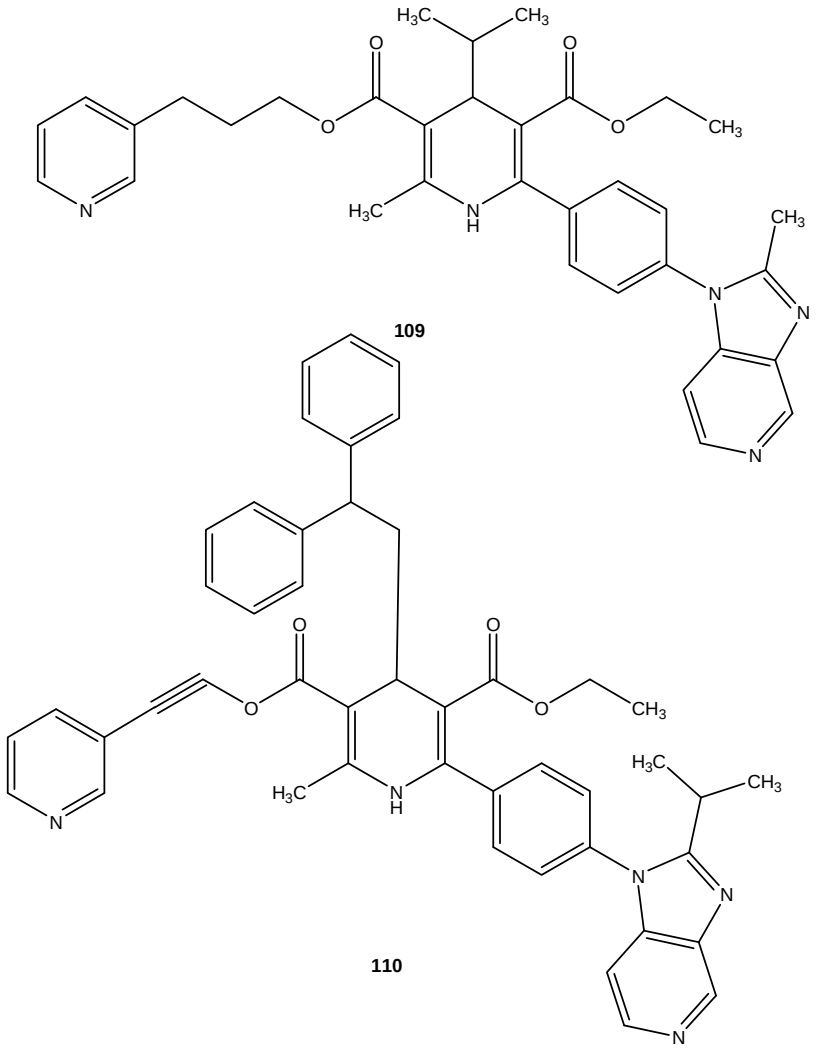


Figure-26. Structures of **109** and **110**

Additionally, a series of novel *N*-acyloxy-1,4-DHPs **111** have been synthesized and evaluated as Pgp inhibitors in an *in vitro*. QSAR were also established to identify significance and regiospecific influence of certain functional groups.[135]

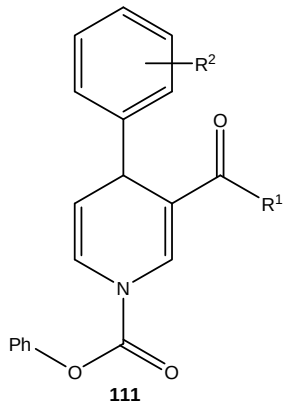


Figure-27. Structure of compound **111**

Modification at position 2 of 1,4-DHP such as in DHPs of formula **112, 113** and **114** increases very significantly the sensitivity of cancer cells to anticancer agents as well as the sensitivity of cancer cell that have acquired a resistance to different anticancer agents, but at the same time exhibits only weak calcium channel blocking properties, which suppress their pharmacological hypotensive effect and make it possible for them to be used in anticancer therapy without causing undesirable side effects.[136]

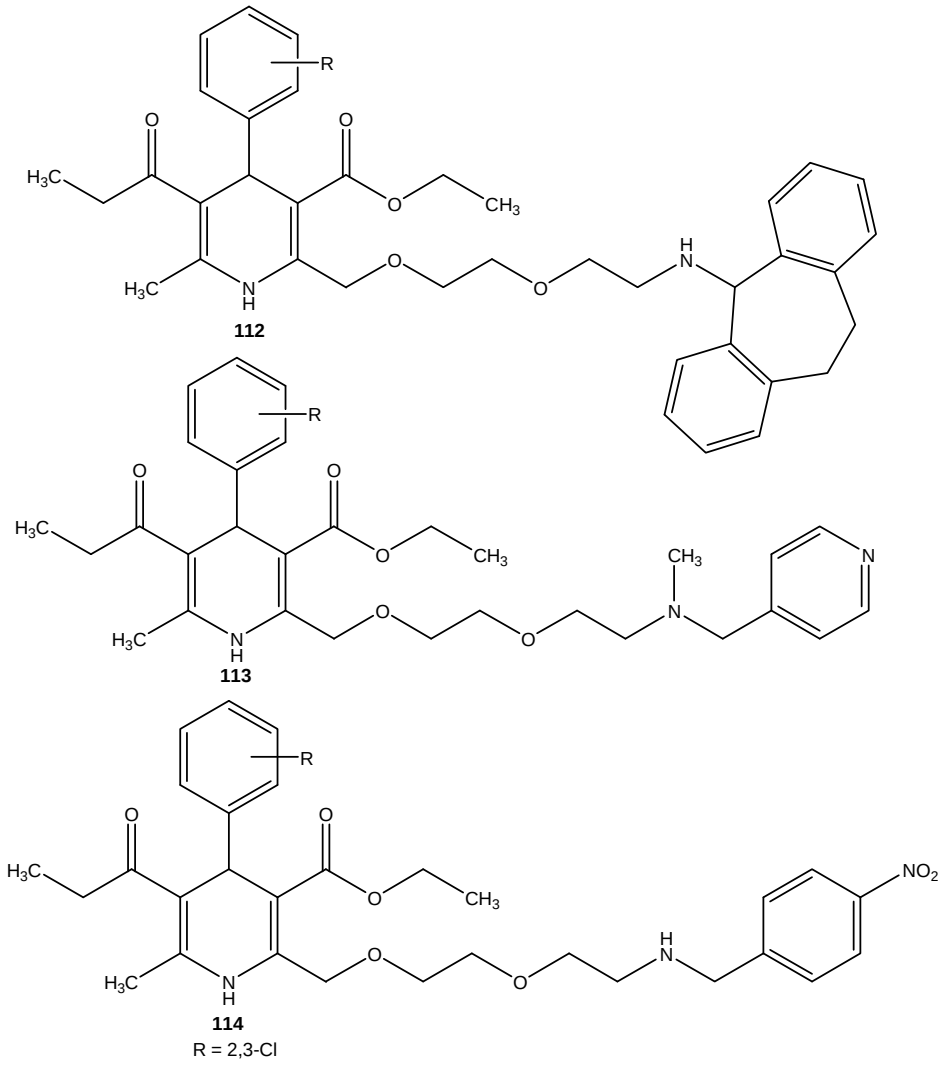


Figure-28. Structures of **112, 113** and **114** DHPs

1.7 Mitotic Kinase Egs5 Inhibitors as Anticancer Agents:

Drugs that target the mitotic spindle are among the most effective cancer therapeutics currently in use. Vinca alkaloids, which promote microtubule depolymerization, and taxanes (paclitaxel and taxotere), which stabilize microtubules, inhibit spindle function by disrupting microtubule dynamics, leading to mitotic arrest and apoptosis.[137,138] Mitotic arrest is mediated by the spindle checkpoint, which is activated by microtubule-targeted drugs.

Recently, inhibiting the mitotic kinesin Eg5 [also known as kinesin spindle protein (KSP)], which is required for the formation of a bipolar spindle, has gained significant attention as an alternative strategy to interfere with spindle function.[139-141] Blockage of Eg5 function with selective inhibitors, results in the characteristic monoastral phenotype, mitotic arrest, and apoptosis in various tumor cell lines[142-144] (Figure-29). Similar to microtubule poisons, inhibition of Eg5 leads to activation of the spindle checkpoint.[145] The spindle checkpoint prevents chromosome missegregation and aneuploidy by ensuring the accurate segregation of sister chromatids to the dividing daughter cells during mitosis.[146-149] The spindle checkpoint remains active until all chromosome kinetochores are properly attached to the bipolar spindle and chromosomes are aligned at the metaphase plate. Proper function of the spindle checkpoint requires the concerted action of several checkpoint proteins, which include BubR1, Bub1, Bub3, Mad1, and Mad2. Several of these components have been shown to preferentially localize to unattached chromosomes. The active checkpoint generates a ‘wait anaphase signal’ to inhibit the anaphase-promoting complex. Inhibition of the anaphase-promoting complex prevents the degradation of several key mitotic proteins, which must be degraded for anaphase initiation to occur. The presence of unattached chromosomes or a lack of spindle tension that is normally generated by bipolar chromosome attachment results in continued checkpoint activation, mitotic arrest, and eventually programmed cell death.[150-153]

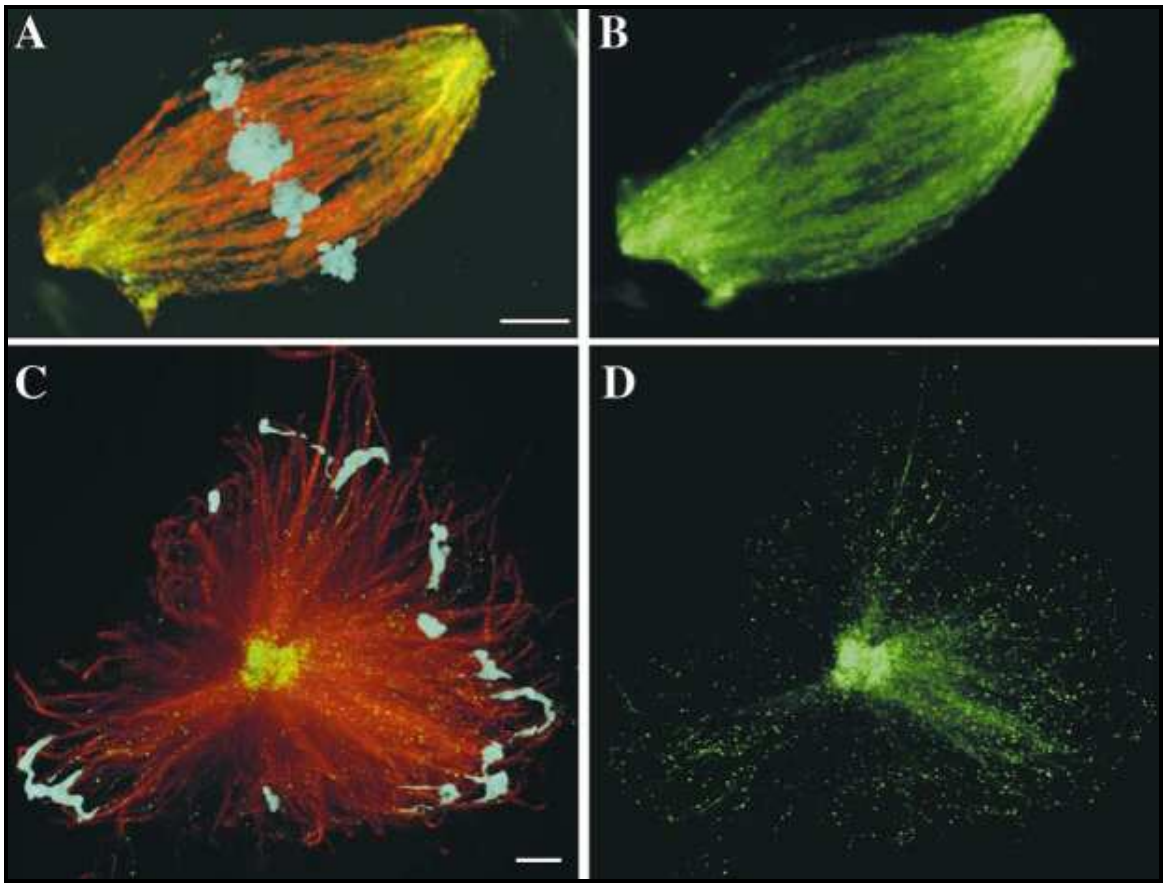
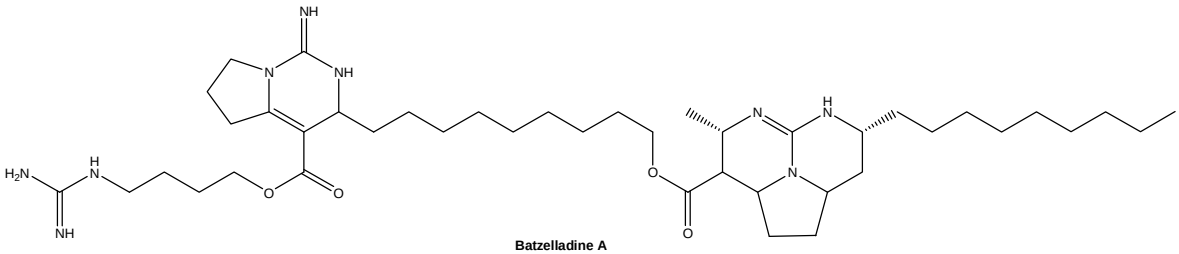


Figure-29. Immunolocalization of Eg5 in bipolar and monoastral spindles. At the completion of the cycled spindle assembly reaction, the spindles were diluted, fixed, layered over glycerol cushions, and spun onto coverslips. The samples were then processed for immunofluorescence. (A) An overlay shows the chromatin (blue), tubulin (red), and Eg5 (green) in a bipolar spindle assembled *in vitro*. (B) Eg5 alone. The protein is localized along microtubules and shows enrichment at the spindle poles. (C) Addition of 50 mM monastrol to assembly reactions results in the formation of monoastral spindles. An overlay of the chromatin (blue), tubulin (red), and Eg5 (green) is shown. (D) Eg5 is immunolocalized along microtubules and is concentrated at the center of the monoaster. Bars: 5 mm.

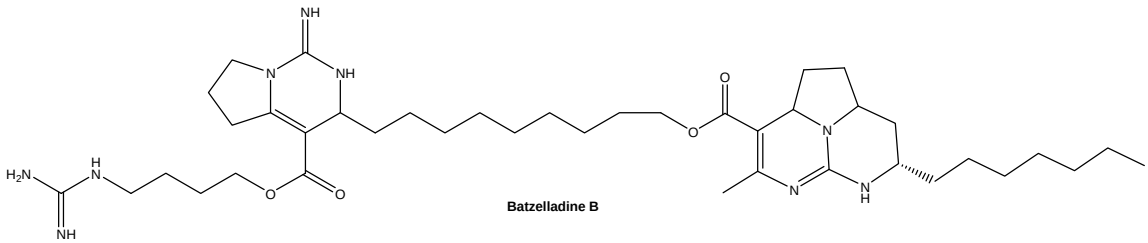
Recent studies have shown a correlation between defects in the spindle checkpoint and chromosomal instability, which is frequently observed in tumor cell lines.[154-160] In addition to an association between defects in the spindle checkpoint and chromosomal instability, spindle checkpoint defects are also associated with the susceptibility of tumor cells to induction of mitotic arrest and apoptosis by microtubule-targeted agents, such as paclitaxel and nocodazole. Several studies have shown that impairment of spindle checkpoint function leads to a reduction in the level of mitotic arrest and apoptosis normally induced by antimicrotubule drugs.[161-163]

Other studies, however, have concluded that inactivation of the spindle checkpoint sensitizes cells to apoptosis induced by antimicrotubule drugs.[164-165] The relationship between the spindle assembly checkpoint and inhibition of the mitotic kinesin motor protein Eg5 is just beginning to be elucidated. In a recent study, Tao *et al.*,[166] and Gregory *et al.*,[167] suggest that induction of apoptosis by an Eg5 inhibitor requires sustained mitotic arrest, followed by adaptation and slippage into the next G1 phase.

In recent years, dihydropyrimidinones and their derivatives have occupied an important place in natural and synthetic organic chemistry mainly due to their wide range of biological activities[168-169], notably as calcium channel blockers.[170,171] Additionally, the structurally related marine alkaloids batzelladine A **115** and B **116** were shown to be the first low molecular weight natural products to inhibit the binding of HIV gp-120 to CD4 cells, so disclosing new vistas towards the development of AIDS therapy.[172]



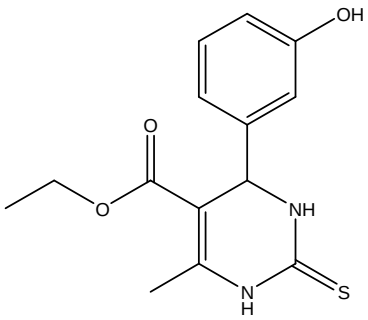
Batzelladine A



Batzelladine B

More recently, ethyl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate, also known as monastrol **117**, was identified as a novel low molecular weight cell-permeable molecule for the development of potentially new anticancer drugs.[173] This compound specifically affects the cell division (mitosis) by a new mechanism, which does not involve the binding to tubulin in contrast with the natural taxanes, vinca alkaloids and epothilones. It has been established that the activity of monastrol is based on the specific and reversible inhibition of the motility of mitotic kinesin Eg5, a motor protein required for bipolar spindle formation during mitosis.[174-178] Moreover, Maliga *et al.*,[179] have demonstrated that monastrol inhibits the motor activity of Eg5 by inhibiting ATP hydrolysis through an allosteric mechanism, whereas the corresponding 4-hydroxyphenyl derivative is a weak Eg5 inhibitor and that (*S*)- monastrol

[(*S*)] is the biologically active enantiomer, indicating a more potent and specific Eg5 inhibitor.



Monastrol 117

Although many reports have been dedicated to elucidate the mechanism of action of monastrol as mitotic inhibitor in the cell cycle,[180-182] few examples concerning the anticancer activity[183-186] were reported. Recently, Leizerman and coworkers described the differential effects of monastrol on AGS and HT-29 cell lines in comparison with taxol.[187]

Further, Russowsky *et al.*,[188] investigated firstly the differential anti-proliferative activity of monastrol **117** and its oxo-analogue, named oxo-monastrol **118**, as well as the thio-analogues **119a-123a** and the corresponding oxo-analogues **119b-123b** (all compounds in the racemic form) on seven human cancer cell lines.

Monastrol and the thio-derivatives **120a**, **121a** and **123a** displayed relevant antiproliferative properties with 3,4-methylenedioxy derivative **123a** being approximately more than 3 times more potent than monastrol against colon cancer (HT-29) cell line.

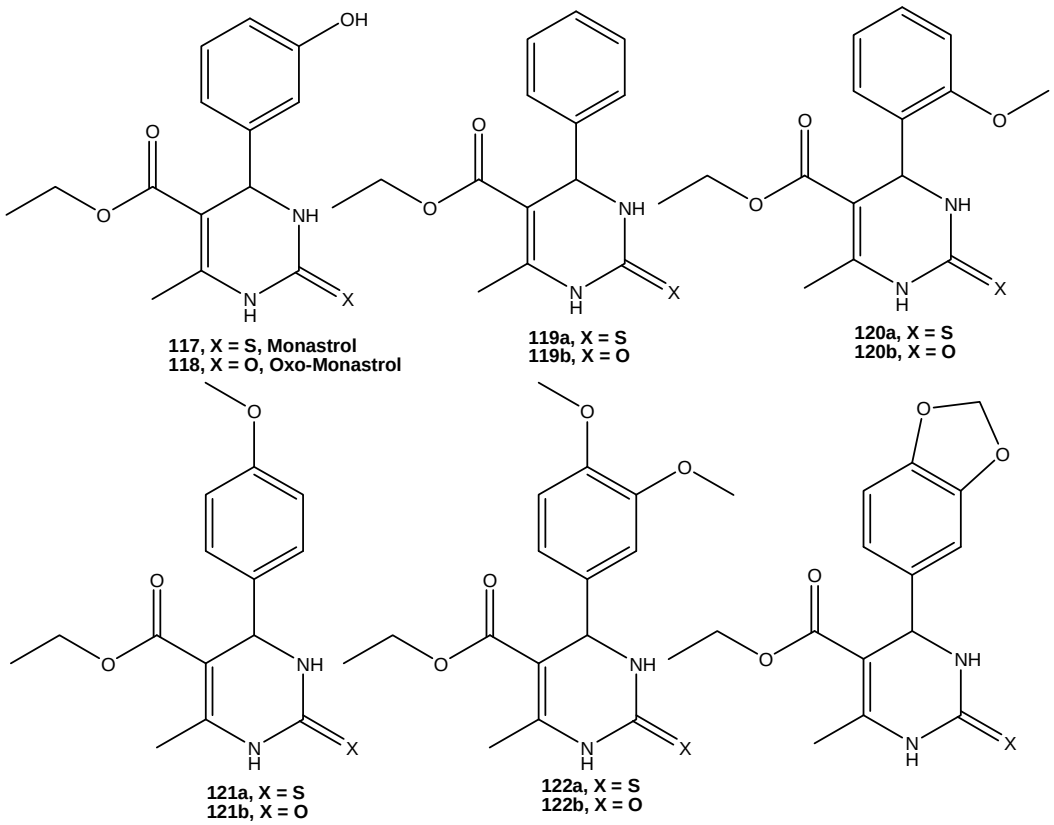
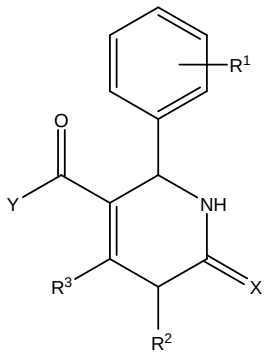


Figure-30. Monastrol **117**, oxo-Monastrol **118**, thio-analogues **119a-123a** and oxo-analogues **119b-123b**.

Further, Lopez *et al.*,[189] has synthesized some novel derivatives **124** of monastrol and describe their anticancer activity. Most of the synthesized compounds were very potent in inhibition of Eg5 by inhibiting ATP hydrolysis through an allosteric mechanism.



R^1 = H, 3-OH, 4-OH, 4-OCH_3, 3-Cl, 3-OCH_3, 2-F, 2-Cl, 3-F
X = O, S
Y = C_2H_5O, $C_6H_5C_2H_2O$, CH_3, C_6H_5, 2-FC_6H_4, 3-Cl-C_6H_4, *etc*
R^2 = H, CH_3, CH_2=$CHCH_2$
R^3 = CH_3, CF_3, $(CH_3)_2CH$,C_2H_5

124

Figure. 31. Structure of some anticancer DHPM derivatives.

Some of the 5-benzoyl substituted derivatives in particular **125**, **126**, **127**, **128**, **129** and **130** were very potent cytotoxic agents with IC_{50} values of 14.7, 1.10, 0.25, 0.15, 5.0 and 0.8 respectively.

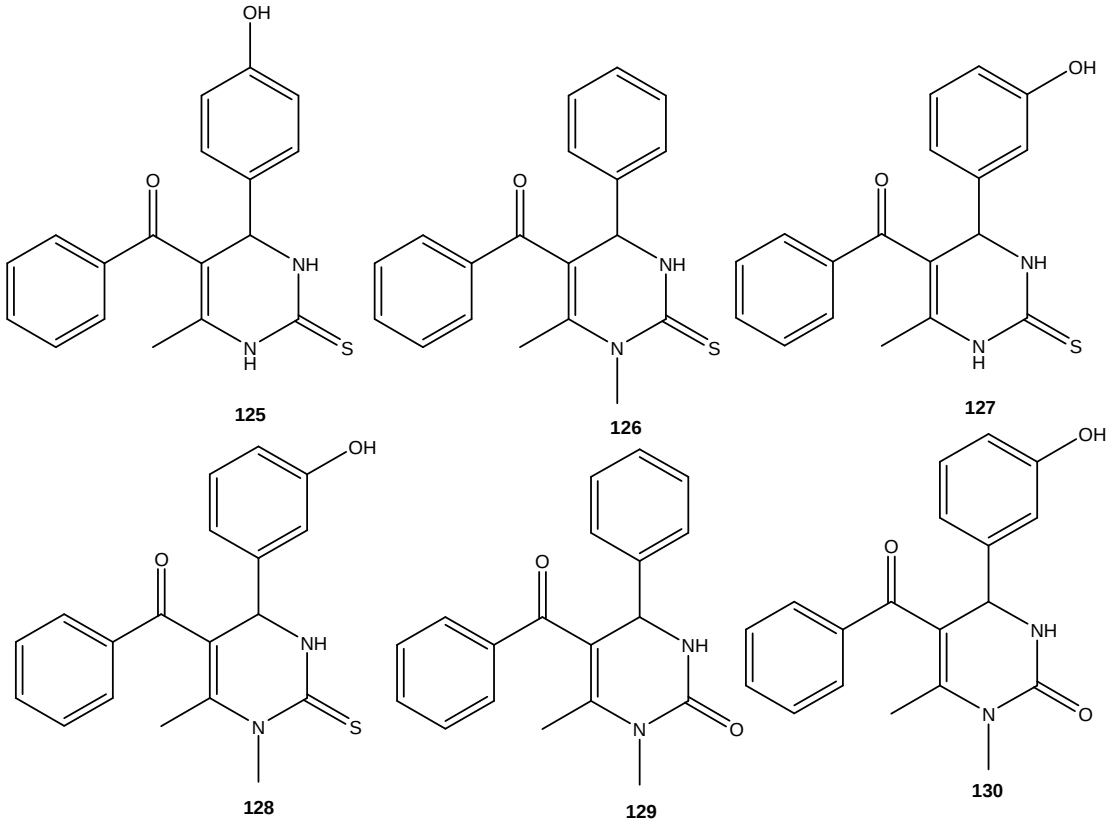


Figure-32. Structure of some potent 5-benzoyl substituted DHPM derivatives.

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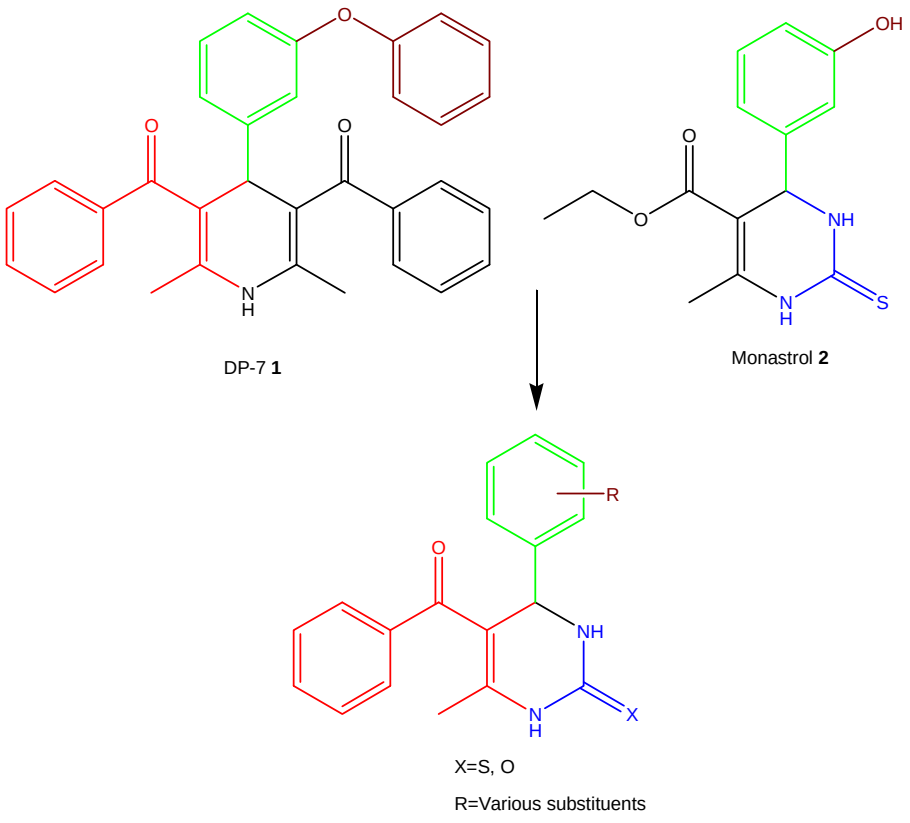
2. Aim of the Present Work

2. Aim of the Present Work

Literature survey reveals that DP-7 **1** (dihydropyridine derivative) is a potent multi drug reverting agent[1-3] and on the other hand, monastrol **2** (dihydropyrimidine derivative) is potent inhibitor of Eg5, that inhibits ATP hydrolysis through an allosteric mechanism.[4-5] Thus, it was thought to hybridize the structural features of these two potent anticancer molecules so that multidrug reverting activity as well as Eg5 inhibitor activity can be obtained by a single molecule. The structure of the hybridized molecule is given in Scheme-1. These hybridized molecules are aza analogues of the DP-7, bearing various substitutions on the 4th position of the dihydropyrimidine ring.

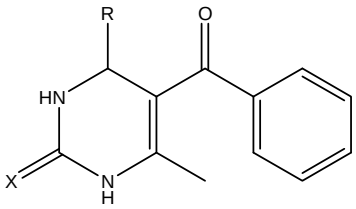


Scheme-1

Dihydropyrimidines (aza analogues of dihydropyridines) are well documented in the literature and various methods of their synthesis have been reported through Biginelli's reaction. The original Biginelli's reaction is a three-component reaction between ethyl acetoacetate, urea or thiourea and an aldehyde, under Bronsted acidic catalysis that affords 3,4-dihydropyrimidin 2(1*H*)-ones.[6] However, this reaction suffers from the harsh conditions, long reaction times and frequently low yields. Although there are many methods for the preparation of dihydropyrimidinones[7,8], we were particularly interested in multicomponent process which allows rapid access to large number of derivatives in very

short time period. Chiral versions of multi-step[9] or multicomponent[10-13] synthesis of dihydropyrimidinones were recently reported. The use of Lewis acids as catalyst has been extensively explored.[14-20] Recently, it has been demonstrated that the 3,4-dihydropyrimidin-2(1*H*)-ones can be easily synthesized by the multicomponent cyclocondensation of ethyl acetoacetate, urea and aldehydes under $SnCl_2.2H_2O$[21] and $In(OTf)_3$ catalysis.[22]

In the present report, we had used conc. HCl as catalyst for the synthesis of dihydropyrimidine derivatives (**VII*i-xxx***), using simple protocol and avoiding used of expensive catalysts.



R = Ph, 4-Cl Ph, 3-Cl Ph, 2-Cl Ph, 4-CN Ph, 3-Br Ph, 3-OH Ph, 3-NO_2
X = O, S

VII*i-xxx*

The newly synthesized derivatives have been evaluated for their Multi Drug Reverting activity on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry.

2.1 References

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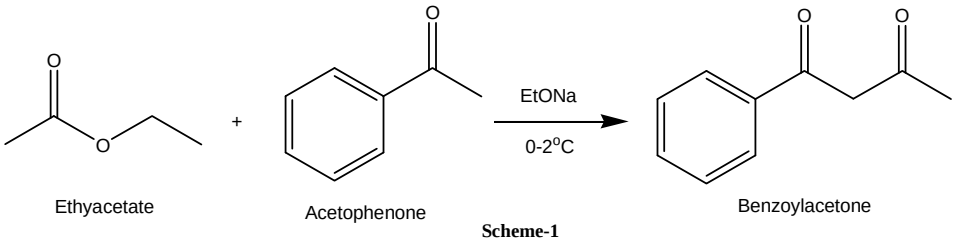
3. Results and Discussion

3. Results and Discussion

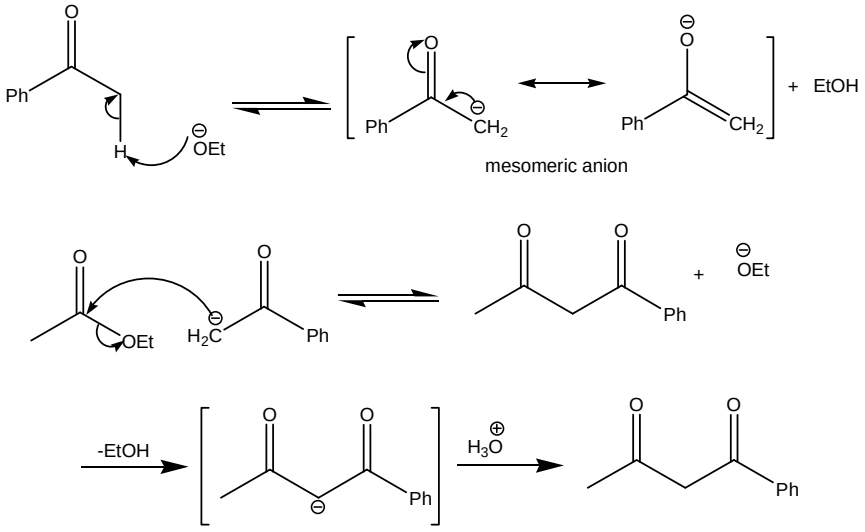
3.1 Synthesis of Starting Material: Benzoylacetone

Benzoylacetone was synthesized as per literature method[1] by reacting acetophenone and ethylacetate in the presence of strong alkali such as sodium hydride or freshly prepared sodium methoxide under chilling conditions as given in the Scheme 1.



Scheme-1

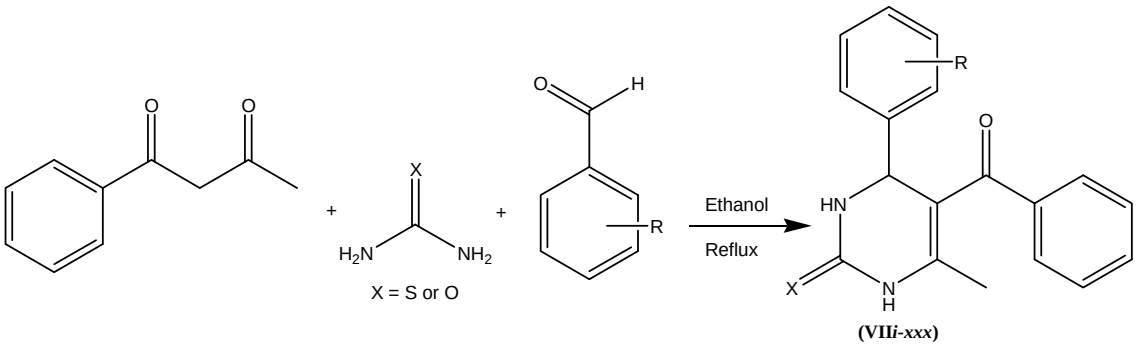
Acylation of ketones with esters requires the presence of a strong base under anhydrous conditions. Ketones, where only one unique mesomeric carbanion formed (*e.g.* symmetrical ketones or alkyl aryl ketones) yield a single regioisomer. The reaction is illustrated by the formation of benzoylacetone from acetophenone and ethyl acetate and may be outlined mechanistically in Scheme-2.



Scheme-2: Proposed mechanis for the synthesis of bezoylacetone

3.2 Synthesis of Target Dihydropyrimidine Compounds (VII*i-xxx*)

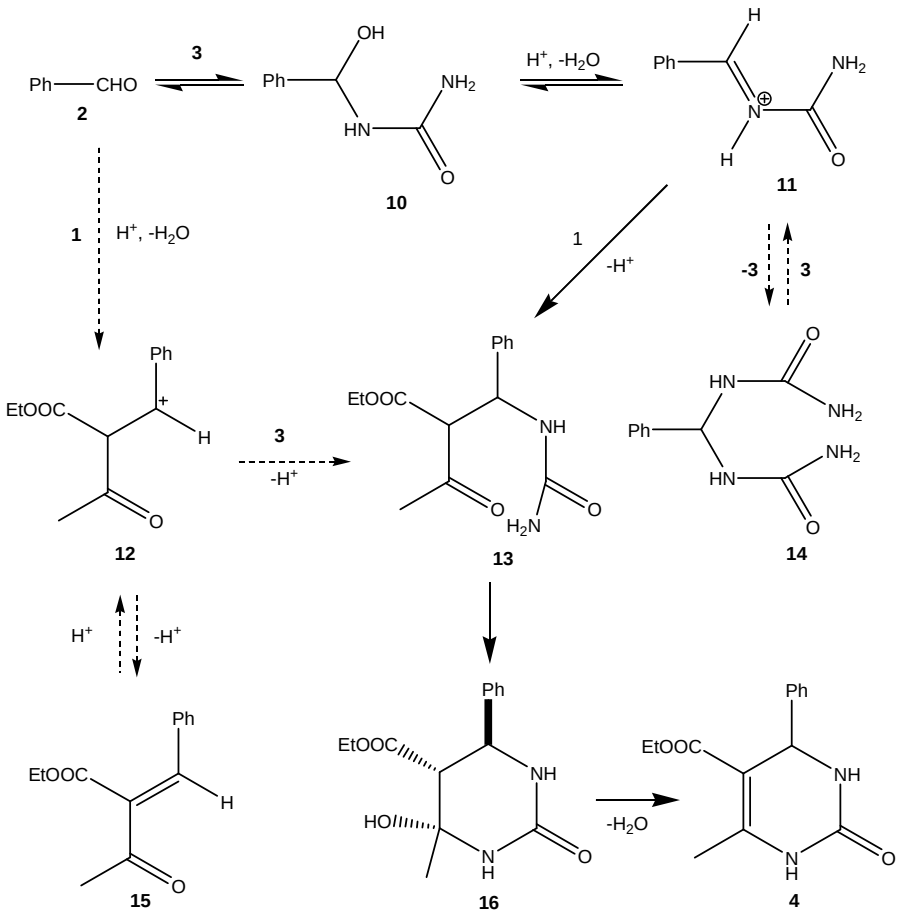
The target compounds were synthesized following classical Biginelli reaction by reacting 1,3-diketon (benzoylacetone), urea or thiourea and substituted aromatic aldehyde as a single pot reaction in the presence of catalytic amount of conc. HCl as given in the Scheme-2.



(VII*i-xxx*)

Scheme-3. Synthesis of 4-phenyl(sub)dihydropyrimidines

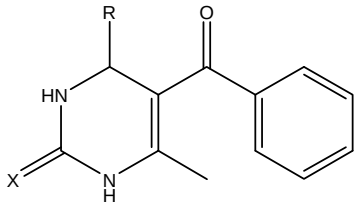
The mechanism of the Biginelli reaction has been the subject of some debate over the past decades. Early work by Folkers and Johnson suggested that bisureide **14**, *i.e.*, the primary bimolecular condensation product of benzaldehyde **2** and urea **3**, is the first intermediate in this reaction.[2] In 1973, Sweet and Fissekis proposed a different pathway and suggested that carbenium ion **12**, produced by an acid-catalyzed aldol reaction of benzaldehyde **2** with ethyl acetoacetate **1**, is formed in the first and limiting step of the Biginelli condensation (**2→12→13**).[3] In 1997, mechanism was reinvestigated using $^{1}H/^{13}C$ NMR spectroscopy and trapping experiments and it has been established that the key step in this sequence involves the acid-catalyzed formation of an *N*-acyliminium ion intermediate of type **11** from the aldehyde **2** and urea **3** precursors.[4] Interception of the iminium ion **11** by ethyl acetoacetate **1**, presumably through its enol tautomer, produces an open-chain ureide **13** which subsequently cyclizes to hexahydropyrimidine **16**. Acid-catalyzed elimination of water from **16** ultimately leads to the final DHPM product **4**. The reaction mechanism can therefore be classified as an *R*-amidoalkylation, or more specifically as an *R*-ureidoalkylation.[5] The alternative "carbenium ion mechanism" **2→12→13**[3] does not constitute a major pathway; however, small amounts of enone **15** are sometimes observed as byproduct.[4] Although the highly reactive *N*-cyliminium ion species **11** could not be isolated or directly observed, further evidence for the proposed mechanism was obtained by isolation of intermediates **17** and **18**, employing sterically bulky[6] or electron-deficient acetoacetates,[7] respectively. The relative stereochemistry in hexahydropyrimidine **18** was established by an X-ray analysis.[7] In fact, a number of hexahydropyrimidines closely related to **18** could be synthesized by using perfluorinated 1,3-dicarbonyl compounds or *β*-keto esters as building blocks in the Biginelli condensation.[8]



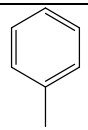
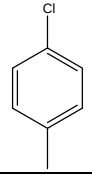
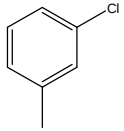
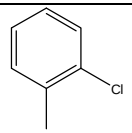
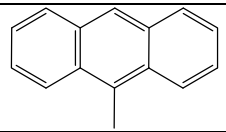
Scheme-4. Mechanism of Biginelli reaction

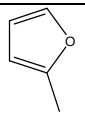
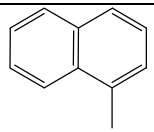
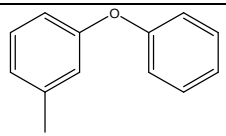
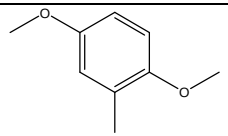
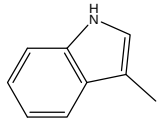
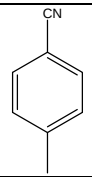
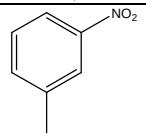
Using Biginelli protocol following hybridized DHPM derivatives has been synthesized.

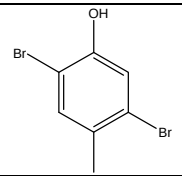
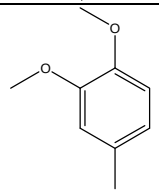
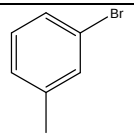
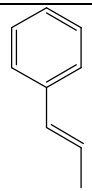
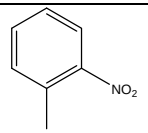
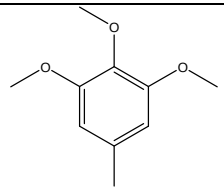
Table-44. Physical data of the newly synthesized derivatives

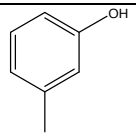
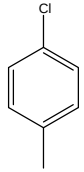
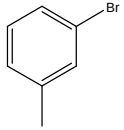
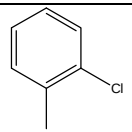
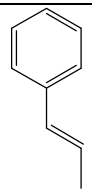
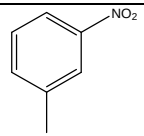


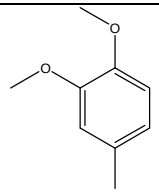
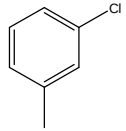
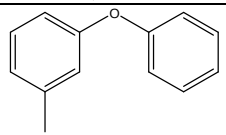
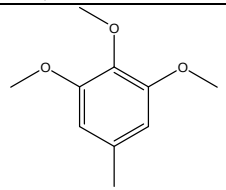
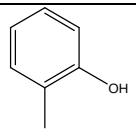
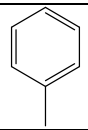
(VII*i-xxx*)

S. No	R	X	Mol Formula (Sol. of Recryst.)	M.P. (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
VII*i*		O	$C_{18}H_{16}N_2O_2$ (E)	190-192	3269(γ_{NH}), 2915(γ_{C-H}), 1710(γ_{CO}), 1680(γ_{CONH}).	--	--
VII*ii*		O	$C_{18}H_{15}ClN_2O_2$ (E)	228-230	3307(γ_{NH}), 1706(γ_{CO}), 1677(γ_{CONH}), 1586(γ_{CH}).	326[M^+], 311, 215, 185.	1.72 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 2.96), 7.34 (br, s, 1H, N*H* of 1), 7.22-7.49 (m, 9H, Ar-*H*), 8.94 (s, 1H, N*H* at 3).
VII*iii*		O	$C_{18}H_{15}ClN_2O_2$ (E)	214-216	3273(γ_{NH}), 1712(γ_{CO}), 1674(γ_{CONH}), 1600(γ_{CH}).	326[M^+], 311, 307, 291, 215, 185.	1.72 (s, 3H, C*H*$_3$), 5.43 (d, 1H, *H* at 4, *J* = 3.04), 7.59 (br, s, 1H, N*H* at 1), 7.17-7.50 (m, 9H, Ar-*H*), 9.06 (s, 1H, N*H* at 3).
VII*iv*		O	$C_{18}H_{15}ClN_2O_2$ (E)	248-250	3284(γ_{NH}), 2951(γ_{CH}), 1650(γ_{CONH}).	--	1.79 (s, 3H, C*H*$_3$), 5.87 (d, 1H, *H* at 4, *J* = 2.56), 7.02 (br, s, 1H, N*H* at 1), 7.19-7.50 (m, 9H, Ar-*H*), 9.04 (s, 1H, N*H* at 3).
II*v*		O	$C_{26}H_{20}N_2O_2$ (E)	232-234	2986(γ_{CH}), 1652(γ_{CONH}).	--	--

S. No	R	X	Mol Formula (Sol. of Recryst.)	M.P. (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
VII*vi*		O	$C_{16}H_14N_2O_3$ (E)	>280	3411(γ_{NH}), 2965(γ_{CH}), 1710(γ_{CO}), 1652(γ_{CONH}).	--	--
VII*vii*		O	$C_{22}H_{18}N_2O_2$ (E)	226-228	3369(γ_{NH}), 2990(γ_{CH}), 1714(γ_{CO}).	--	--
VII*viii*		O	$C_{24}H_{20}N_2O_3$ (E)	168-170	3278(γ_{NH}), 2920(γ_{CH}), 1697(γ_{CO}), 1674(γ_{CONH}).	--	1.72 (s, 3H, C*H*$_3$), 5.53 (d, 1H, *H* at 4, *J* = 2.68), 6.71 (s, 1H, N*H* at 1), 6.81-7.49 (m, 14 H, Ar-*H*), 8.60 (s, 1H, N*H* at 3).
VII*ix*		O	$C_{20}H_{20}N_2O_4$ (E)	206-208	3233(γ_{NH}), 2941(γ_{CH}, 1697(γ_{CO}).	--	--
VII*x*		O	$C_{20}H_{17}N_3O_2$ (E)	268-270	3405(γ_{NH}), 3099(γ_{CH}), 1706(γ_{CO}).	--	--
VII*xi*		O	$C_{19}H_{15}N_3O_2$ (E)	192-194	3296(γ_{NH}), 2232(γ_{CN}), 1677(γ_{CONH}).	--	1.70 (s, 3H, C*H*$_3$), 5.50 (d, 1H, *H* at 4, *J* = 3.24), 7.71 (br., 1H, N*H* at 1), 7.37-7.63 (m, 9H, Ar-*H*), 9.16 (s, 1H, N*H* at 3).
VII*xii*		O	$C_{18}H_{15}N_3O_4$ (E)	240-242	3229(γ_{NH}), 1710(γ_{CO}), 1685(γ_{CONH}).	--	--

S. No	R	X	Mol Formula (Sol. of Recryst.)	M.P. (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
VII*xiii*		O	$C_{18}H_{14}Br_2N_2O_3$ (E)	240-242	3383(γ_{NH}), 2955(γ_{CH}), 1712(γ_{CO}), 1689(γ_{CONH}).	--	--
VII*xiv*		O	$C_{20}H_{20}N_2O_4$ (E)	234-236	3372(γ_{NH}), 1656(γ_{CONH}).	--	--
VII*xv*		O	$C_{18}H_{15}BrN_2O_2$ (E)	218-220	3290(γ_{NH}), 2946(γ_{CH}), 1708(γ_{CO}), 1673(γ_{CONH}).	--	--
VII*xvi*		O	$C_{20}H_{18}N_2O_2$ (E)	184-186	3232(γ_{NH}), 2933(γ_{CH}), 1721(γ_{CO}), 1678(γ_{CONH}).	--	--
VII*xvii*		O	$C_{18}H_{15}N_3O_4$ (E)	222-224	3297(γ_{NH}), 1691(γ_{CO}), 1657(γ_{CONH}).	--	--
VII*xviii*		O	$C_{21}H_{22}N_2O_5$ (E)	222-224	3297(γ_{NH}), 2940(γ_{CO}), 1702(γ_{CO}).	--	1.78 (s, 3H, C*H*$_3$), 3.75 (s, 9H, (OC*H*$_3$)$_3$), 5.48 (d, 1H, *H* at 4, *J* = 2.90), 6.48 (s, 2H, Ar-*H* of Phenyl), 6.96 (br, 1H, N*H* at 1), 7.39-7.52 (m, 5H, Ar-*H*), 8.76 (s, 1H, N*H* at 3).

S. No	R	X	Mol Formula (Sol. of Recryst.)	M.P. (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
VII*ix*		O	$C_{18}H_{16}N_2O_3$ (E)	256-258	3378(γ_{OH}), 3282(γ_{NH}), 1715(γ_{CO}), 1644(γ_{CONH}).	--	--
VII*xx*		S	$C_{18}H_{15}ClN_2OS$ (E)	244-245	3280(γ_{NH}), 2935(γ_{CH}), 1588, 1550(γ_{CH}).	342[M^+], 337, 231, 105.	1.76 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 3.36), 7.21-7.59 (m, 9H, Ar-*H*), 9.29 (s, 1H, N*H* at 1), 9.98 (s, 1H, N*H* at 3).
VII*xxi*		S	$C_{18}H_{15}BrN_2OS$ (E)	198-200	3282(γ_{NH}), 3070(γ_{CH}), 1606, 1575(γ_{CH}).	388[M^+], 371, 281, 231, 172, 144.	1.78 (s, 3H, C*H*$_3$), 5.49 (d, 1H, *H* at 4, *J* = 3.2), 7.15-7.51 (m, 9H, Ar-*H*), 8.90 (s, 1H, N*H* at 1), 9.61 (s, 1H, N*H* at 3).
VII*xxii*		S	$C_{18}H_{15}ClN_2OS$ (E)	202-203	3289(γ_{NH}), 3020(γ_{CH}), 1585(γ_{CH}).	--	1.82 (s, 3H, C*H*$_3$), 5.88 (d, 1H, *H* at 4, *J* = 2.76), 7.19-7.55 (m, 9H, Ar-*H*), 8.73 (s, 1H, N*H* at 1), 10.04 (s, 1H, N*H* at 3)
VII*xxiii*		S	$C_{20}H_{18}N_2OS$ (E)	220-222	3207(γ_{NH}), 3081(γ_{CH}), 1587(γ_{CH}).	--	1.76 (s, 3H, C*H*$_3$), 5.01 (dd, 1H, *H* at 4, *J* = 3.7), 6.20 (dd., 1H, *H* at a, *J* = 6.2), 6.39 (dd., 1H, *H* at b, *J* = 15.88), 7.21-7.62 (m, 10H, Ar-*H*), 8.84 (s, 1H, N*H* at 1), 9.77 (s, 1H, N*H* at 3).
VII*xxiv*		S	$C_{18}H_{15}N_3O_3S$ (E)	236-238	3286(γ_{NH}), 3008(γ_{CH}), 1602(γ_{CH}).	--	--

S. No	R	X	Mol Formula (Sol. of Recryst.)	M.P. (^oC)	IR (cm^{-1}) (KBr)	Mass (m/e)	NMR (δppm) (DMSO-d_6)
VII*xxv*		S	$C_{20}H_{20}N_2O_3S$ (E)	240-242	3288(γ_{NH}), 2991(γ_{CH}), 1612, 1571(γ_{CH}).	--	1.78 (s, 3H, C*H*$_3$), 3.78 (s, 3H, -OC*H*$_3$), 3.81 (s, 3H, -OC*H*$_3$), 5.42 (d, 1H, *H* at 4, *J* = 3.12), 6.77-6.80 (m, 3H, Ar-*H* phenyl at 4), 7.37-7.51 (m, 5H, Ar-*H* of benzoyl), 9.16 (s, 1H, N*H* at 1), 9.90 (s, 1H, N*H* at 3)
VII*xxvi*		S	$C_{18}H_{15}ClN_2OS$ (E)	188-190	3250(γ_{NH}), 3009(γ_{CH}), 1524(γ_{CH}).	--	--
VII*xxvii*		S	$C_{24}H_{20}N_2O_2S$ (E)	208-210	3287(γ_{NH}), 3092(γ_{CH}), 1573(γ_{CH}).	--	1.75 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 3.24), 6.68-7.61 (m, 14H, Ar-*H*), 9.25 (s, 1H, N*H* at 1), 9.95 (s, 1H, N*H* at 3).
VII*xxviii*		S	$C_{21}H_{22}N_2O_4S$ (E)	228-230	3282(γ_{NH}), 2994(γ_{CH}), 1608(γ_{CH}).	--	--
VII*xxix*		S	$C_{18}H_{16}N_2O_2S$ (E)	248-250	3206(γ_{NH}), 2991(γ_{CH}), 1514(γ_{CH}).	--	--
VII*xxx*		S	$C_{18}H_{16}N_2OS$ (E)	221-220	3283(γ_{NH}), 3170(γ_{CH}), 1587(γ_{CH}).	--	--

E = Ethanol

3.3 Spectral Discussion:

The 4-phenyl(substituted)dihydropyrimidinones are colored solid (some dark brown, yellow, bright orange and buff white), with high melting points generally above 200^{o}C. These compounds are soluble in DMF and practically insoluble in methanol, hexane or cold ethanol.

Infra Red (IR) Spectra

The NH stretch in dihydropyrimidines was observed at 3400-3200 cm^{-1}. The characteristic carbonyl group (C=O) was observed between 1720-1660 cm^{-1}. The dihydropyrimidinones showed the ring skeleton vibrations at 1630-1600, 1590-1550, 1520-1550, 1495-1470 cm^{-1}.

The ^{1}H NMR Spectra

The ^{1}H NMR spectra of dihydropyrimidines were taken in DMSO-d_6. The compounds studies, showed characteristic peaks corresponding to the protons of different groups and functionalities in the molecules. The 2-methylene protons appear as a singlet at around 1 to 2 ppm. The NH protons present in all the compounds were observed as a singlet above 9 ppm. All the aromatic protons present in the molecules were observed as a multiplet at 7-8 ppm. The chiral proton at postion-4 was observed as a doublet between 5 to 6 ppm.

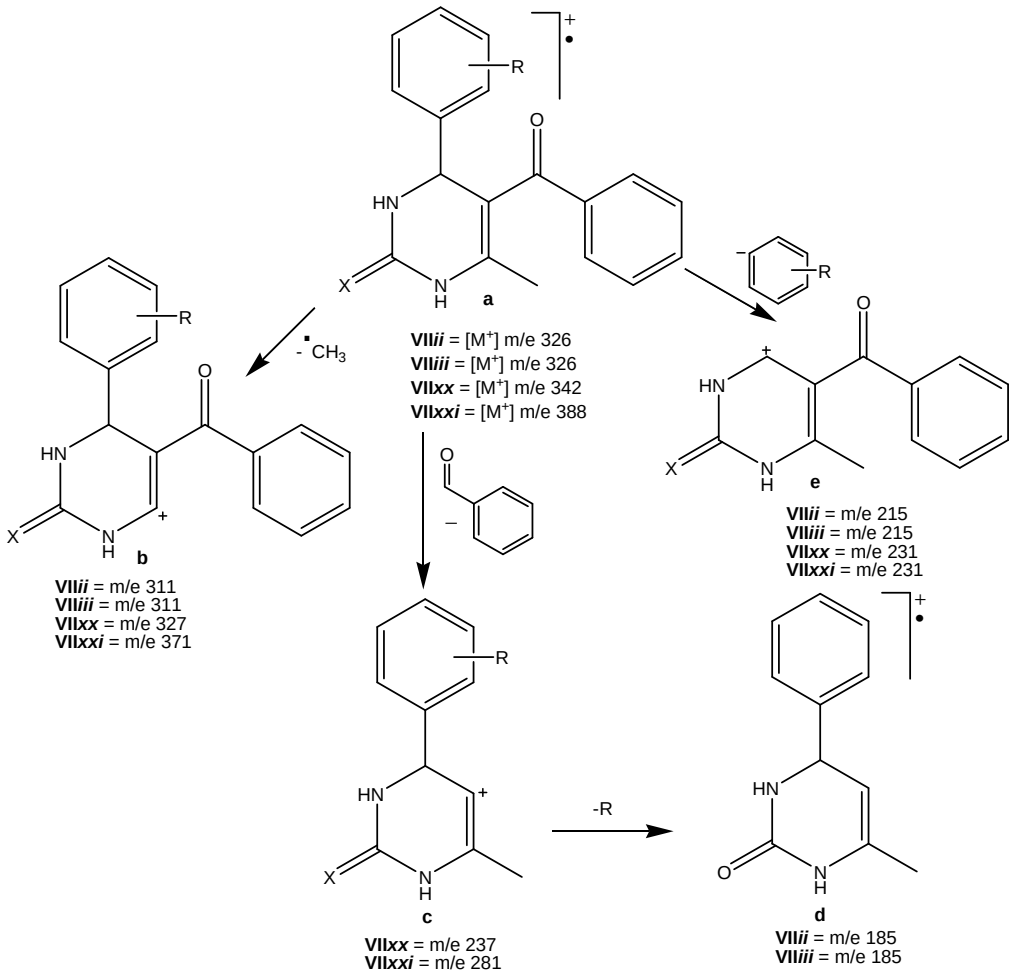
^{13}C NMR Spectra:

^{13}C NMR spectra were recorded on Bruker AC 400 MHz instrument using DMSO-d_6 as the solvent and TMS (Tetramethyl silane) as respective internal standard. All the compounds showed characteristic peaks corresponding to the different carbons present in the molecules.

The Mass Spectra

The fragmentation pattern of the synthesized DHPM, under electron impact ionization has been studied. Many prominent fragment ion peaks were revealed in the mass spectra of these compounds. The mass spectrum DHPM clearly showing the molecular ion peak (**a**), corresponding to their molecular weight. The major mode of fragmentation is loss of methyl ion to give daughter ion (**b**) and ejection of 3-bezoyl group to give second daughter ion (**c**). The daughter ion (**c**) loses substitutions of 4-phenyl group to give fragment (**d**). Molecular ion (**a**) also gives third daughter ion (**e**) by the ejaculation of 4-

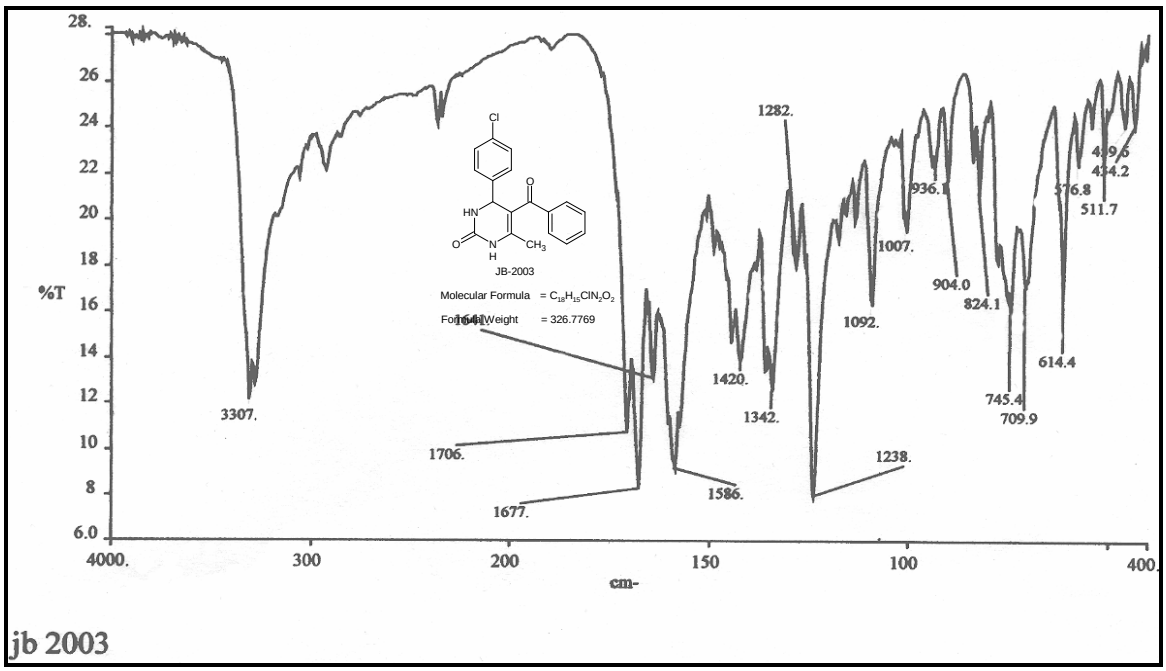
phenyl ring. The common fragmentation pattern of newly synthesized DHPM derivatives is given in Scheme-5 (Table-1).



Scheme-5

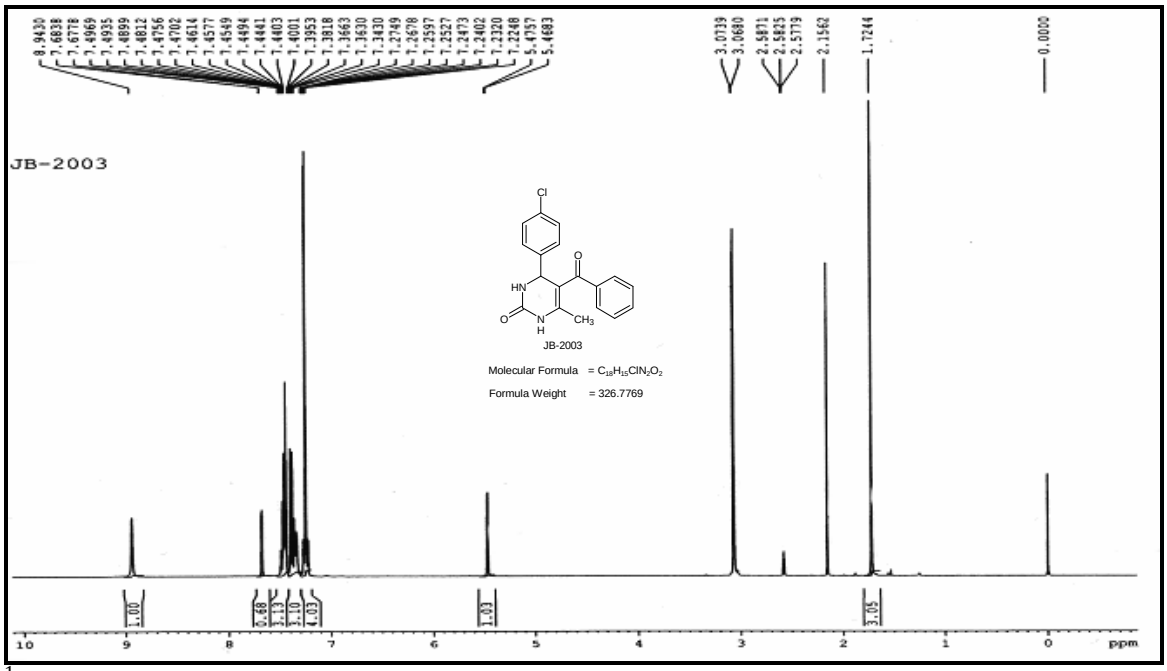
Specimen IR spectra of some DHPMs:

1. IR spectrum of 5-benzoyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*ii*)**



IR (KBr) cm^{-1}: 3307(γ_{NH}), 1706(γ_{CO}), 1677(γ_{CONH}), 745($\gamma_{C\text{-}Cl}$).

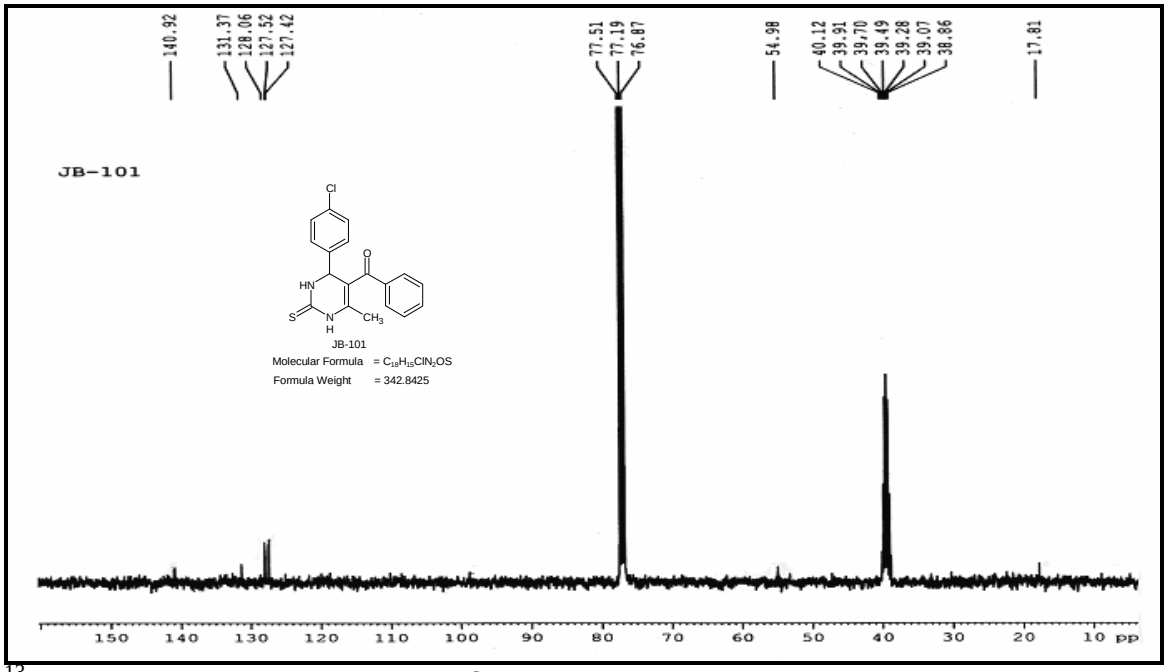
2. ^{1}H NMR spectrum of 5-benzoyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one **(VII*ii*)**



^{1}H NMR 400 MHz (DMSO-d_6, δppm): 1.72 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 2.96), 7.34 (br, s, 1H, N*H* of 1), 7.22-7.49 (m, 9H, Ar-*H*), 8.94 (s, 1H, N*H* at 3).

Specimen ^{13}C NMR spectra of some DHPMs:

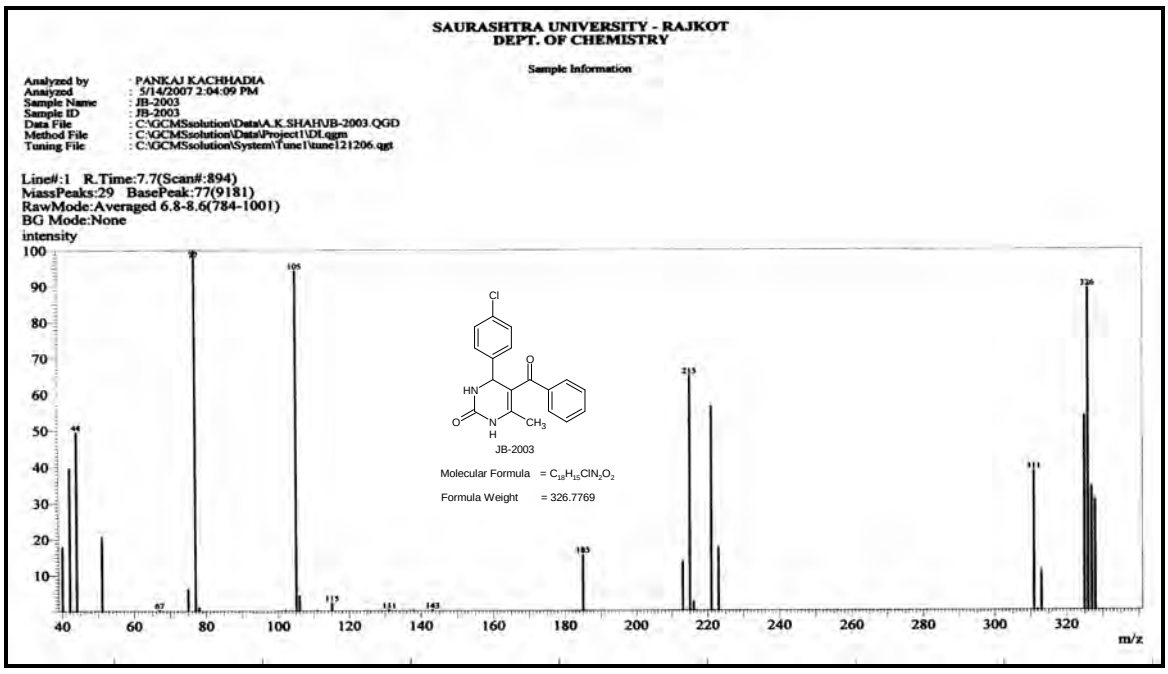
3. ^{13}C NMR spectrum of [4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl)methanone **(VII*xx*)**



^{13}C NMR 400 MHz (DMSO-d_6, δppm): 17.81, 54.98, 76.87, 77.19, 77.51, 127.42, 127.52, 128.06, 131.37, 140.92.

Specimen Mass spectra of some DHPMs:

4. Mass spectrum of 5-benzoyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*ii*)**



Mass m/e: [M^+] 326, 311, 215, 185, 105, 77.

3.4 MDR reversal effects of Newly Synthesized DHPM Series (VII*i-xxx*)

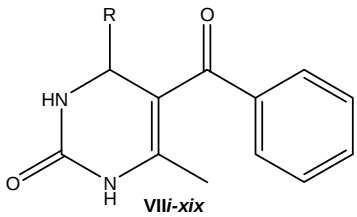
MDR reversal effects of DHPM Series (VII*i-xxx*) on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry.[9-10]

The cells were adjusted to a density of 2×10^6/ml, resuspended in serum-free McCoy's 5A medium and distributed in 0.5 ml aliquots into Eppendorf centrifuge tubes. The tested compounds were added at various concentrations in different volumes (2.0-20.0 μl) of the 1.0-10.0 mg/ml stock solutions, and the samples were incubated for 10 min at room temperature. Next, 10 μl (5.2 μM final concentration) of the indicator rhodamine 123 was added to the samples and the cells were incubated for a further 20 min at 37°C, washed twice and resuspended in 0.5 ml PBS for analysis. The fluorescence of the cell population was measured with a Beckton Dickinson FACScan flow cytometer. Verapamil was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared with the untreated cells. An activity ratio R was calculated via the following equation, on the basis of the measured fluorescence values:

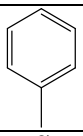
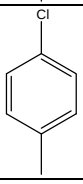
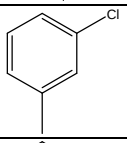
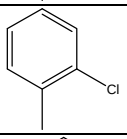
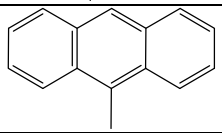
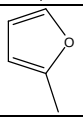
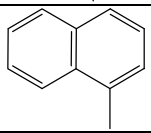
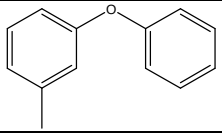
$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\ treated \,/\ parental\,control}$$

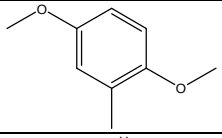
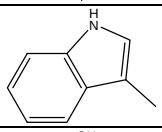
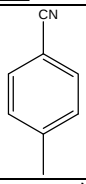
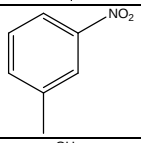
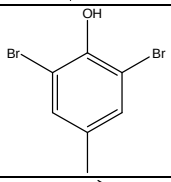
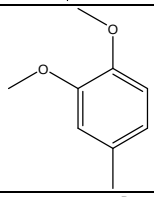
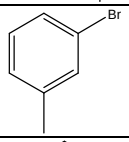
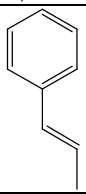
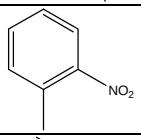
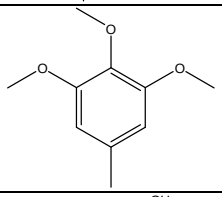
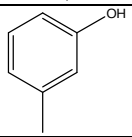
Results of this protocol have been elucidated in the Table-45.

Table-45. Antiproliferative activity results of newly synthesized DHPM derivatives (VII*i-xxx*).



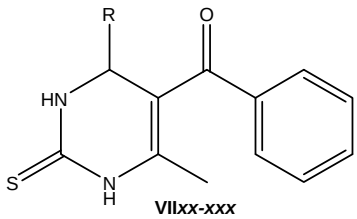
VII*i-xix*

	Samples	µM	dye	FSC	SSC	FL-1	FAR	Peak Ch
1	**PAR**	-	R123	458,30	172,15	941,45	-	865
2	**PAR**	-	R123	459,37	173,16	970,00	-	1036
3	**MDR**	-	R123	514,50	220,60	9,56	-	8
MDR mean				484,91	213,55	**8,91**	-	-
	Verapamil	21,99	R123	510,85	222,13	27,43	3,07	16
VII*i*		4	R123	512,10	212,07	9,78	1,09	9
VII*ii*		4	R123	520,45	220,83	8,58	0,96	8
VII*iii*		4	R123	502,01	219,11	7,98	0,89	7
VII*iv*		4	R123	503,71	210,74	8,74	0,98	7
VII*v*		4	R123	494,06	212,48	40,75	4,57	31
VII*vi*		4	R123	481,11	206,48	11,29	1,26	10
VII*vii*		*4*	*R123*	*461,05*	*207,98*	*266,15*	*29,87*	*257*
VII*viii*		4	R123	478,71	203,54	10,96	1,23	10

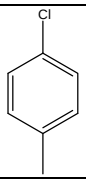
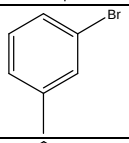
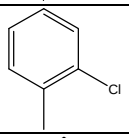
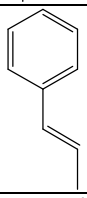
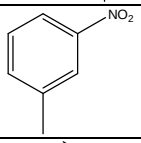
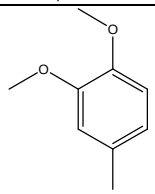
VII*ix*		*4*	*R123*	*471,36*	*201,90*	*328,41*	*36,85*	*302*
VII*x*		*4*	*R123*	*456,30*	*199,22*	*699,99*	*78,56*	*661*
VII*xi*		4	R123	470,90	200,91	18,83	2,11	18
VII*xii*		4	R123	448,05	201,36	12,40	1,39	10
VII*xiii*		4	R123	463,37	204,56	7,35	0,82	6
VII*xiv*		4	R123	455,91	202,17	10,28	1,15	8
VII*xv*		4	R123	458,85	201,31	10,30	1,15	9
VII*xvi*		4	R123	467,70	202,83	8,90	0,99	8
VII*xvii*		4	R123	464,03	198,74	8,44	0,94	7
VII*xviii*		4	R123	459,71	201,39	8,62	0,96	7
VII*xix*		4	R123	563,26	221,33	6,38	0,97	6
	DMSO control 20 µl		R123	473,83	205,24	8,65	0,97	8
	MDR	-	R123	455,32	206,50	8,25	-	8

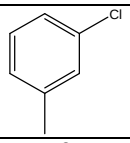
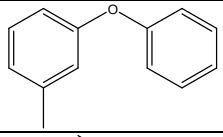
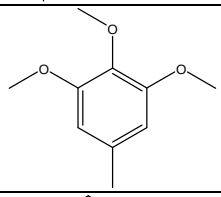
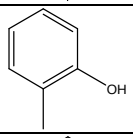
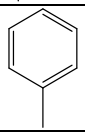
FAR: Fluorescence Activity Ratio

Table-45. Antiproliferative activity results of newly synthesized DHPM derivatives (VII*i-xxx*) (Contd.)



VII*xx-xxx*

	Samples	µM	dye	FSC	SSC	FL-1	FAR	Peak Ch
1	**PAR**	-	R123	509,50	196,92	963,39	-	956
2	**PAR**	-	R123	517,57	201,82	979,59	-	1074
3	**MDR**	-	R123	568,14	234,98	6,93	-	7
MDR mean				571,32	237,35	**6,56**	-	-
	Verapamil	21,99	R123	568,16	234,96	27,99	4,26	14
VII*xx*		4	R123	569,75	226,45	6,79	1,03	6
VII*xxi*		4	R123	568,15	227,89	7,25	1,11	6
VII*xxii*		4	R123	571,86	226,69	7,67	1,17	6
VII*xxiii*		4	R123	559,66	224,18	8,44	1,28	7
VII*xxiv*		4	R123	562,04	226,11	5,79	0,88	5
VII*xxv*		4	R123	557,19	221,92	8,40	1,28	6

VII*xxvi*		4	R123	565,01	225,60	7,95	1,21	7
VII*xxvii*		*4*	*R123*	*567,04*	*221,23*	*24,17*	*3,68*	*11*
VII*xxviii*		4	R123	564,75	222,69	6,11	0,93	5
VII*xxix*		4	R123	568,09	225,82	6,78	1,03	6
VII*xxx*		4	R123	571,58	222,31	6,39	0,97	5
	DMSO control 20 µl		R123	580,10	226,24	6,52	0,99	5
	MDR	-	R123	574,51	239,73	6,19	-	5

FAR: Fluorescence Activity Ratio

3.5 Discussion

In the present report, hybrid derivatives (thio and oxo analogues) of DP-7 (dihydropyridine) and monastrol (dihydropyrimidines) were synthesized to get the dual action in cancer chemotherapy. The newly synthesized molecules were screened on MDR1-gene transfected mouse lymphoma cell line (l5178 y) for MDR reversal effects at non toxic concentrations.

MDR reversal assay has gained importance in view of many cancerous cells developing multiple drug resistance (MDR) due to incorporation of MDR-1 gene coding of Pgp, a glycoprotein involved in MDR. The glycoprotein Pgp is driven by ATP and is responsible for efflux of drug from the cancerous cells leading to MDR. Therefore, MDR reversal agents are being exploited as potential anticancer agents.[9-10]

The four compounds from this series were very effective, namely 5-benzoyl-6-methyl-4-(1-naphthyl)-3,4-dihydropyrimidin-2(1*H*)-one (**VII*vii*)**, 4-(2,5-dimethoxyphenyl)-6-methyl-5-(1-phenylvinyl)-3,4-dihydropyrimidin-2(1*H*)-one (**VII*ix*),** 5-benzoyl-4-(3*H*-indol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (**VII*x*)** and [6-methyl-4-(3-phenoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl](phenyl)methanone (**VII*xxvii*)** when measured in 4 micromol/ml/1million cells. These compounds had moderate effect on the MDR reversal efflux pump activity.

The substitution of 3-phenyloxy ring on 4th phenyl ring in case of (**VII*viii*)** resulted an ineffective compounds while dimethoxy, indolyl and napthyl substituents are very active as resistance modifiers The differences in biological effects can be explained by steric differences in binding abilities of compounds to Pgp. Direct evidence of the nature of effective binding of the four most effective compounds to Pgp could be obtained by X-ray diffraction of co-crystallizations.

3.6 Reference:

1. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G. and Tatchell, A. R. In *Vogel's Text Book of Practical Organic Chemistry*, 5th edition, Addison-Wesley Longman: Harlow, 1998, pp 634-635.
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4. Kappe, C. O. *J. Org. Chem.* **1997**, *62,* 7201.
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6. Hu, E. H.; Sidler, D. R.; Dolling, U.-H. *J. Org. Chem.* **1998**, *63,* 3454.
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8. Saloutin, V. I.; Burgat, Ya. V.; Kuzueva, O. G.; Kappe, C. O.; Chupakhin, O. N. *J. Fluorine Chem.* **2000**, *103,* 17.
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4. Experimental

4. Experimental

All the chemicals used in the synthesis were of laboratory grade. The melting points were determined in open capillary method on Veego (VMP-D) electronic apparatus and are uncorrected.

The IR spectra of synthesized compounds were recorded on Shimadzu 8400-S FT-IR, as well as, Perkin Elmer BX_2 FT-IR Spectrophotometer in potassium bromide discs.

^{1}H NMR spectra were recorded on a Bruker AC 400 MHz FT-NMR spectrometer using TMS (Tetramethyl silane) as an internal standard and DMSO-d_6 as a solvent at SAIF, Punjab University, Chandigarh.

Mass spectra were obtained by Electron Impact method on (GCMS-QP2010 spectrometer) using 70 eV ionizing beam and using direct insertion probe.

To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated silica plates (Merck Silicagel F_{254}) using hexane-ethyl acetate-glacial acetic acid as the solvent systems and the spots were visualized by exposure to iodine vapors or under ultra violet (UV) light at 254 nm and 360 nm.

4.1 Synthesis of Starting Materials

4.1.1 Preparation of preparation of benzoylacetone[1]

A suspension of 11.0 gm (0.5 mole) of granulated sodium metal in dry xylene (50 ml) was prepared and was transferred to a 1-litre three neck flask and the xylene was decanted. The flask was kept in water bath with a stirrer and 100 ml of ethanol was added to the flask. The mixture was continued to reflux till all the sodium was reacted. The residual sodium ethoxide containing flask was then surrounded with ice and 176.0 gm (2.0 mole) of pure, dry ethyl acetate was added. The stirring was started and 60.0 gm (0.5 mole) of acetophenone was added drop wise. The reaction commences with the precipitation of sodium salt of benzoylacetone. The stirring was continued for 2 hrs and was kept in ice box overnight; the ppt. obtained were filtered and washed with diethyl ether and dried. The dried solid was dissolved in cold water and finally acidified with

glacial acetic acid to get crude benzoylacetone. Crude benzoylacetone was purified by distillation under reduced pressure and obtained as colorless crystalline needles. m.p. 61^{o}C, (m.p. 62^{o}C)[1], 100.0 gm, yield 62%.

4.1.2 Synthesis of target Dihydropyrimidines

1. Synthesis of 5-benzoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*i*) from benzyolacetone

A mixture of benzoylacetone (0.0094 mole; 1.0 gm), benzaldehyde (0.0092 mole; 1.5 gm), urea (0.0111 mole; 0.67gm) and ethanol (10 ml) were taken in a round bottom flask and the contents were dissolved by gently heating the flask. Conc. HCl (1-2 drops) was added to the flask and then refluxed for 6 hrs. After cooling at room temperature, the precipitated solid product was filtered and washed with methanol and then recrystallized from ethanol to afford 5-benzoyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*i*)**.

M.P. : 190-192^{o}C; Yield: 73%.
Mol. Formula : $C_{18}H_{16}N_2O_2$; Mol. Wt. 292.12
IR (KBr) cm^{-1} : 3269(γ_{NH}), 2915(γ_{C-H}), 1710(γ_{CO}), 1680(γ_{CONH}).

2. Synthesis of 5-benzoyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*ii*) from benzyolacetone

A mixture of benzoylacetone (0.00852 mole; 1.38 gm), 4-chlorobenzaldehyde (0.0071 mole; 1 gm) and urea (0.0106 mole; 0.639 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*ii*).**

M.P. : 228-230^{o}C; Yield 64%.
Mol. Formula : $C_{18}H_{15}ClN_2O_2$; Mol. Wt. 326.78
IR (KBr) cm^{-1} : 3307(γ_{NH}), 1706(γ_{CO}), 1677(γ_{CONH}), 1586(γ_{CH}).
^{1}H NMR(DMSO-d_6)δppm : 1.72 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 2.96), 7.34 (br, s, 1H, N*H* of 1), 7.22-7.49 (m, 9H, Ar-*H*), 8.94 (s, 1H, N*H* at 3).

MS m/e : 326[M^+], 311, 215, 185.

3. Synthesis of 5-benzoyl-4-(3-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*iii*) from benzyolacetone

A mixture of benzoylacetone (0.00852 mole; 1.38 gm), 3-cholrobenzaldehyde (0.0071 mole; 1 gm) and urea (0.0106 mole; 0.639 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-4-(3-chlorophenyl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one **(VII*iii*)**.

M.P. : 214-216^{o}C; Yield 67%.

Mol. Formula : $C_{18}H_{15}ClN_2O_2$; Mol. Wt. 326.78

IR(KBr) cm^{-1} : 3273(γ_{NH}), 1712(γ_{CO}), 1674(γ_{CONH}), 1600(γ_{CH})

^{1}H NMR (DMSO-d_6)δppm : 1.72 (s, 3H, C*H*$_3$), 5.43 (d, 1H, *H* at 4, *J* = 3.04), 7.59 (br, s, 1H, N*H* at 1), 7.17-7.50 (m, 9H, Ar-*H*), 9.06 (s, 1H, N*H* at 3).

MS m/e : 326[M^+], 311, 307, 291, 215, 185.

4. Synthesis of 5-benzoyl-4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*iv*) from benzoylacetone.

A mixture of benzoylacetone (0.00852 mole; 1.38 gm), 2-cholrobenzaldehyde (0.0071 mole; 1 gm) and urea (0.0106 mole; 0.639 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-4-(2-chlorophenyl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one **(VII*iv*)**.

M.P. : 248-250^{o}C; Yield: 71%.

Mol. Formula : $C_{18}H_{15}ClN_2O_2$; Mol. Wt. 326.78

IR(KBr) cm^{-1} **:** 3284(γ_{NH}), 2951(γ_{CH}), 1650(γ_{CONH})

^{1}H NMR(DMSO-d_6)δppm **:** 1.79 (s, 3H, C*H*$_3$), 5.87 (d, 1H, *H* at 4, *J* = 2.56), 7.02 (br, s, 1H, N*H* at 1), 7.19-7.50 (m, 9H, Ar-*H*), 9.04 (s, 1H, N*H* at 3).

5. Synthesis of 4-(9-anthryl)-5-benzoyl-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*v*) from benzyolacetone

A mixture of benzoylacetone (0.0058 mole; 0.94 gm), anthrylaldehyde (0.0048 mole; 1 gm) and urea (0.0048 mole; 0.43 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 4-(9-anthryl)-5-benzoyl-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*v*)**.

M.P. : 232-234^{o}C (decompose); Yield: 74%.

Mol. Formula : $C_{26}H_{22}N_2O_2$; Mol. Wt. 394.47

IR(KBr) cm^{-1} : 2986(γ_{CH}), 1652(γ_{CONH}).

6. Synthesis of 5-benzoyl-4-(2-furyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*vi*) from benzoylacetone

A mixture of benzoylacetone (0.0124 mole; 2.02 gm), furfurylaldehyde (0.0104 mole; 1 gm) and urea (0.015 mole; 0.93 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-4-(2-furyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*vi*)**.

M.P. : > 280^{o}C; Yield: 56%.

Mol. Formula : $C_{16}H_{14}N_2O_3$; Mol. Wt. 282.29

IR(KBr) cm^{-1} : 3411(γ_{NH}), 2965(γ_{CH}), 1710(γ_{CO}), 1652(γ_{CONH}).

7. Synthesis of 5-benzoyl-6-methyl-4-(1-naphthyl)-3,4-dihydropyrimidin-2(1*H*)-one from benzoylacetone (VII*vii*).

A mixture of benzoylacetone (0.0076 mole; 1.2 gm), naphthaldehyde (0.0064 mole; 1 gm) and urea (0.0096 mole; 0.57 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(1-naphthyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*vii*)**.

M.P. : 226-228^{o}C; Yield 62%.

Mol. Formula : $C_{22}H_{18}N_2O_2$; Mol. Wt. 342.39

IR(KBr) cm^{-1} : 3369(γ_{NH}), 2990(γ_{CH}), 1714(γ_{CO}).

8. Synthesis of 5-benzoyl-6-methyl-4-(3-phenoxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one from benzoylacetone (VII*viii*).

A mixture of benzoylacetone (0.0060 mole; 0.98 gm), 3-phenoxybenzaldehyde (0.0050 mole; 1 gm) and urea (0.0075 mole; 0.45 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(3-phenoxyphenyl)-3,4-dihydro-pyrimidin-2(1*H*)-one **(VII*viii*)**.

M.P. : 168-170^{o}C; Yield: 68%.

Mol. Formula : $C_{24}H_{20}N_2O_3$; Mol. Wt.: 384.43

IR(KBr) cm^{-1} : 3278(γ_{NH}), 2920(γ_{CH}), 1697(γ_{CO}), 1674(γ_{CONH}).

^{1}H NMR(DMSO-d_6)δppm **:** 1.72 (s, 3H, C*H*$_3$), 5.53 (d, 1H, *H* at 4, *J* = 2.68), 6.71 (s, 1H, N*H* at 1), 6.81-7.49 (m, 14 H, Ar-*H*), 8.60 (s, 1H, N*H* at 3).

9. Synthesis of 4-(2,5-dimethoxyphenyl)-6-methyl-5-(1-phenylvinyl)-3,4-dihydro-pyrimidin-2(1*H*)-one, (VII*ix*) from benzoylacetone.

A mixture of benzoylacetone (0.0072 mole; 1.17 gm), 2-5-dimethoxybenzaldehyde (0.0060 mole; 1 gm) and urea (0.009 mole; 0.54 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 4-(2,5-dimethoxyphenyl)-6-methyl-5-(1-phenylvinyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*ix*)**.

M.P. : 206-208^{o}C; Yield: 72%.

Mol. Formula : $C_{20}H_{20}N_2O_4$; Mol. Wt. 352.38

IR(KBr) cm^{-1} : 3233(γ_{NH}), 2941(γ_{CH}, 1697(γ_{CO}).

10. Synthesis of 5-benzoyl-4-(3*H*-indol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*x*) from benzoylacetone

A mixture of benzoylacetone (0.0068 mole; 1.32 gm), indol-3-carboxylaldehyde (0.0068 mole; 1 gm) and urea (0.0103 mole; 0.61 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(3*H*-indol-3-yl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*x*)**.

M.P. : 268-270^{o}C; Yield 76%.

Mol. Formula : $C_{20}H_{17}N_3O_2$; Mol. Wt.: 331.37

IR(KBr) cm^{-1} : 3405(γ_{NH}), 3099(γ_{CH}), 1706(γ_{CO}).

11. Synthesis of 4-(5-benzoyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)-benzonitrile (VII*xi*) from benzoylacetone

A mixture of benzoylacetone (0.0091 mole; 1.48 gm), 4-cynobenzaldehyde (0.0076 mole; 1 gm) and urea (0.011 mole; 0.68 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 4-(5-benzoyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)benzonitrile **(VII*xi*)**.

M.P. : 192-194^{o}C; Yield 59%.

Mol. Formula : $C_{19}H_{15}N_3O_2$; Mol. Wt.: 317.34

IR(KBr) cm^{-1} : 3296(γ_{NH}), 2232(γ_{CN}), 1677(γ_{CONH}).

^{1}H NMR(DMSO-d_6)δppm : 1.70 (s, 3H, C*H*$_3$), 5.50 (d, 1H, *H* at 4, *J* = 3.24), 7.71 (br., 1H, N*H* at 1), 7.37-7.63 (m, 9H, Ar-*H*), 9.16 (s, 1H, N*H* at 3).

12. Synthesis of 5-benzoyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one from benzoylacetone (VII*xii*)

A mixture of benzoylacetone (0.0079 mole; 1.28 gm), 3-nitrobenzaldehyde (0.0066 mole; 1 gm) and urea (0.009 mole; 0.59 gm) in ethanol (10 ml) were reacted to as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xii*).**

M.P. : 240-242^{o}C; Yield 68%.

Mol. Formula : $C_{18}H_{15}N_3O_4$; Mol. Wt. 337.33

IR(KBr) cm^{-1} : 3229(γ_{NH}), 1710(γ_{CO}), 1685(γ_{CONH}).

13. Syntheis of 5-benzoyl-4-(3,5-dibromo-4-hydroxyphenyl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one (VII*xiii*) from benzoylacetone

A mixture of benzyolacetone (0.0043 mole; 0.69 gm), 3,5-dibromo-4-hydroxy-benzaldehyde (0.0035 mole; 1 gm) and urea (0.0052 mole; 0.315 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of 5-benzoyl-6-methyl-4-(3,5-dibromo-4-hydroxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xiii*)**.

M.P. : 240-242^{o}C; Yield 66%.

Mol. Formula : $C_{18}H_{14}Br_2N_2O_3$; Mol. Wt. 466.12

IR(KBr) cm^{-1} : 3383(γ_{NH}), 2955(γ_{CH}), 1712(γ_{CO}), 1689(γ_{CONH}).

14. Synthesis of 5-benzoyl-4-(3,4-dimethoxyphenyl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one from benzoylacetone (VII*xiv*)

A mixture of benzoylacetone (0.0072 mole; 1.1 gm), 3,4-dimethoxybenzaldehyde (0.0060 mole; 1 gm) and urea (0.009 mole; 0.54 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(3,4-dimethoxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xiv*)**.

M.P. : 234-236^{o}C; Yield 77%.

Mol. Formula : $C_{20}H_{20}N_2O_4$; Mol. Wt. 352.38

IR(KBr) cm^{-1} : 3372(γ_{NH}), 1656(γ_{CONH}).

15. Synthesis of 5-benzoyl-4-(3-bromophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*xv*) from benzoylacetone

A mixture of benzoylacetone (0.0064 mole; 1.05 gm), 3-bromobenzaldehyde (0.0054 mole; 1 gm) and urea (0.0081 mole; 0.48 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-(3-bromophenyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xv*)**.

M.P. : 218-220^{o}C; Yield 69%.

Mol. Formula : $C_{18}H_{15}BrN_2O_2$; Mol. Wt. 371.23

IR(KBr) cm^{-1} : 3290(γ_{NH}), 2946(γ_{CH}), 1708(γ_{CO}), 1673(γ_{CONH}).

16. Synthesis of 5-benzoyl-6-methyl-4-[(*E*)-2-phenylvinyl]-3,4-dihydropyrimidin-2(1*H*)-one (VII*xvi*) from benzoylacetone

A mixture of benzoylacetone (0.0090 mole; 1.47 gm), cinnamaldehyde (0.0075 mole; 1 gm) and urea (0.0112 mole; 0.67 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get 5-benzoyl-6-methyl-4-[(*E*)-2-phenylvinyl]-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xvi*)**.

M.P. : 184-186^{o}C; Yield 71%.

Mol. Formula : $C_{20}H_{18}N_2O_2$; Mol. Wt. 318.37

IR(KBr) cm^{-1} : 3232(γ_{NH}), 2933(γ_{CH}), 1721(γ_{CO}), 1678(γ_{CONH}).

17. Synthesis of 5-benzoyl-4-(2-nitrophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (VII*xvii*) from benzoylacetone

A mixture of benzoylacetone (0.0072 mole; 1.17 gm), 2-nitrobenzaldehyde (0.0060 mole; 1 gm) and urea (0.006 mole; 0.54 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to 5-benzoyl-4-(2-nitrophenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xvii*)**.

M.P. : 222-224^{o}C; Yield 72%.

Mol. Formula : $C_{18}H_{15}N_3O4$; Mol. Wt. 337.33

IR(KBr) cm^{-1} : 3297(γ_{NH}), 1691(γ_{CO}), 1657(γ_{CONH}).

18. Synthesis of 5-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)-3,4-dihydro-pyrimidin-2(1*H*)-one (VII*xviii*) from benzoylacetone

A mixture of benzoylacetone (0.006 mole; 0.97 gm), 3,4,5-trimethoxybenzaldehyde (0.0050 mole; 1 gm) and urea (0.0075 mole; 0.45 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of 5-benzoyl-6-methyl-4-(3,4,5-trimethoxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one **(VII*xviii*)**.

M.P. : 222-224^{o}C; Yield 57%.

Mol. Formula : $C_{21}H_{22}N_2O_5$; Mol. Wt. 382.41

IR(KBr) cm^{-1} : 3297(γ_{NH}), 2940(γ_{CO}), 1702(γ_{CO}).

^{1}H NMR(DMSO-d_6)δppm : 1.78 (s, 3H, C*H*$_3$), 3.75 (s, 9H, (OC*H*$_3$)$_3$), 5.48 (d, 1H, *H* at 4, *J* = 2.90), 6.48 (s, 2H, Ar-*H* of Phenyl), 6.96 (br, 1H, N*H* at 1), 7.39-7.52 (m, 5H, Ar-*H*), 8.76 (s, 1H, N*H* at 3).

19. Synthesis of 5-benzoyl-4-(3-hydroxyphenyl)-6-methyl-3,4-dihydro-1*H*-pyrimidin-2-one (VII*xix*) from benzoylacetone

A mixture of benzoylacetone (0.006 mole; 0.97 gm), 3-hydroxybenzaldehyde (0.0050 mole; 1 gm) and urea (0.0075 mole; 0.45 gm) in ethanol (10 ml) were reacted as per

procedure for the compound **(VII*i*)** to get of 5-benzoyl-4-(3-hydroxyphenyl)-6-methyl-3,4-dihydro-1*H*-pyrimidin-2-one **(VII*xix*)**.

M.P. : 256-2258^{o}C; Yield 57%.
Mol. Formula : $C_{18}H_{16}N_2O_3$; Mol. Wt. 308.33
IR(KBr) cm^{-1} : 3378(γ_{OH}), 3282(γ_{NH}), 1715(γ_{CO}), 1644(γ_{CONH}).

20. Synthesis of [4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl)methanone (VII*xx*) from benzoylacetone

A mixture of benzoylacetone (0.0087 mole; 1.38 gm), 4-chlorobenzaldehyde (0.0071 mole; 1 gm) and thiourea (0.010 mole; 0.80 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(4-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xx*)**.

M.P. : 244-245^{o}C; Yield 81%.
Mol. Formula : $C_{18}H_{15}ClN_2OS$; Mol. Wt. 342.84
IR(KBr) cm^{-1} : 3280(γ_{NH}), 2935(γ_{CH}), 1588, 1550(γ_{CH}).
^{1}H NMR(DMSO-d_6)δppm : 1.76 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 3.36), 7.21-7.59 (m, 9H, Ar-*H*), 9.29 (s, 1H, N*H* at 1), 9.98 (s, 1H, N*H* at 3).
^{13}C NMR(DMSO-d_6)δppm : 17.81, 54.98, 76.87, 77.19, 77.51, 127.42, 127.52, 128.06, 131.37, 140.92.
MS m/e : 342[M^{+}], 327, 231, 105.

21. Synthesis of [4-(3-bromophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone (VII*xxi*) from benzoylacetone.

A mixture of benzoylacetone (0.0064 mole; 1.05 gm), 3-bromobenzaldehyde (0.0054 mole; 1 gm) and thiourea (0.0081 mole; 0.61 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(3-bromophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xxi*)**.

M.P. : 198-200^{o}C; Yield 64%.
Mol. Formula : $C_{18}H_{15}BrN_2OS$; Mol. Wt. 387.29
IR(KBr) cm^{-1} : 3282(γ_{NH}), 3070(γ_{CH}), 1606, 1575(γ_{CH}).

^{1}H NMR(DMSO-d_6)δppm : 1.78 (s, 3H, C*H*$_3$), 5.49 (d, 1H, *H* at 4, *J* = 3.2), 7.15-7.51 (m, 9H, Ar-*H*), 8.90 (s, 1H, N*H* at 1), 9.61 (s, 1H, N*H* at 3).

^{13}C NMR(DMSO-d_6)δppm : 17.88, 55.44, 110.21, 124.80, 127.51, 128.04, 129.14, 130.37, 131.50, 144.50.

MS m/e : 388[M^+], 371, 281, 231, 172, 144.

22. Synthesis of [4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl](phenyl) methanone (VII*xxii*) from benzoylacetone

A mixture of benzoylacetone (0.0085 mole; 1.38 gm), 2-chlorobenzaldehyde (0.0071 mole; 1 gm) and thiourea (0.0106 mole; 0.80 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xxii*)**.

M.P. : 202-203^{o}C; Yield 72%.

Mol. Formula : $C_{18}H_{15}ClN_2OS$; Mol. Wt. 342.84

IR(KBr) cm^{-1} : 3289(γ_{NH}), 3020(γ_{CH}), 1585(γ_{CH}).

^{1}H NMR(DMSO-d_6)δppm : 1.82 (s, 3H, C*H*$_3$), 5.88 (d, 1H, *H* at 4, *J* = 2.76), 7.19-7.55 (m, 9H, Ar-*H*), 8.73 (s, 1H, N*H* at 1), 10.04 (s, 1H, N*H* at 3).

23. Synthesis of {6-methyl-4-[(*E*)-2-phenylvinyl]-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}(phenyl)methanone (VII*xxiii*) from benzoylacetone

A mixture of benzoylacetone (0.0090 mole; 1.47 gm), cinnamaldehyde (0.0075 mole; 1gm) and thiourea (0.011 mole; 0.85 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get {6-methyl-4-[(*E*)-2-phenylvinyl]-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl}(phenyl)-methanone **(VII*xxiii*)**.

M.P. : 220-222^{o}C; Yield 78%.

Mol. Formula : $C_{20}H_{18}N_2OS$; Mol. Wt. 334.43

IR(KBr) cm^{-1} : 3207(γ_{NH}), 3081(γ_{CH}), 1587(γ_{CH}).

^{1}H NMR(DMSO-d_6)δppm : 1.76 (s, 3H, C*H*$_3$), 5.01 (dd, 1H, *H* at 4, *J* = 3.7), 6.20 (dd., 1H, *H* at a, *J* = 6.2), 6.39 (dd., 1H, *H* at b, *J* = 15.88), 7.21-7.62 (m, 10H, Ar-*H*), 8.84 (s, 1H, N*H* at 1), 9.77 (s, 1H, N*H* at 3).

24. Synthesis of [6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl](phenyl)methanone (VII*xxiv*) from benzoylacetone

A mixture of benzoylacetone (0.0079 mole; 1.28 gm), 3-nitrobenzaldehyde (0.0066 mole; 1 gm) and thiourea (0.009 mole; 0.75 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get [6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xxiv*).**

M.P. : 236-238^{o}C; Yield: 64%.

Mol. Formula : $C_{18}H_{15}N_3O_3S$; Mol. Wt. 353.39

IR(KBr) cm^{-1} : 3286(γ_{NH}), 3008(γ_{CH}), 1602(γ_{CH}).

25. Synthesis of [4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetra-hydro-pyrimidin-5-yl](phenyl)methanone (VII*xxv*) from benzoylacetone.

A mixture of benzoylacetone (0.0072 mole; 1.16 gm), 3,4-dimethoxybenzaldehyde (0.0060 mole; 1gm) and thiourea (0.009 mole; 0.68 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl](phenyl)methanone **(VII*xxv*)** .

M.P. : 240-242^{o}C; Yield: 61%.

Mol. Formula : $C_{20}H_{20}N_2O_3S$; Mol. Wt. 368.45

IR(KBr) cm^{-1} : 3288(γ_{NH}), 2991(γ_{CH}), 1612, 1571(γ_{CH}).

^{1}H NMR(DMSO-d_6)δppm : 1.78 (s, 3H, C*H*$_3$), 3.78 (s, 3H, -OC*H*$_3$), 3.81 (s, 3H, -OC*H*$_3$), 5.42 (d, 1H, *H* at 4, *J* = 3.12), 6.77-6.80 (m, 3H, Ar-*H* phenyl at 4), 7.37-7.51 (m, 5H, Ar-*H* of benzoyl), 9.16 (s, 1H, N*H* at 1), 9.90 (s, 1H, N*H* at 3).

26. Synthesis of [4-(3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl)methanone (VII*xxvi*) from benzoylacetone.

A mixture of benzoylacetone (0.0085 mole; 1.38 gm), 3-chlorobenzaldehyde (0.0071 mole; 1 gm) and thiourea (0.010 mole; 0.80 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(3-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xxvi*).**

M.P. : 188-190^{o}C; Yield 57%.

Mol. Formula : $C_{18}H_{15}ClN_2OS$; Mol. Wt. 342.84

IR (KBr) cm^{-1} : 3250(γ_{NH}), 3009(γ_{CH}), 1524(γ_{CH}).

27. Synthesis of [6-methyl-4-(3-phenoxyphenyl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl)methanone (VII*xxvii*) from benzoylacetone.

A mixture of benzoylacetone (0.0060 mole; 0.98 gm), 3-phenoxyphenylbenzaldehyde (0.0050 mole; 1 gm) and thiourea (0.0075 mole; 0.57 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(3-phenoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl](phenyl) methanone **(VII*xxvii*)**.

M.P. : 208-210^{o}C; Yield 57%.

Mol. Formula : $C_{24}H_{20}N_2O_2S$; Mol. Wt. 400.49

IR(KBr) cm^{-1} : 3287(γ_{NH}), 3092(γ_{CH}), 1573(γ_{CH}).

^{1}H NMR(DMSO-d_6)δppm : 1.75 (s, 3H, C*H*$_3$), 5.46 (d, 1H, *H* at 4, *J* = 3.24), 6.68-7.61 (m, 14H, Ar-*H*), 9.25 (s, 1H, N*H* at 1), 9.95 (s, 1H, N*H* at 3).

28. Synthesis of [6-methyl-2-thioxo-4-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetra-hydro-pyrimidin-5-yl](phenyl)methanone (VII*xxviii*) from benzoylacetone.

A mixture of benzoylacetone (0.0061 mole; 0.99 gm), 3,4,5-trimethoxybenzaldehyde (0.0051 mole; 1 gm) and thiourea (0.0091 mole; 0.69 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(3,4,5-trimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **(VII*xxviii*)**.

M.P. : 228-230^{o}C; Yield 76%.

Mol. Formula : $C_{21}H_{22}N_2O_4S$; Mol. Wt. 398.48

IR(KBr) cm^{-1} : 3282(γ_{NH}), 2994(γ_{CH}), 1608(γ_{CH}).

29. Synthesis of [4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl)methanone (VII*xxix*) from benzoylacetone.

A mixture of benzoylacetone (0.0098 mole; 1.59 gm), 2-hydroxybenzaldehyde (0.0081 mole; 1 gm) and thiourea (0.012 mole; 0.92 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of [4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl]-(phenyl)methanone **(VII*xxix*)**.

M.P. : 248-250^{o}C; Yield 61%.

Mol. Formula : $C_{18}H_{16}N_2O_2S$; Mol. Wt. 324.4

IR(KBr) cm^{-1} : 3206(γ_{NH}), 2991(γ_{CH}), 1514(γ_{CH}).

30. Synthesis of (6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-(phenyl)methanone (VII*xxx*) from benzoylacetone.

A mixture of benzoylacetone (0.011 mole; 1.83 gm), benzaldehyde (0.0094 mole; 1 gm) and thiourea (0.014 mole; 1.07 gm) in ethanol (10 ml) were reacted as per procedure for the compound **(VII*i*)** to get of (6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methanone **(VII*xxx*)**.

M.P. : 218-220^{o}C; Yield 61%.

Mol. Formula : $C_{18}H_{16}N_2OS$; Mol. Wt. 308.4

IR(KBr) cm^{-1} : 3283(γ_{NH}), 3170(γ_{CH}), 1587(γ_{CH}).

4.2 MDR reversal effects of DHPM series on MDR1-gene transfected mouse lymphoma cell line (l 5178 y) by flow cytometry.

Assay for reversal of MDR in tumour cells[2,3]:

The cells were adjusted to a density of 2×10^6/ml, resuspended in serum-free McCoy's 5A medium and distributed in 0.5-ml aliquots into Eppendorf centrifuge tubes. The tested compounds were added at various concentrations in different volumes (2.0-20.0 μl) of the 1.0-10.0 mg/ml stock solutions, and the samples were incubated for 10 min at room temperature. Next, 10 μl (5.2 μM final concentration) of the indicator rhodamine 123 was added to the samples and the cells were incubated for a further 20 min at 37°C, washed twice and resuspended in 0.5 ml PBS for analysis. The fluorescence of the cell population was measured with a Beckton Dickinson FACScan flow cytometer. Verapamil was used as a positive control in the rhodamine 123 exclusion experiments. The percentage mean fluorescence intensity was calculated for the treated MDR and parental cell lines as compared with the untreated cells. An activity ratio R was calculated via the following equation, on the basis of the measured fluorescence values:

$$R = \frac{MDR\,treated \,/\, MDR\,control}{parental\ treated \,/\ parental\,control}$$

4.3 References

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Summary and Conclusion

Summary and Conclusion

Part-I of this thesis deals with "Synthesis, Pharmacological Evaluation and QSAR of some pyrimidylmethylsulfinylbenzimidazoles as potential reversible Proton Pump Inhibitors (PPIs)".

A careful study of the literature on the currently used PPI's especially of the PMSB types indicates some important drawbacks associated with their usage. An investigation into the mechanism of action of these PPI's can throw some light on the probable reasons for these drawbacks. These molecules rearrange in the strongly acidic environment of the parietal cells. Covalent binding of the rearranged inhibitor to the H^+/K^+-ATPase results in inactivation of proton pump. In the covalent binding, a disulfide linkage of the drug is formed with the active site of the cystine-rich H^+/K^+-ATPase (Proton Pump). One of these sites has been identified as cystine-813 (and probably cystine-822) of H^+/K^+-ATPase.

Therefore, it was realized to develop better analogs of the existing PPI's in which the formation of this disulfide intermediate can be avoided, so as to attain reversible proton pump inhibition & thus overcome the drawbacks of the currently available PPI's.

One of the options that has not been tried is the 3-aza analog of pyridine *i.e.* pyrimidine, (which is it's logical bioisoster) in the PMSB nucleus. Thus, one can envisage that this system though can bind to the proton pump; the binding may not as strong as the PMSB pyridine system and may be even loose and reversible. Therefore, a series of pyrimidine analogues of the existing drug PMSB skeleton was planned to be synthesized and evaluated for the proposed work.

Thus, a series of 2-(1*H*-benzimidazol-2-ylsulfinyl)-3*H*-pyrimidin-4-ones and 2-(5-methoxy-1*H*-benzimidazol-2-ylsulfinyl)-3*H*-pyrimidin-4-ones was planned to be synthesized, characterized and evaluated for antiulcer activity, using a suitable animal model.

In all 35 derivatives have been synthesized in this part which are characterized by spectral data. These derivatives were evaluated preliminary for anti-secretary and antiulcer

activity in Pylorus Ligation methods on Wistar rats using method reported by Shay *et al.* Four compounds from this series namely 2-(1*H*-benzimidazol-2-yl)methyl-sulfinyl 5,6,7,8-tetrahydro-benzo-(*b*)sulfinyl[2,3-*d*]pyrimidin-4-(3*H*)-one **IV*i*,** 2-((1*H*-benzo[*d*]imidazol-2-ylsulfinyl)methyl)5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*) one **IV*vii*,** 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-5-phenyl-thieno[2,3-*d*]-pyrimidin-4(3*H*)-one **IV*x*** and 2-{[(5-methoxy-1*H*-benzimidazol-2-yl)sulfinyl]methyl}-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one **IV*xxiii*** were found very potent inhibitors of gastric juice and were able to protect the stomach from the ulcers at the dose of 30 mg/kg. Omeprazole (30 mg/kg) was taken as the standard drug in this study.

QSAR studies of this series reveal that electronic and stearic properties are governing the activities of newly synthesized proton pump inhibitors. Among electronic parameters, Quadrupole2, XCompDipole and Dipole Moment are responsible for governing the activity, while chi4pathcluster, chivV6chain and chi3cluster are the stearic parameters responsible for the activity. Apart from these some alignment independent (AI) descriptors like T_C_O_2, T_2_O_6 and T_O_S_3 are also responsible for the activities of proton pump inhibitors under study.

Part-II of this thesis deals with the "Novel Microwave Assisted Green Chemical Synthesis of Condensed 2-Substitutedpyrimidin-4(3*H*)-ones Under Solvent Free Conditions, their MWI Assisted Facile and Rapid Chlorination and their Multidrug Reverting Activity".

Condensed pyrimidines and quinazolines have shown a wide spectrum of biological activities and have been reported in the literature. Thieno[2,3-*d*]pyrimidines are considered to be bioisosteres of quinazolines. This has lead to the synthesis of various types of condensed pyrimidines, which show a wide range of biological activities.

The synthesis of condensed pyrimidines is a very important process which is subject to improvement, routinely. The regularly employed synthetic methodology involves annulation of the pyrimidine ring on an appropriately substituted heterocycles in which a variety of *o*-aminocarbonyl heterocycles have been cyclocondensed with a host of reagents namely amides, thioamodes, imidates, amidines, *etc*., mostly under basic

conditions to afford variety of condensed pyrimidines, quinazolines, thienopyrimidines, furanopyrimidines, purines, pteridines, pyridopyrimidines, pyrrolopyrimidines, pyrazolopyrimidines, *etc.*

Shishoo *et al.*, have exploited the reactions of a variety of nitriles with a host of *o*-aminocarbonyl substrates, under the influence of dry HCl gas to obtain a wide range of 2-substituted-4-oxo/4-amino/4-chloro & 4-aryl condensedpyrimidines.

Reactions that are adaptable for high speed and throughput syntheses have become an important component of the modern medicinal chemist's library, as a great number of compounds can be produced through such rapid parallel synthetic programs. Synthetic methods that enable the rapid production of an array of heterocycles, useful for the identification of new lead structures are of critical importance to the pharmacological activity.

Encouraging results in the MWI based syntheses of thiophene *o*-aminoesters involving Gewald reaction, as well as, thienopyrimidine bioisosteres of gefitinib under microwave conditions gave an impetus to assess whether, these could be extended to the single pot cyclocondensation of the nitriles with various *o*-aminoester substrates under solvent free conditions using MWI. This was particularly of interest, especially for quickly generating compound libraries of increasing molecular diversity, through the development of synthetic methods that could combine the expediency of microwave energy.

Thus, the aim of the this part was to use microwave irradiation for the synthesis of condensed 2-substitutedpyrimidin-4(3*H*)-ones involving the condensation of variety of nitriles with *o*-aminoesters of thiophene, benzene, dimethoxybenzene and quinazolinone in the presence of catalytic amount of HCl alone or with the Lewis acid, $AlCl_3$ under solvent free conditions for the first time. Further, it was decided to synthesize 4-chloro derivatives of these condensed 2-substituted pyrimidines-4-ones through MWI assisted facile and rapid chlorination and also evaluated them for multi drug reverting activity on resistant cancer cell lines.

In all 52 compounds has been synthesized in this part which were characterized by spectral data. The synthesized derivatives have been screened for their multi drug

reverting activity on MDR1-gene transfected mouse lymphoma cell line (l5178 y). Verapamil was taken as a positive control. Few derivatives, namely 4-chloro-2-(2-chloroethyl)-5-(4-methylphenyl)thieno[2,3-*d*]-pyrimidine **VI*xiv***, 2,5,6-trimethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **V*xxvi***, 4-chloro-2-(2-chloroethyl)-5,6-dimethyl-thieno[2,3-*d*]pyrimidine **VI*xv***, 4-chloro-2-(2-chloroethyl)-5,6,7,8-tetrahydro-benzo[4,5]-thieno[2,3-*d*]pyrimidine **VI*v***, 2-methylquinazolin-4(3*H*)-one **V*xxx***, 2-chloro-methyl-3*H*-benzo[4,5]thieno[3,2-*d*]pyrimidin-4-one **V*xiv***, 9-methoxy-2-chloromethyl-3*H*-benzo-[4,5]thieno[3,2-*d*]pyrimidin-4-one **V*xv***, 2-chloromethyl-3,5,6,7,8,9-hexahydro-10-thia-1,3-diaza-benzo[*a*]azulen-4-one **V*vii***, 7-benzyl-2-chloromethyl-5,6,7,8-tetra-hydro-3*H*-9-thia-1,3,7-triaza-fluoren-4-one **V*x***, 4-chloro-2-chloromethyl-6,7,8,9-tetra-hydro-5*H*-10-thia-1,3-diaza-benzo[*a*]azulene **VI*vi*** and 4-chloro-2-(chloromethyl)-5-(4-methoxy phenyl)thieno[2,3-*d*]pyrimidine **VI*vii*** showed moderate activity in inhibiting the Pgp, which is reflected by fluorescence activity ratio. None of the compounds showed cytotoxic effect in the when measure at 4 micromol/ml/1million cells concentration, which is desirable. This means that the above stated compounds were moderately effective in reversal of MDR efflux pump activity. Verapamil was taken as a standard in this study.

Part-III of the thesis deals with "Synthesis, Characterization and Anticancer Activity of some Aza-analogue of DP-7".

Literature survey reveals that DP-7 (dihydropyridine derivative) is a potent multi drug reverting agent and on the other hand, monastrol (dihydropyrimidine derivative) is potent inhibitor of Eg5 that inhibits ATP hydrolysis through an allosteric mechanism. Thus, it was thought to hybridize the structural features of these two potent anticancer molecules so that multidrug reverting activity as well as Eg5 inhibitor activity can be obtained by a single molecule. These hybridized molecules are aza analogues of the DP-7, bearing various substitutions on the 4^{th} position of the dihydropyrimidine ring.

In all 30 dihydropyrimidine derivatives has been synthesized in this part which were characterized by spectral data. The newly synthesized derivatives were screened on MDR1-gene transfected mouse lymphoma cell line (l5178 y) for MDR reversal effects at non toxic concentrations taking verapamil as a standard. The four compounds namely 5-benzoyl-6-methyl-4-(1-naphthyl)-3,4-dihydropyrimidin-2(1*H*)-one **VII*vii***, 4-(2,5-dimethoxy phenyl)-6-methyl-5-(1-phenylvinyl)-3,4-dihydro-pyrimidin-2(1*H*)-one **VII*ix*,**

5-benzoyl-4-(3*H*-indol-3-yl)-6-methyl-3,4-dihydro-pyrimidin-2(1*H*)-one **VII*x*** and [6-methyl-4-(3-phenoxyphenyl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl](phenyl) methanone **VII*xxvii*** were very effective in inhibiting the Pgp mediated resistance of cancer cell line, when measured in 4 micromol/ml/1million cells. Verapamil was taken as a standard in this study.

Publications and Presentations

Publication from the Thesis

1. Kishor S. Jain, **Jitender B. Bariwal.** "Pyrimidylmethylsulfinylbenzimidazoles as Reversible Proton Pump Inhibitors" (**Applied for Indian Patent**).
2. Kishor S. Jain, Anamik K. Shah, **Jitender Bariwal**, Suhas M. Shelke, Amol P. Kale, Jayshree R. Jagtap and Ashok V. Bhosale. "Recent advances in proton pump inhibitors and management of acid-peptic disorders." *Bioorg. Med. Chem.* **2007**, *15***,** 1181.
3. Kishor S. Jain, **Jitender B. Bariwal**, Manisha S. Phoujdar, Rakesh D. Amrutkar, Manoj K. Munde, Riyaj S. Tamboli, Samrat A. Khedkar, Rahul H. Khiste, Nikhil C. Vidyasagar, Vinit V. Dabholkar and Muthu K. Kathiravan. "A Novel Microwave Assisted Green Chemical Synthesis of Condensed 2-Substituted pyrimidin-4(3*H*)-ones under solvent free conditions". (Submitted to *J. Heterocycl. Chem.*, June 2008)
4. **Jitender B. Bariwal,** Anamik K. Shah, Muthu K. Kathiravan and Kishor S. Jain. "Impact of Microwave Assisted Heating on the Combinatorial and Parallel Syntheses of Compound Libraries for New Drug Discovery Research". (Communicated for publication in *Eur. J. Med. Chem.*)
5. Anamik Shah, **Jitender Bariwal**, Joseph Molnar, Masami Kawase, Noboru Motohashi. In *Advanced Dihydropyridines as Novel Multidrug Resistance Modifiers and Reversing Agents*. Book series of "**Topics in Heterocyclic Chemistry**", Ed Noboru Motohashi, Springer-Verlag, Berlin Heidelberg, Published online: 2007. DOI 10.1007/7081_2007_095.
6. **J. B. Bariwal**, A. K. Shah, M. K. Kathiravan, R. S. Somani, J. R. Jagtap and K. S. Jain. "Synthesis and Antiulcer Activity of Novel Pyrimidylthiomethyl- and Pyrimidylsulfinylmethyl Benzimidazoles as Potential Reversible Proton Pump Inhibitors." (Accepted for publication in *Indian J. Pharm. Edu. Res.*).
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Other Publication

1. Kishor S. Jain, **Jitender B. Bariwal**, Muthu M. Kathiravan, Manisha S. Phoujdar, Rajkumari S. Sahne, Bishram S. Chauhan, Anamik K. Shah and Mange Ram Yadav. "Recent advances in selective α_1-adrenoreceptor antagonists as antihypertensive agents." *Bioorg. Med. Chem.* **2008,** *16*, 4759.
2. Manisha S. Phoujdar, Muthu K. Kathiravan, **Jitender B. Bariwal**, Anamik K. Shah and Kishor S. Jain. "Microwave-based synthesis of novel thienopyrimidine bioisosteres of gefitinib." *Tetrahedron Lett.* **2008**, *49*, 1269.
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6. S. V. Londhe, S. V. Mulgund, T. S. Chitre, P. S. Mallade, **J. B. Bariwal** and K. S. Jain. "Hyphenated Techniques in Analytical World." (Accepted for publication in ***Indian J. Pharm. Edu. Res.***).

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1. **Jitender Bariwal,** Jalpa Trivedi, Hrishikesh Acharya and Anamik Shah, Synthesis and Anti-microbial Activity of 4-(Substitutedhenyl)-Bis (4-Fluorophenyl)-2,6-Dimethyl-1,4 Dihydropyridine-3,5 Dicarboxamides, International Conference on Advances in Drug Discovery Research, 11th ISCB conference (1SCBC-2007), Aurangabad, February 24-26, 2007 Poster No. **141**. **(Awarded best Poster of the conference in the field of Chemical Sciences)**

2. **Jitender Bariwal,** K. Raval, R. Khunt, N. Vekariya, C. Dholakiya, D. Thakker, and A. Shah. "Synthesis Structural Modification and Biological Evaluation of 1, 4 Diphenyl substituted-1,4,5,6-tetrahydropyridine, Abstract no. 086, 10th International Conference of ISCB on "Drug Discovery: Prospective and Challenges", Feb 24-25, 2006, CDRI, Lucknow, India.
3. **Jitender Bariwal,** R. Kakadiya, V. Virsodiya, C. Dholakiya, A. Parecha, and A. Shah. "Synthesis and Crystal Structure of Dimethyl 2,6-Dimethyl-4-(2-nitrophenyl)-*N*-(2-methoxyphenyl-1,4-dihydropyridine-3,5-dicarboxylate. Abstract no. 087, 10th International Conference of ISCB on "Drug Discovery: Prospective and Challenges", Feb 24-25, 2006, CDRI, Lucknow, India.
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7. K.S. Jain, B.B. Chavan, M.R. Dumbre, M.K. Kathiravan, V.S. Veer, V.S. Bendre, P.S. Gadewar, S.R. Patil and **J. Bariwal,** "Design, Synthesis and Evaluation of Novel Mutual Prodrugs of LM-1554 and its active analog with Vit. B_3 for Antihyperlipaemic activity" Abstract no. BP64, 57th Indian Pharmaceutical Congress 2005, Hyderabad, India. **(Awarded best paper of the session).**

8. K. S. Jain, M. K. Kathiravan, T. S. Chitre, M. S. Phoujdar, **J. Bariwal**, A. K. Shah, "Total Microwave Base Synthesis of Novel Thienopyrimidine Analogues of Gefitinib." Eighth Tetrahedron Symposium, 26-29 June, 2007, Berlin, Germany, Poster No. P3.90.