"Synthesis and Characterization of Pharmacologically Active Compounds"

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BY

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UNDER THE GUIDANCE OF

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Statement under *O. Ph. D. 7* of Saurashtra University

The work included in the thesis is done by me under the supervision of Prof. Anamik K. Shah and the contribution made thereof is my own work.

Date:

Place: **Shrey P. Parekh**

Certificate

This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by Mr. Shrey P. Parekh has been the result of work carried out under my supervision and is a good contribution in the field of synthetic medicinal chemistry and analytical Chemistry.

Date:

Place: Prof. Anamik K. Shah

Dedicated To
My Beloved Mummy-Papa
And
My Beloved Brother

Acknowledgement

It is a moment of gratification and pride to look back with a sense of contentment at the long traveled path, to be able to recapture some of the fine moments, to be able to think of the infinite number of people, some who were with me from the beginning, some who joined me at some stage during the journey, whose kindness, love and blessings has brought me to this day. I wish to thank each one of them from the bottom of my heart.

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GENERAL REMARKS

1. Melting points were recorded by open capillary method and are uncorrected.
2. Infrared spectra were recorded on Shimadzu FT IR-8400 (Diffuse reflectance attachment) using KBr. Spectra were calibrated against the polystyrene absorption at 1610 cm^{-1}.
3. ^{1}H & ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer. Making a solution of samples in DMSO d_6 and $CDCl_3$ solvents using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned, and are given in the δ scale. The standard abbreviations s, d, t, q, m, dd, dt, br s refer to singlet, doublet, triplet, quartet, multiplet, doublet of a doublet, doublet of a triplet, AB quartet and broad singlet respectively.
4. Mass spectra were recorded on Shimadzu GC MS-QP 2010 spectrometer operating at 70 eV using direct injection probe technique.
5. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel-G F_{254} aluminium plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light.
6. The chemicals used for the synthesis of intermediates and end products were purchased from Spectrochem, Sisco Research Laboratories (SRL), Thomas Baker, Sd fine chemicals, Loba chemie and SU-Lab.
7. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions
8. All evaporation of solvents was carried out under reduced pressure on Heidolph LABOROTA-400-efficient.
9. % Yield reported are isolated yields of material judged homogeneous by TLC and before recrystallization.
10. The structures and names of all compounds given in the experimental section and in physical data table were generated using ChemBio Draw Ultra 10.0.
11. Elemental analysis was carried out on Vario EL Carlo Erba 1108.

ABBREVIATIONS

ABC	ATP Binding Cassette
AcOH	Acetic Acid
ActD	Actinomycin D
ADHD	Attention-deficit hyperactivity disorder
AIDS	Acquired Immuno Deficiency Syndrome
$AlCl_3$	Aluminum chloride
Ar	Aromatic
ARC	Aids Related Complex
ATP	Adenosine Triphosphate
Av.	Average
BBB	Blood-brain barrier
BMI	Body mass index
BMS	Bristol-Myers Squibb
BP	Boiling Point
BSEP	Bile salt excretory pump
BuLi	Butyllithium
CAMP	Cyclic Adenosine Mono Phosphate
$CDCl_3$	deuterated chloroform
CNS	Central Nervous System
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CoMFA	Comparative Molecular Field Analysis
CoMSIA	Comparative Molecular Similarity Index Analysis
Conc.	Concentrated
Cox	Cyclooxygenase
CS_2CO_3	Cesium carbonate
$CuCl_2$	Cupric chloride
CuI	Copper(I) iodide
D.M.	Demineralized Water
DHP	Dihydropyridine
DMAP	Dimethylamino Pyridine

DMF	Di Methyl Formamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxy Ribo Nucleic Acid
DSCG	Disodium cromoglycate
EBP	Enhancer binding protein
Equiv	Equivalent
EtOH	Ethanol
Exp No	Experiment No
FACS	Fluorescence-activated cell sorting
FAR	Fluorescence Activity Ratio
FDA	Food and Drug Administration
FT-IR	Fourier Transform Infrared
FVP	Flash Vacuum Pyrolysis
GABA	Gama-amino butyric acid
GC	Gas Chromatography
GC -MS	Gas Chromatography Mass Spectra
H_2SO_4	Sulphuric acid
HBF_4	Tetrafluoroboric acid
HCl	Hydrochloric acid
HDL	High-density lipoprotein
HIV	Human Immunodeficiency Virus
HPLC	High-performance liquid chromatography
Hr.	Hour
HSV	Herpes Simplex Virus
HTLV 1	Human T-Lymphotropic Virus type 1
Hz	Hertz
I_2	Iodine
IC_{50}	Inhibitory Concentration
IND	Investigational New Drug Application
IR	Infra Red
K_2CO_3	Potassium Carbonate
K_3PO_4	Potassium Phosphate
KBr	Potassium Bromide

$KMNO_4$	Potassium Permanganate
KOH	Potassium Hydroxide
LC	LiquidChromatography
LDL	Low density lipoproteins
LSD	Lysergic acid diethylamide
m	Meta
MAOIs	Monoamine Oxidase inhibitors
MAOS	Microwave-Assisted Organic Synthesis
MDC	Dichloro methane
MDR	Multi Drug Resistance
MF	Molecular Formula
MHz	Mega Hurtz
MIN	Minutes
mM	Mili moles.
MP	Melting Point
MS	Mass Spectra
MW	Microwave
MW	Mili Watt
MW	Molecular Weight
MWI	Micro Wave Irradiation
MXR	Mitoxantrone resistance protein
Na_2CO_3	Sodium Carbonate
NADPH	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium Bicarbonate
$NaNO_2$	Sodium nitrite
NaOH	Sodium Hydroxide
NaOtBu	Sodium tert Butoxide
NCEs	New Chemical Entities
NDA	New Drug Application
NEt_3	N'tetramethyl ethylene diamine
NH_2NH_2	Hydrazine Hydrazide
nm	Nano Meter
NMDA	N-methyl-D-aspartate

NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
o	Ortho
OD	Optical Density
p	Para
$Pd(OAc)_2$	Palladium diacetate
PhMe	Toluene
PIFA	Phenyliodine bis(trifluoroacetate)
$POCl_3$	Phosphorous Oxychloride
PPAR	Peroxisome proliferators-activated receptor
ppm	Parts Per Million
$PtCl_2$	Platinum dichloride
QSAR	Quantitative Structural Activity Relationship
R&D	Rsearch and development
R.T.	Room Temperature
R_f	Retention Factor
RH	Relative Humidity
RNA	Ribo Nucleic Acid
RT	Reverse Transcriptase
SAR	Structure Activity Relationship
SD	Standard Deviation
SDS	Sodium dodecyl sulphate
SNRIs	Serotonin-norepinephrine reuptake inhibitors
SREBP	Steroid regulatory element binding protein
SSRIs	Selective serotonin reuptake inhibitors
TB	Tuberculosis Basilus
TCAs	Tricyclic antidepressants
TeCAs	Tetracyclic antidepressants
TFAA	Trifluoroacetic anhydride
THF	Tetrahydro Furan
THF	Tetrahydrofuran
$TiCl_4$	Titanium tetrachloride
TLC	Thin Layer Chromatography

TMD	Transmembrane domains
TMEDA	Tetramethylethylenediamine
TMH	Transmembrane helices
TMS	Tetra Methyl Silane
U.S. FDA	United states of food and drug administration
UV	Ultra Violet
VH Reagent	Vilsmeier–Haack Reagent
VLDL	Very low density lipoproteins
VPA	Valproate
W	Watt
w/v	Weight/Volume
WCR	World Cancer Report
$Zn(OTf)_2$	Zinc Triflate
$ZnCl_2$	Zinc Chloride

PART-I

CHAPTER - 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Organic chemists synthesize hundreds of new heterocyclic compounds every week. In most cases the chemist has specific reasons for synthesizing a particular compound, usually based on theoretical considerations, medicinal chemistry, biological mechanisms or a combination of all three.

The heterocyclic compounds are very widely distributed in nature and are very essential to living organisms. They play a vital role in the metabolism of all the living cells. Among large number of heterocycles found in nature, nitrogen heterocycles are the most abundant specially those containing oxygen or sulphur[1] due to their wide distribution in nucleic acid illustration and their involvement in almost every physiological process of plants and animals.

Heterocyclic compounds possess great applicability in industry as well as in our life in various ways. For example most of the sugars and their derivatives, including vitamin C[2], exist largely in the form of five membered (Furanosied structure) or six membered (Pyranosied structure) ring containing one oxygen atom. Further, most members of the vitamin B group possess heterocyclic rings containing nitrogen. e.g., vitamin B_6 (Pyridoxine),[3, 4] which is a derivative of the pyridine essential in amino acid metabolism.

The heterocyclic compounds also occupy key position in the area of drugs and pharmaceuticals. Almost 80% of the drugs in clinical use are based on heterocyclic constitution because they have specific chemical reactivity. Majority of the large number of drugs being introduced in pharmacopeias in recent year are heterocyclic compounds. A wide variety of modern drugs such as chlordiazepoxide (tranquillizer),[5,6] imipromine (antidepressant),[7] guanethidine (antihypertensive),[8] indapamide (diuretic and antihypertensive)[9, 10], etc. Many non-steroidal drugs such as ketoprofen [11], fenoprofen and flurbiprofen[12] are well known anti-inflammatory agents; these derivatives were found to more potent with fewer side effects. Many antibiotics including penicillin[13], cephalosporin[14], norfloxacin[15], streptomycin[16, 17] etc., also contain heterocyclic ring.

Many veterinary products like parental and morantel are the drug of choice as broad spectrum anthelmintics[18]. The herbicides Atrazine and Simazine are well known examples of heterocyclic agrochemicals[19, 20]. Plant pigments such as indigo[21], hemoglobin[22] and anthiocyanins[23], chlorophyll[24] has contributed much to color chemistry and all these contain heterocyclic ring. Further, many other heterocyclic coloring matters are in use since prehistoric times. Further, the heterocyclic tetra Selene fulvalene was the first ionic molecular crystal to demonstrate superconductivity.[25]

1.2 MEDICINAL CHEMISTRY

The art of medicinal chemistry – the science that strives to identify, create or modify molecules for therapeutic application has enriched greatly from developments in the areas of organic chemistry, biology, biophysical/biochemical methods and computational tools. While opportunities are enormous, advancing a drug candidate from bench top to clinic is associated with challenges as well and a good understanding on both these aspects would significantly accelerate drug discovery process. Really, the unprecedented increase in human life expectancy, which has almost doubled in a hundred years, is mainly due to drugs and to those who discovered them.

A race has been going on between the diseases and scientific investigations. Most of the infectious diseases like tuberculosis, typhoid, malaria, infective hepatitis, tetanus, cholera, etc., which kills lacks of people every year in underdeveloped or developing countries, have been completely wiped off from the developed nations. However, these have gradually been replaced by heart disease, cancer and HIV infection which are now considered as the biggest killers.

1.2.1 HISTORY AND EVOLUTION OF MEDICINAL CHEMISTRY[26]

Medicinal chemistry's roots can be found in the fertile mix of ancient folk medicine and early natural product chemistry and hence its name. As appreciation for the links between chemical structure and observed biological activity grew, medicinal chemistry began to emerge about 150 years ago as a distinct discipline intending to explore these relationships via chemical modification and structural mimicry of nature's materials, particularly with an eye towards enhancing the efficacy of substances thought to be of therapeutic value.[27]

Just as in all fields of science, the history of medicinal chemistry is comprised of the ideas, knowledge and available tools that have advanced contemporary knowledge. The spectacular advances in medicinal chemistry over the years are no exception. Burger[28] stated that "the great advances of medicinal chemistry have been achieved by two types of investigators: those with the genius of prophetic logic, who have opened a new field by interpreting correctly a few well-placed experiments, whether they pertained to the design or the mechanism of action of drugs and those who have varied patiently the chemical structures of physiologically active compounds until a useful drug could be evolved as a tool in medicine."

1.2.2 DEVELOPMENTS LEADING TO VARIOUS DRUG CLASSES

Psychiatrists have been using agents that are active in the central nervous system for hundreds of years. Stimulants and depressants were used to modify the mood and mental states of psychiatric patients. Amphetamine, sedatives and hypnotics were used to stimulate or depress the mental states of patients. The synthesis of chlorpromazine by Charpentier ultimately caused a revolution in the treatment of schizophrenia, but who really discovered chlorpromazine? Charpentier, who first synthesized the molecule in 1950 at Rhone-Poulenc's research laboratory; Simon Courvoisier, who reported distinctive effects on animal behavior; Henri Laborit, a French military surgeon who first noticed distinctive psychotropic effects in man; or Pierre Deniker and Jean Delay, French psychiatrists who clearly outlined what has now become its accepted use in psychiatry and without whose endorsement

and prestige, Rhone-Poulenc might never have developed it further as an antipsychotic. Because of the bitter disputes over the discovery of chlorpromazine, no Nobel Prize was awarded for what has been the single most important breakthrough in psychiatric treatment.

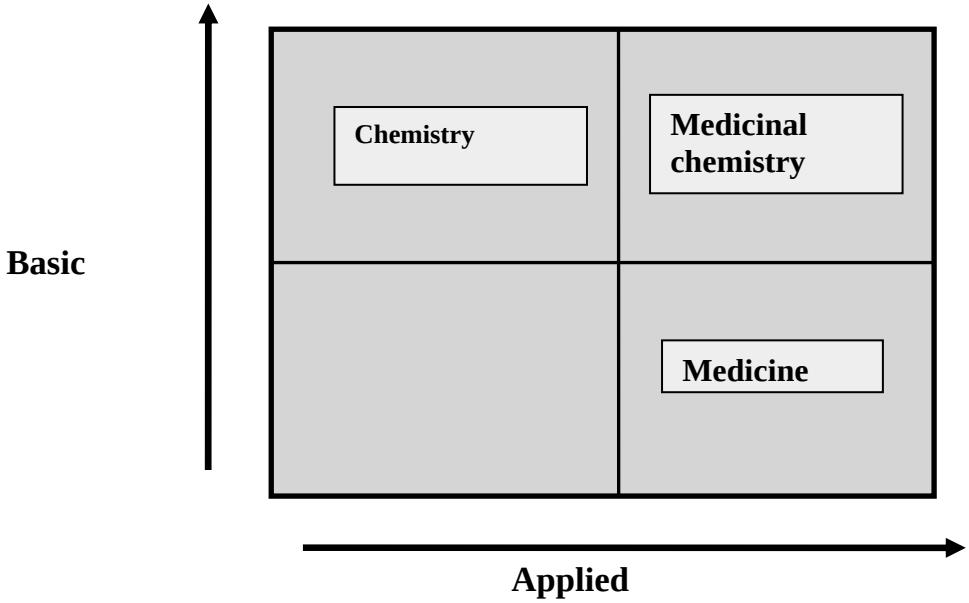
The first pure hormone to be isolated from an endocrine gland was epinephrine, which led to further molecular modifications in the area of sympathomimetic amines. Subsequently, nor epinephrine was identified from sympathetic nerves. The development of chromatographic techniques allowed the isolation and characterization of a multitude of hormones from a single gland. In 1914, biochemist Edward Kendall isolated thyroxin (T_4) from the thyroid gland. He subsequently won the Nobel Prize in physiology or medicine in 1950 for his discovery of the activity of cortisone. Less than 50 years after the discovery of oxytocin in 1904 by Sir Henry Dale found that an extract from the human pituitary gland contracted the uterus of a pregnant cat. Biochemist Du Vigneud synthesized the cyclic peptide hormone. His work resulted in the Nobel Prize in chemistry in 1955.

Frederick G. Banting and Charles H. Best, working in the laboratory of John J. R. McLeod at the University of Toronto, isolated the polypeptide hormone insulin and began its testing in dogs. By 1922, researchers, with the help of James B. Collip and the pharmaceutical industry, were able to purify and produce animal-based insulin in large quantities. Insulin soon became a major product for Eli Lilly & Co. and Novo Nordisk, a Danish pharmaceutical company. In 1923, McLeod and Banting were awarded Nobel Prize in Medicine or Physiology and after much controversy; they shared the prize with Collip and Best. For the next 60 years, cattle and pigs were the major sources of insulin. In 1978, the biotech company Genentech and the City of Hope National Medical Center produced human insulin in the laboratory using recombinant DNA technology. By 1982, Lilly's Humulin became the first genetically engineered drug to be approved by the U.S. Food and Drug Administration (FDA).

1.2.3 EVOLVING DRUG DISCOVERY AND DEVELOPMENT PROCESS

Working definition for medicinal chemistry

A working definition simply states that medicinal chemistry uses physical organic principles to understand the interaction of small molecular displays with the biological realm. Physical organic principles encompass overall conformational considerations, chemical properties and molecular electrostatic potentials, as well as distinctly localized stereo chemical, hydrophilic, electronic and steric parameters. Small molecular displays should be thought of in terms of low molecular weight compounds (e.g., usually less than 1 kg) that are typically of a xenobiotic origin and thus not in terms of biotechnologically derived polymers.



[**Fig. 1: Nonlinear relationship of medicinal chemistry to basic and applied research**]

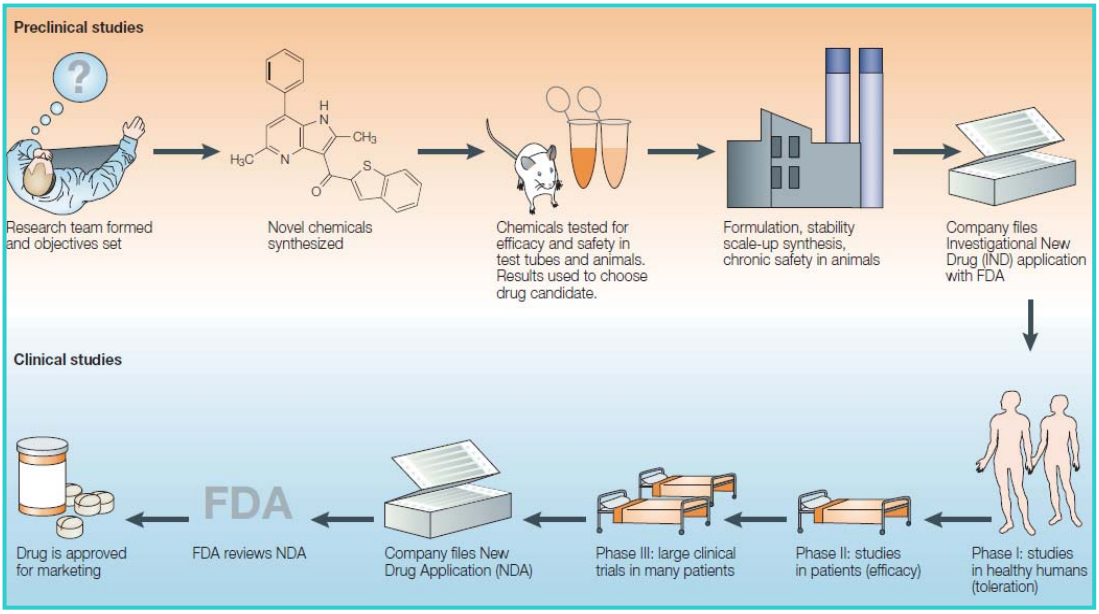
The technologies that can be deployed as tools to study these interactions at medicinal chemistry's fundamental level of understanding are, by intent, dissociated from medicinal chemistry's definition. Presently, such tools include biotechnology-related methods such as site-directed mutagenesis, combinatorial chemistry methods, provided that the latter are coupled with knowledge generating structural databases and long-standing synthetic chemistry manipulations that can be conducted in a

systematic manner on either one of the interacting species in order to explore SARs. Finally, it should be appreciated that this definition merges both the basic and applied natures of medicinal chemistry's scientific activities into a key mix of endeavors for which a new research paradigm **(Fig. 1)** has also been recently proposed as being a significant trend,[29] even if potentially "dangerous" in that it could compromise the longer-term pursuit of fundamental knowledge by bringing applied science decision criteria into the funding programs that have previously supported pure, basic science.[30]

1.2.4 THE PROCESS OF DRUG DISCOVERY

Inventing and developing a new medicine is a long, complex, costly and highly risky process that has few peers in the commercial world. Research and development (R&D) for most of the medicines available today has required 12–24 years for a single new medicine, from starting a project to the launch of a drug product **(Fig. 2)**. In addition, many expensive, long-term research projects completely fail to produce a marketable medicine. The cost for this overall process has escalated sharply to an estimated US $1.4 billion for a single new drug.[31] The funds to support this research usually come from the income of the private pharmaceutical company that sponsors the work. In research ('R'; discovery) phase, only a fraction of the scientific hypotheses that form the basis for a project actually yield a drug candidate for development. In the drug development ('D') phase, experience has shown that only approximately 1 out of 15–25 drug candidates survives the detailed safety and efficacy testing (in animals and humans) required for it to become a marketed product. And for the few drug candidates that successfully become marketed products, some will not recover their costs of development in the competitive marketplace and only approximately one in three will become a major commercial product. Clearly, this is a high-stake, long-term and risky activity, but the potential benefits to the millions of patients with serious diseases provide a constant motivating force. At virtually every phase-from project initiation to discovery, development and planning for marketing for a new drug-modern medicinal chemist can have a role.

Stages in the drug discovery process



[Fig. 2: The process of new drug discovery]

The drug discovery process begins with the identification of a medical need, including a judgment on the adequacy of existing therapies (if there are any). From this analysis, together with an appraisal of the current knowledge about the target disease, will come hypotheses on how to possibly improve therapy — that is, what efficacy, safety or mechanistically novel improvements will advance the method of drug treatment for patients with the target disease. On the basis of these hypotheses, specific objectives will be set for the project. Then, testing selected chemicals in appropriate biological tests can begin. Key subsequent steps in the process include detecting relevant biological activity (a 'hit') for a structurally novel compound *in vitro*, then finding a related compound with *in vivo* activity in an appropriate animal model, followed by maximizing this activity through the preparation of analogous structures and finally selecting one compound as the drug development candidate. This drug candidate then undergoes toxicological testing in animals, as required by law. If the compound passes all these tests, all the accumulated research data are assembled and submitted as an Investigational New Drug Application (IND) to the Food and Drug Administration (FDA) in the United States (or comparable agency in other countries) before clinical trials are initiated. In the clinic, there is sequential evaluation in normal human volunteers of toleration (Phase I), efficacy and dose range in patients (Phase II), followed by widespread trials in thousands of appropriate

patients to develop a broad database of efficacy and safety. For the few (4–7%) drug candidates that survive this series of development trials, a New Drug Application (NDA) that contains all the accumulated research data is filed for thorough review by the experts at the FDA. Only with their approval can the new drug be offered to doctors and their patients to treat the disease for which it was designed.

1.2.5 DRUG METABOLISM

The human body is an example of an exquisitely designed, extremely complex machine that functions day-in and day-out to allow for survival of the organism in response to a never-ending onslaught of external challenges. When one considers the enormous variety of environmental stressors to which the body is continually subjected, it is not surprising to anticipate the existence of a multitude of checks and balances associated with its physiological and biochemical systems.

Humans are exposed throughout their lifetime to a large variety of drugs and nonessential exogenous (foreign) compounds (collectively termed “xenobiotic”) that may pose health hazards. Most drugs and other xenobiotic are metabolized by enzymes normally associated with the metabolism of endogenous constituents (e.g., steroids and biogenic amines). The liver is the major site of drug metabolism, although other xenobiotic-metabolizing enzymes are found in nervous tissue, kidney, lung, plasma and the gastrointestinal tract.

Among the more active extra hepatic tissues capable of metabolizing drugs is the intestinal mucosa, kidney and lung. The ability of the liver and extra hepatic tissues to metabolize substances to either pharmacologically inactive or bioactive metabolites before reaching systemic blood levels is termed “first-pass metabolism”. Other metabolism reactions occurring in the gastrointestinal tract are associated with bacteria and other microflora of the tract. The bacterial flora can affect metabolism through the production of toxic metabolites, formation of carcinogens from inactive precursors, detoxication and exhibition of species differences in drug metabolism, exhibition of individual differences in drug metabolism, production of pharmacologically active metabolites from inactive precursors and production of

metabolites not formed by animal tissues. The pathways of xenobiotic metabolism are divided into two major categories.

Phase I reactions (Biotransformation)

This type includes oxidation, hydroxylation, reduction and hydrolysis. In these enzymatic reactions, a new functional group is introduced into the substrate molecule, an existing functional group is modified or a functional group or acceptor site for Phase II transfer reactions is exposed, making the xenobiotic more polar and therefore, more readily excreted.

Phase II reactions (Conjugation)

These reactions are enzymatic syntheses whereby a functional group, such as alcohol, phenol or amine, is masked by the addition of a new group, such as acetyl, sulfate, glucoronic acid or certain amino acids, which further increases the polarity of the drug or xenobiotic. Most substances undergo both Phase I and Phase II reactions sequentially.

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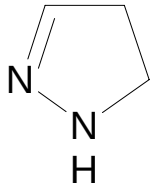
CHAPTER - 2

SYNTHESIS OF BENZOFURAN-2-YL (4,5-DIHYDRO-3,5-SUBSTITUTED DIPHENYLPYRAZOL-1- YL) METHANONE

2.1 INTRODUCTION: PYRAZOLES

The chemistry of pyrazoles has been reviewed by Jarobe in 1967. Pyrazoles have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them. Pyrazole have been studied extensively because of ready accessibility, diverse chemical reactivity, broad spectrum of biological activity and varieties of industrial applications.

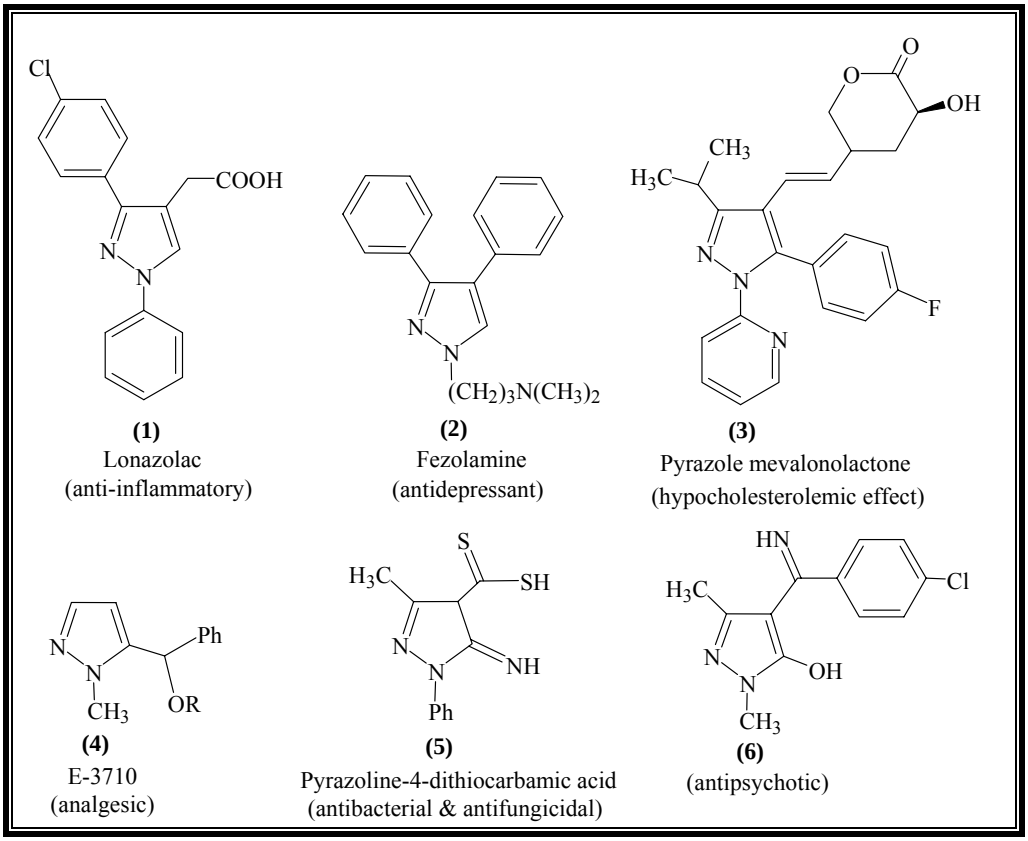
Pyrazole has three possible tautomeric structures. But 2-pyrazole (**1)** consist a unique class of nitrogen containing five member heterocycles.

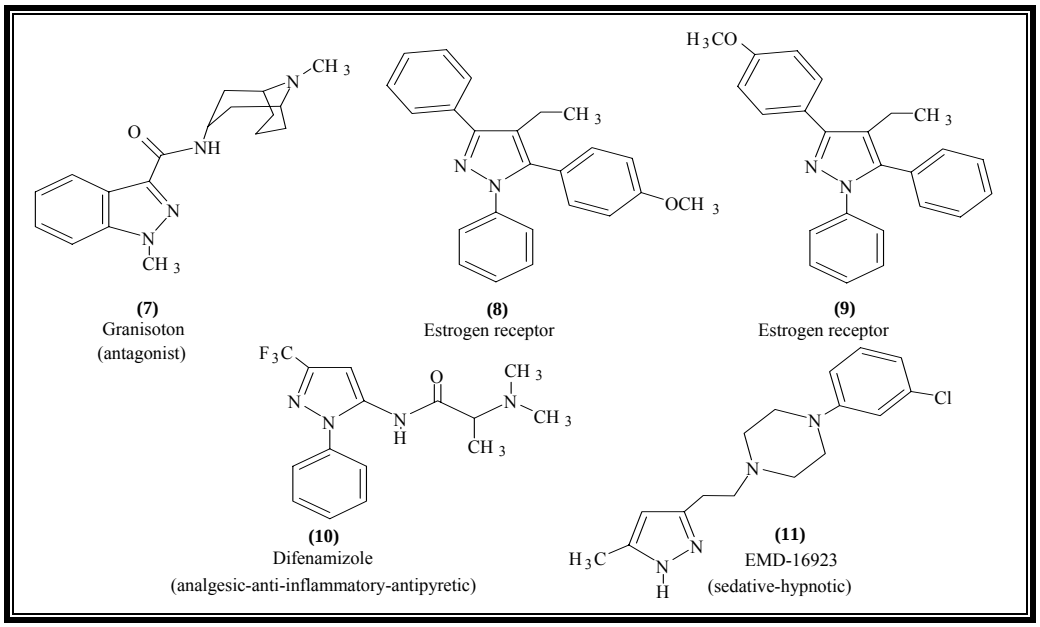


(1)

As evident from the literature in recent years a significant portion of research work in heterocyclic chemistry has been devoted to pyrazoles containing different alkyl, aryl and heteroaryl groups as substituents.

Pyrazoles belong to the family of azoles, i.e. five-member ring containing only nitrogen and carbon atoms, ranging from pyrrole to pentazole. According to Albert's classification, they are π-excessive, *N*-hetero aromatic derivatives and according to Kauffmann's arenology principle, as a substituted carbon, they are analogues of amines and as substituted nitrogen they are analogues of halogens, i.e. pseudo halogens. Synthesis of pyrazole and its *N*-aryl analogs has been a subject of consistent interest because of the wide applications of such heterocycles in pharmaceutical as well as in agrochemical industry.[1-3] numerous compounds containing pyrazole moiety have been shown to exhibit anti-hyperglycemic, analgesic, anti-inflammatory, anti-pyretic, anti-bacterial and sedative-hypnotic activities. [4-6]

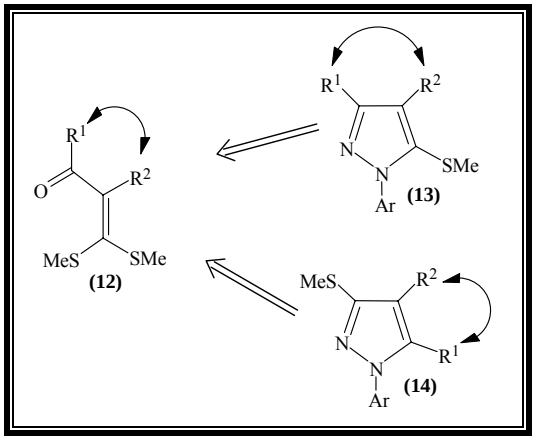




1-Phenylpyrazole moiety is present in several drug candidates for the treatment of various diseases such as cyclooxygenase-2 (Cox-2) inhibitors, IL-1 synthesis inhibitors and protein kinas inhibitors etc.[7-10] Similarly, few of the 1,5-diarylpyrazole derivatives have been shown to exhibit non-nucleoside HIV-1 reverse transcriptase inhibitor activity[11] along with Cox-2 inhibitor.[9,10] The corresponding

1,3,5-triaryl-4-alkylpyrazoles have been recently identified as the efficient legends for estrogen receptor displaying high binding affinities and selective transcriptional efficacy for ERI subtype.[12-16] Therefore, continuous efforts have been devoted for the development of more general and efficient methods for the synthesis of this class of compounds.

During the course of ongoing interest in development of efficient general synthetic routes for five and six-membered heterocycles utilizing α-ox ketene dithioacetals **(12)** as a versatile 3-carbon building blocks,[17-19] it has been shown in these studies, that it is possible to tune the reactivity of these ambient electrophiles



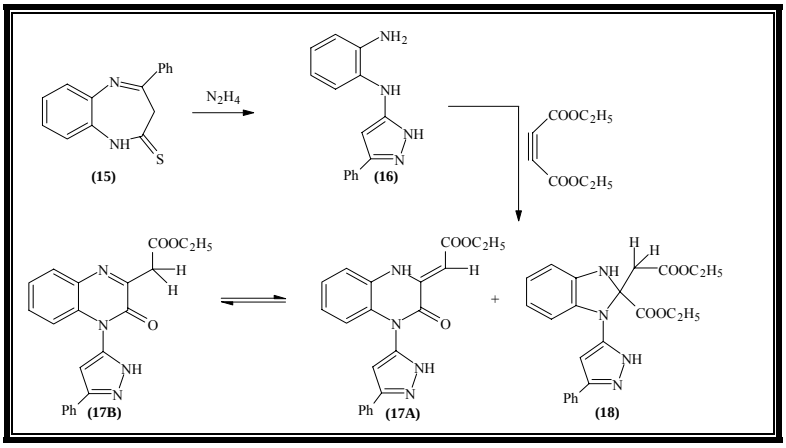
towards unsymmetrical heterobionucleophiles such as hydroxylamine by variation of reaction conditions to afford either 3-aryl-5-(methylthio) or 5-aryl-3-(methylthio)isoxazoles in highly regiocontrolled fashion.[20] Recently, Junjappa H and Ila H have shown in their subsequent aromatic[21-23] and heteroaromatic[24] annulations studies, the possibility of achieving regioselective addition (1,4- addition *vs.* 1,2-addition) to α-ox ketene dithioacetals **(12)** with various carbanionic species by altering their nucleophilic nature (hard/soft nucleophiles). In continuation of these studies, more interest was taken in probing the reaction of these α-oxoketene dithioacetals **(12)** with aryl hydrazine with a view to develop a regioselective synthesis of isomeric 1-aryl-3,4-substituted-5-(methylthio)- and 1-aryl-4,5-substituted-3-(methylthio)-pyrazoles **(13)** and **(14)** by manipulating the reaction conditions.

2.1.1 SYNTHETIC STUDY OF PYRAZOLES

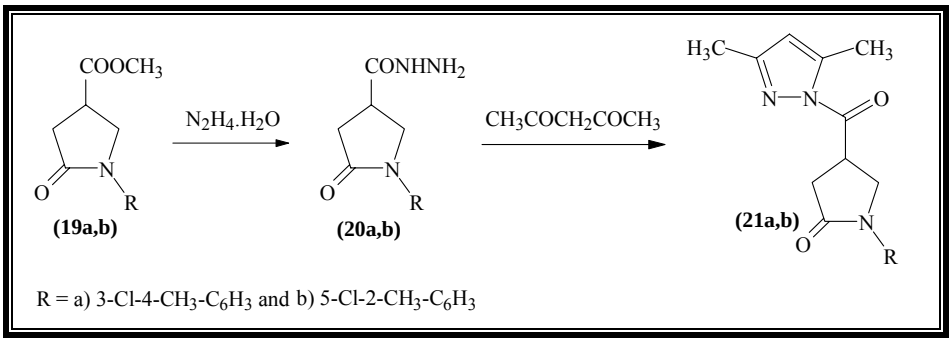
Most of the methods for the synthesis of pyrazoles involve approaches based on either **(i)** cyclocondensation of 1,3-dicarbonyl compounds and their equivalent 1,3-dienophilic synthons such as propargylic ketenes, dialkylamino/alkoxy/chloro ketones with arylhydrazines or **(ii)** intermolecular [2+3] cycloadditions of 1,3-dipoles to alkynes.[25-35]

However, the appealing generality of these methods are somewhat vitiated due to the frequent formation of regioisomeric mixtures of unsymmetrical pyrazoles in these reactions. Therefore, several elegant methods for the synthesis of asymmetrically substituted pyrazoles have been reported in the literature. Before presenting the results of our work, a brief literature survey on some of the recent syntheses of pyrazoles and its derivatives has been discussed. Among these methods, few selected recent examples have been highlighted in the following.

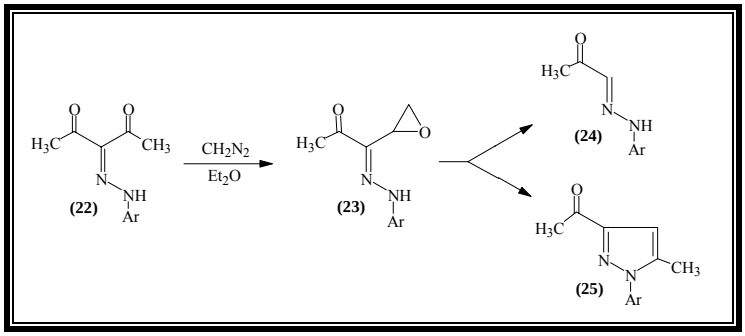
Essassi *et al*.[36] have reported that 4-phenyl-1, 5-benzodiazepine-2-thione **(15)**, reacts with hydrazine to give 3-*N*-(2-aminophenyl amino)-5-phenylpyrazole **(16)**. The reaction of **(16)** with diethyl acetylenedicarboxylate in ethanol under stirring at room temperature afforded ethyl-2-(2-ethoxy-2-oxoethyl)-1-(3-phenyl-1*H*-pyrazol-5-yl)-2,3-dihydro-1*H*-benzimidazole-2- carboxylate **(18)** (resulting from the attack of the NH_2 and NH groups of **(16)** on the triple bond of acetylenedicarboxylate) and **(17)** in good yield. There exists a tautomeric equilibrium between the enamine form **A** and the methyleneimine form **B** as shown in the reaction scheme.



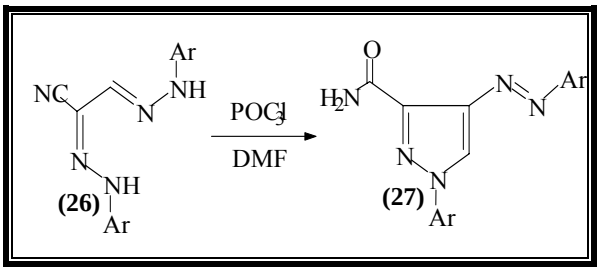
Mikulskiene *et al*.[37] have synthesized some new 1-aryl-4-[(3, 5–dimethylpyrazole-1-yl) carbonyl]-2-pyrrolidinones **(21a, b)** by the condensation of hydrazide **(20a, b)** with 2,4-pentanedione in 2-propanol in the presence of a catalytic amount of hydrochloric acid.



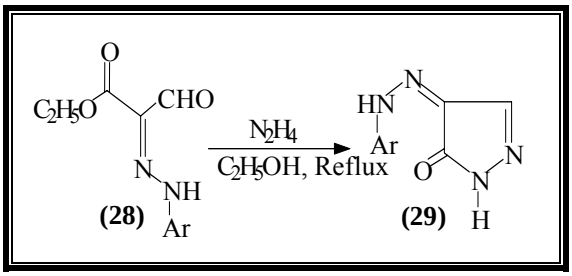
Elassar *et al*.[38] have discussed the chemistry of carbofunctionally substituted hydrazones and reported some important methods for the synthesis of pyrazole derivatives. The reaction of 3-(2-phenylhydrazono) pentane-2, 4-dione **(22)** with diazomethane who afforded pyrazole **(25)** in addition to **(23)** and **(24)**.[39,40] Although reaction sequence looks logical, this result should be checked again and reaction product should be characterized spectroscopically.



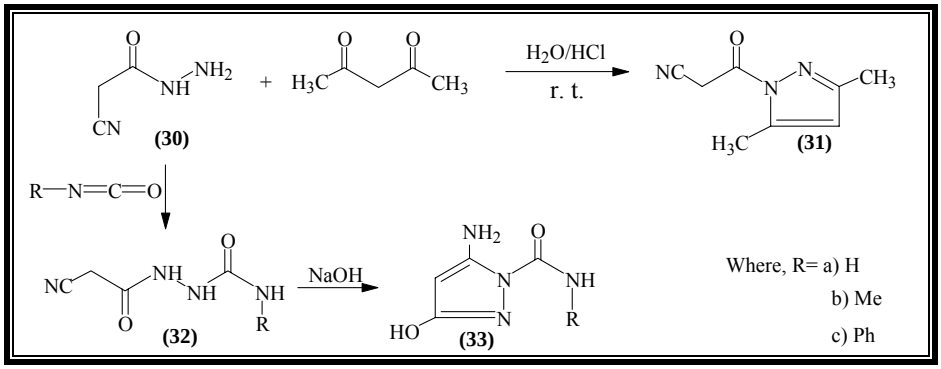
Patel *et al*.[41] have reported successful Vilsmeier formylation of **(26)** followed by cyclization and hydrolysis to yield pyrazole **(27)**.



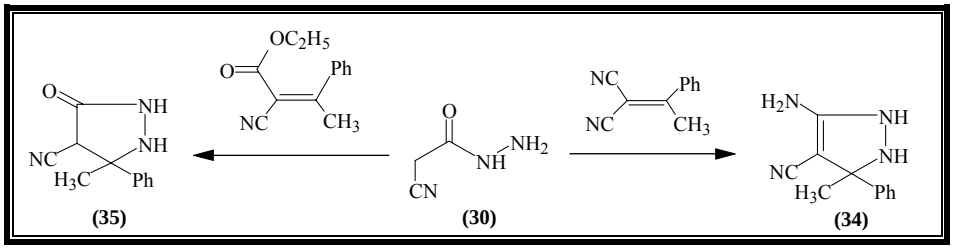
The ester **(28)** afforded arylazopyrazoles **(29)** upon treatment with hydrazine hydrate in refluxing ethanol.[42, 43]



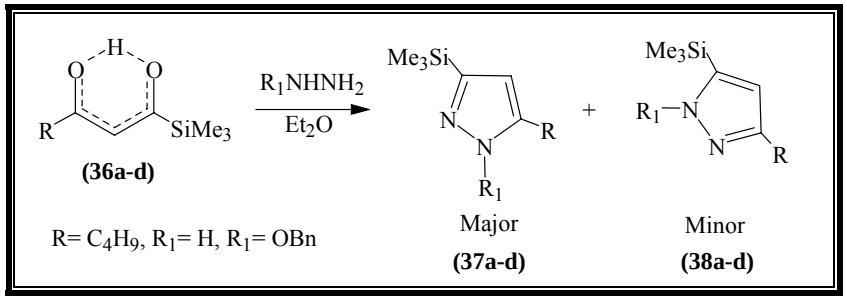
Bondock *et al*.[44] have reported the synthesis of 1-cyanoacetyl-3, 5-dimethyl pyrazole[45] **(31)** by the treatment of **(30)** in water containing a catalytic amount of conc. HCl with acetyl acetone at room temperature. The reaction of **(30)** with alkyl-isocyanate yields alkyl-carbamoyl derivative **(32)** that cyclized into pyrazole derivative **(33)** on treatment with 2N sodium hydroxide.[46]



Elagdi *et al*.[47] have reported the reaction of 2-(1-phenylethylidene) malononitrile with **(30)** furnished pyrazoline derivative **(34)**. Pyrazolidinone derivative **(35)** was obtained by treatment of **(30)** with ethyl 2-cyano-3-phenylbut -2-enoate.



Plantier-Royon *et al*.[48] have developed a novel method for the synthesis of 3-trialkylsilyl pyrazoles from *β*-oxoacylsilanes. This methodology was further extended to the synthesis of silylated pyrazoles bearing a carbohydrate moiety.



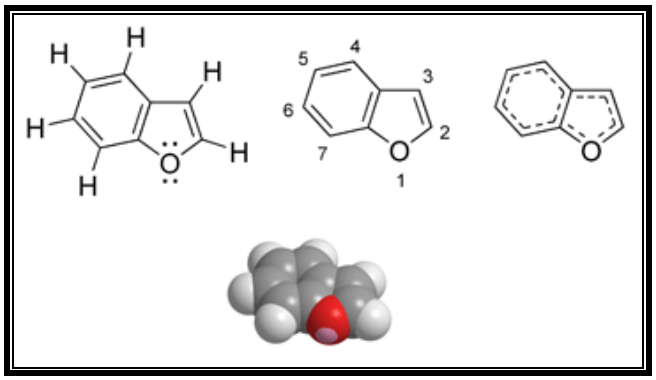
2.1.2 PHARMACOLOGICAL-SIGNIFICANCE OF PYRAZOLES

Pyrazoles belong to an important class of heterocycles due to their biological and pharmacological activities[49,50] such as anti-inflammatory,[51] herbicidal,[52] fungicidal,[53] bactericidal,[53] plant growth inhibitory,[52] antipyretic,[54] and protein kinase inhibitory activities.[55] Also, they are the key starting materials for the synthesis of commercial aryl/hetero-arylazopyrazolone dyes.[56,57]

Many other authors have reported other biological activities.[58-85]

2.2 BENZOFURANS

Benzofuran is the heterocyclic compound consisting of fused benzene and furan rings. This colorless solid is a component of coal tar. Benzofuran is the "parent" of many related compounds with more complex structures. For example, psoralen is a benzofuran derivative that occurs in several plants.

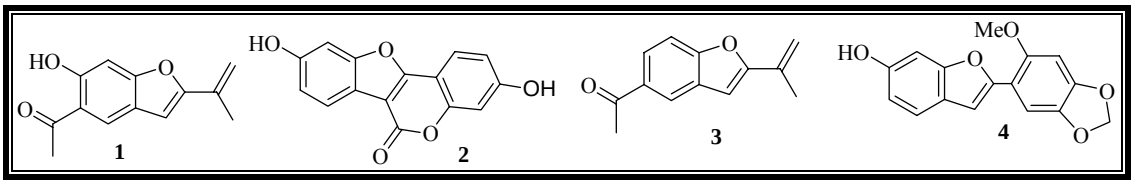


2.2.1 PHYSICAL PROPERTIES

Benzofuran is oily liquid. Its Molecular formula is C_8H_6O and molecular weight is 118.13 g mol^{-1} Its Boiling-point is 173 °C, 446 K, 343 °F and Melting-point is -18 °C, 255 K, -0 °F it do not dissolve in water, soluble in ethanol, ethyl ether and benzene. With the steam can be volatile and could be chemical decomposition and other oxidants.

2.2 INTRODUCTION

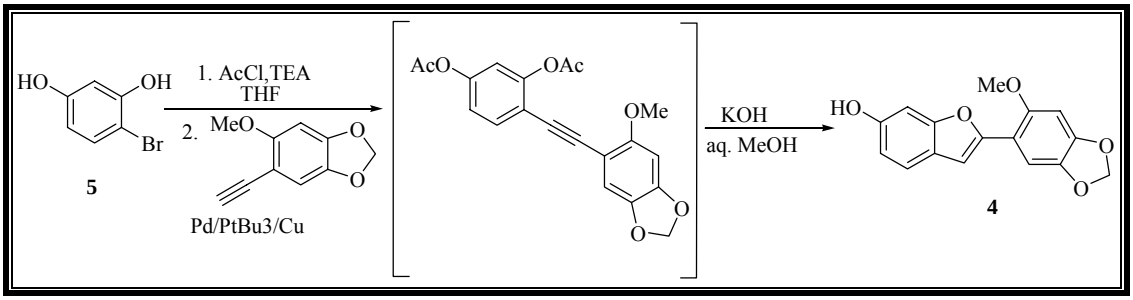
In nature's collection of biologically active heterocycles, benzo[*b*]furan derivatives[86-89] constitutes a major group. They are usually important constituents of plant extracts used in traditional medicine,[87] and some of them also play an important role in the natural defense mechanisms of their plants. These compounds include hydroxylated benzofurans such as Euparin[90] (**1**, Figure 1.), Coumestrol[91] (**2**), dehydrotremetone[92] (**3**), or Cicerfuran[93] (**4**). Due to this effect, a synthetic route--leading to hydroxylated benzofurans would be of general interest.



(Fig.-1)

An obvious approach would utilize the cross-coupling of the benzofuran and C2-substituent moieties as demonstrated for analogous systems by Timári.[94] Unfortunately, extension of this procedure would require the selective preparation of differently substituted benzofurans, a task that has not been solved generally yet. An alternate approach would achieve the formation of the benzofuran moiety through the ring closure of an *o*-alknylphenol, [95-100] available through the coupling of an aryl halide and the appropriate aryl acetylene.

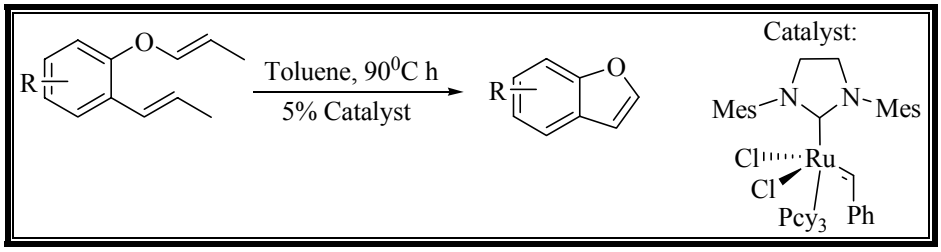
In spite of the large number of publications describing the conversion of 2-halophenols to benzo[*b*]furans,[95-100] the preparation of hydroxylated benzofurans has received only limited attention so far.[97,99,100] By analogy, such a process would start from a halogenated dihydroxybenzene. These compounds usually fail to undergo Sonogashira coupling and the successful procedures on similar systems usually utilize the selective protection of the different hydroxyl groups or an equimolar amount of preformed organo copper reagent.[100] A recent publication described the use of acetyl protecting groups that allowed for the efficient, 'one-pot' conversion of bromorezorcine (**5**, Figure 2.) to Cicerfuran (**4**).[101]



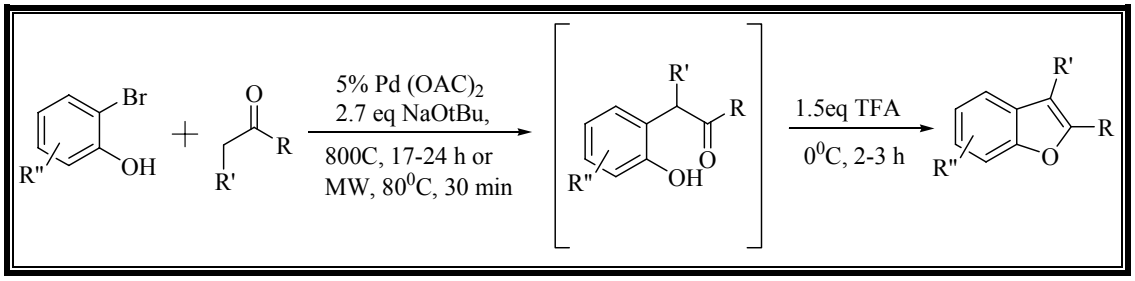
(Fig.-2)

After the discovery of disodium cromoglycate (DSCG), an efficient drug which is useful in treatment of human asthma, several isosteric or structurally related bisheterocyclic systems have been synthesized.[102-104] DSCG-like and bisbenzofuran containing structures have been synthesized and characterized by Sunder and Peet.[105] The condensation products of quinine that incorporate the structures of the 2,8-dimethoxydibenzofuran dimer, the trimer, 2,11-dimethoxybenzo-[1,2-b;3,4-b']bis(benzofuran),[106] and the open tetramer dibenzo[b,b']furo[3,2-e;4,5-e']bis(benzofuran) derivatives[107] have been synthesized and their X-ray crystal structures have been clarified, but overall there is limited literature on bisbenzofuran itself and bisbenzofuran containing compounds. According to our literature survey, no reports concerning oxime ether containing bisbenzofuran rings have been published since 1970. On the other hand, limited biological activity studies on bis- benzofuran containing compounds were found. [104, 106and 108] As it is known that, oxime ethers have found many uses in recent years as nonsteroidal anti-inflammatory drugs, [109] beta-adrenergic, [110] mold inhibitory active compound in poultry science,[111] anti-protozoan,[112] and insect growth regulator [113] and as various materials with steroidal effects.[114]

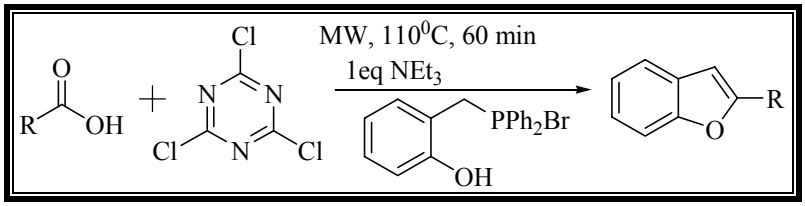
2.2.2 SYNTHESIS OF BENZOFURANS



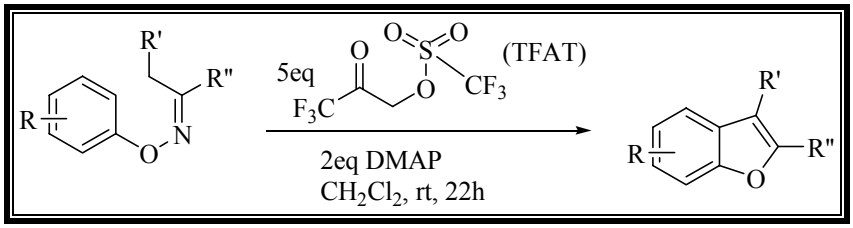
Substituted benzofurans were synthesized from their corresponding substituted 1-allyl-2-allyloxybenzenes using ruthenium-catalyzed *C*- and *O*-allyl isomerization followed by ring-closingmetathesis.[115]



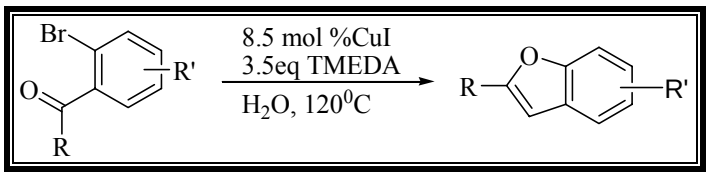
A one-pot synthesis of benzofurans which utilizes a palladium-catalyzed enolate arylation demonstrates broad substrate scope and provides differentially substituted Benzofurans in moderate yields. The utility of the method is further demonstrated by the synthesis of the natural product eupomatenoid 6 in three steps.[116]



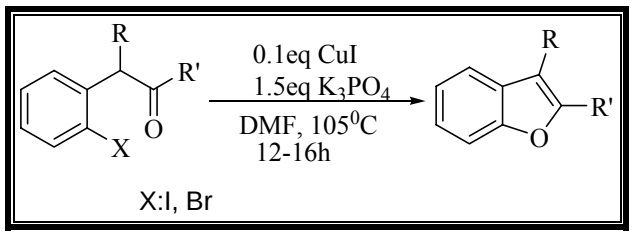
An effective and mild microwave-assisted route to 2-substituted benzofurans directly from carboxylic acids allows the preparation of α-alkyl-2-benzofuranmethanamines from *N*-protected α-amino acids without racemization in good yields.[117]



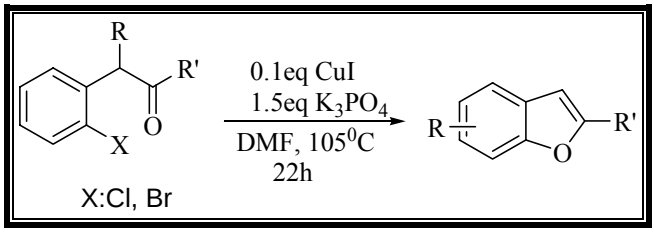
TFAA induces a [3,3]-sigmatropic rearrangement of *N*-trifluoroacetyl-ene-hydroxyl amines for the synthesis of dihydrobenzofurans, whereas reactions with TFAT-DMAP gives benzofurans. The synthetic utility is demonstrated by the short synthesis of natural benzofurans without protection of the hydroxyl group.[118]



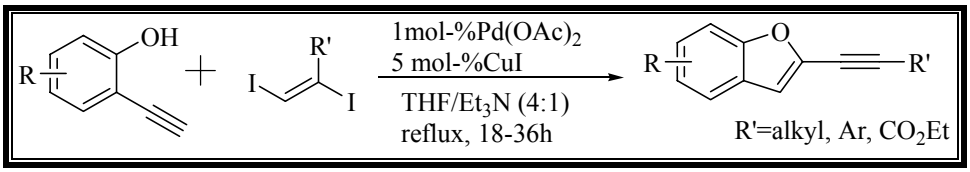
Copper-TMEDA catalyzes the transformation of readily available ketone derivatives into the corresponding benzo[b]furans in good to excellent yields. The sustainable protocol uses water as the solvent without organic co-solvents and one example of catalyst reutilization is also presented.[119]



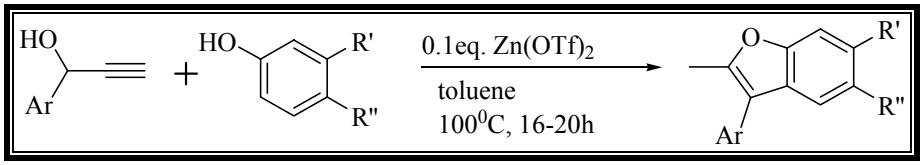
An efficient CuI-catalyzed ring closure of 2-haloaromatic ketones gives a wide variety of benzo[*b*] furans.[120]



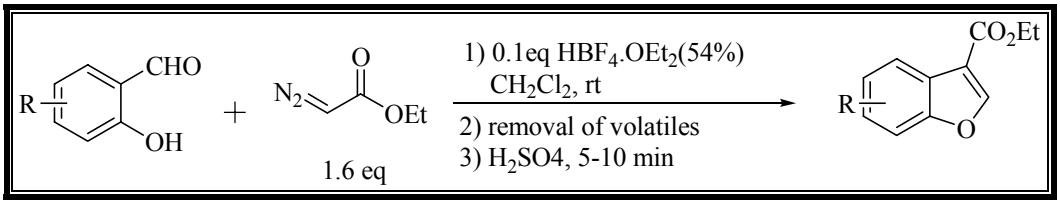
An efficient, indirect anti-Markovnikov hydration of asymmetrically substituted terminal and internal alkynes is based on $TiCl_4$-catalyzed hydroamination reactions. Its application to *ortho*-alkynylhaloarenes, followed by a copper-catalyzed *O*-arylation, provides substituted benzo[b]furans.[121]



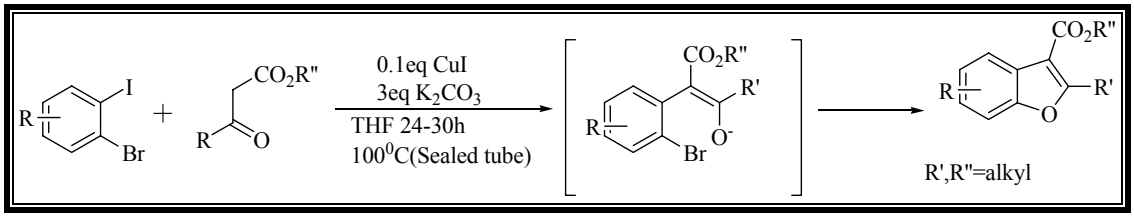
In the presence of palladium (II) acetate and copper (I) iodide, unsymmetrical buta-1,3-diynes were selectively obtained from the reaction of (*E*)-1,2-diiodoalkenes with terminal alkynes in moderate to good yields at room temperature. Using the same conditions, the coupling of 2-ethynylphenol with (*E*)-1, 2-diiodoalkenes followed by a cyclization at 100°C gives ethynylbenzofurans.[122]



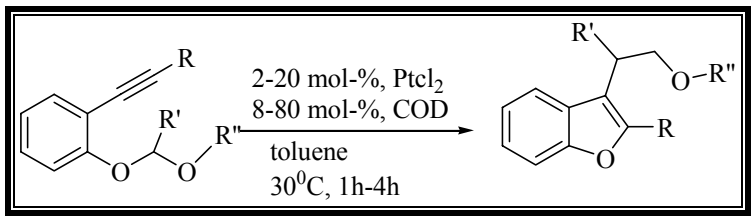
$Zn(OTf)_2$ catalyzed the cyclization of propargyl alcohols with anilines and phenols in toluene at 100°C without additive and gave various indole and benzofuran products with different structures. The cyclization of propargyl alcohols and amides gave oxazoles. Mechanisms for the different substitution patterns are discussed.[123]



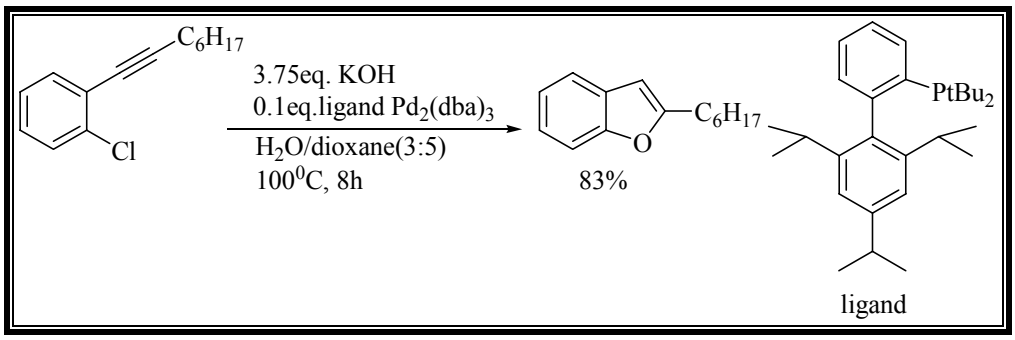
A convenient high-yielding, efficient, selective and simple one-pot procedure for the synthesis of 3-ethoxycarbonylbenzofurans from commercially available salisaldehyde and ethyl diazoacetate has been developed.[124]



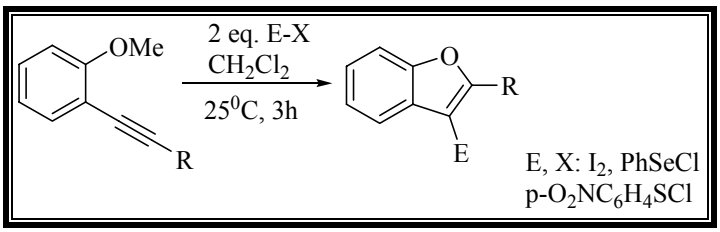
CuI-catalyzed coupling of 1-bromo-2-iodobenzenes with β-keto esters in THF at 100°C provides 2,3-disubstituted benzofurans. This domino transformation involves an intermolecular -C-C- bond formation and a subsequent intramolecular –C=O bond formation process.[125]



The Pt-catalyzed cyclization of *o*-alkynylphenyl acetals produces3-(-alkoxyalkyl) benzofurans in good to high yields. The mechanism is discussed.[126]



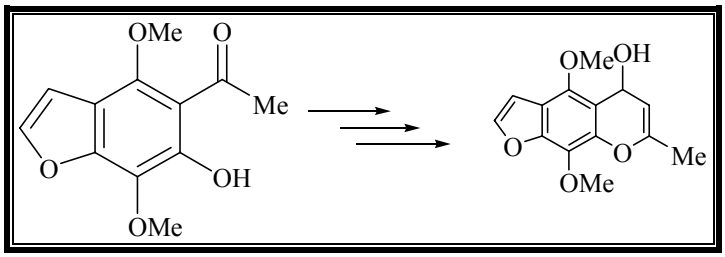
A one-pot method for the preparation of alkyl aryl ethers from aryl halides and the Preparation of substituted benzofurans via a Pd-catalyzed phenol formation/cycli--zation protocols starting from 2-chloroaryl alkynes are described.[127]



2,3-Disubstituted benzo[*b*]furans are readily prepared under very mild reaction conditions by the Sonogashira coupling of various *o*-iodoanisoles and terminal alkynes, followed by an electrophilic cyclization. Aryl- and vinylic-substituted alkynes give cyclization products in excellent yields.[128]

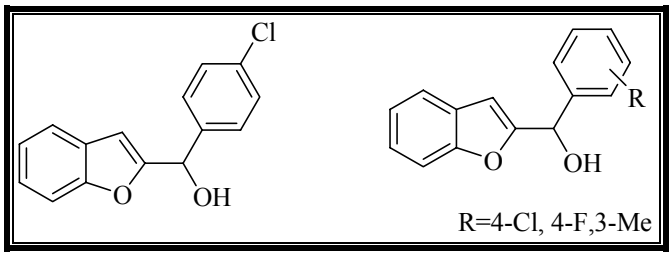
2.2.3 BIOLOGICALLY RELEVANT 2-SUBSTITUTED BENZOFURANS

Khellin (Fig. **1**) is one of several furochromones that can be isolated from *Ammi visnaga L.,* a perennial herbaceous plant that grows wild in many Eastern Mediterranean countries. Recently, khellin, along with several analogues, was found to possess desirable lipid-altering activity and is a coronary vasodilator: i.e., lowering the atherogenic VLDL + LDL-cholesterol fraction and elevating the anti-atherogenic (i.e., protective against atherosclerosis) HDL-cholesterol fraction, in animal models [129] as well as in man.



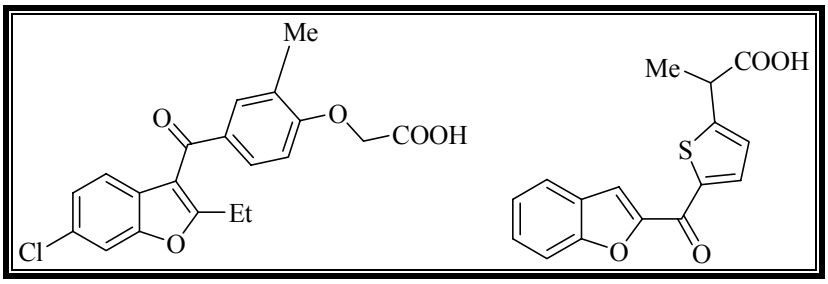
(Fig-1.)

Cloridarol (Fig. **2**) was used in for treatment of lipidemia and as an anticoagulant.[130] Race mates of 2- benzofuranyl carbinols like have been shown to display antifungal and aromatase inhibiting activities. These carbinols were prepared in racemic form in good yields and recently synthesis of aryl 2-benzofuranyl in high enantiomeric purity was reported.[131]



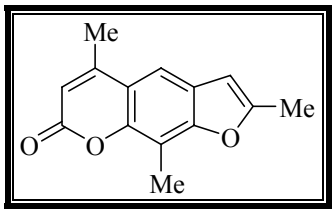
(Fig-2.)

Other benzofuran drugs have similar structures: compound promotes the excretion of uric acid and is useful for treating gout.[132] Thiophene (Fig. **3**) has analgesic activity.[133]



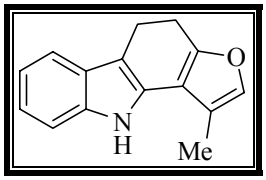
(Fig-3.)

Furaprofen (-methyl-3-phenyl-7-benzofuranacetic acid)[134] has a similar activity than the well-known Ibuprofen (2- [4-(2-methylpropyl) phenyl] propanoicacid). Trioxsalen[135] (Fig. **4**) is a synthetic psoralen[136] that offers skin protection against UVB erythema and is an orally active tanning and pigmentation agent useful in the treatment of vitiligo.



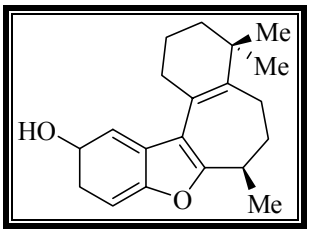
(Fig-4.)

A variety of polycyclic benzofurans have anticancer activity: indole (Fig. **5**) has antitumor and antiviral activity.[137]



(Fig-5.)

A chiral optically active benzofuran derivative was isolated from the sponge *Dysidea frondosa* together with other derivatives such as Frondosin A-D, each of them inhibiting the binding of IL-8 to its receptor in the low micromolar range.[138] Frondosin B (Fig. **6**) is recovered in nature in an enantiomeric form. A more recent paper reported the total synthesis and determination of the absolute configuration of Frondosin B.[139]



(Fig-6.)

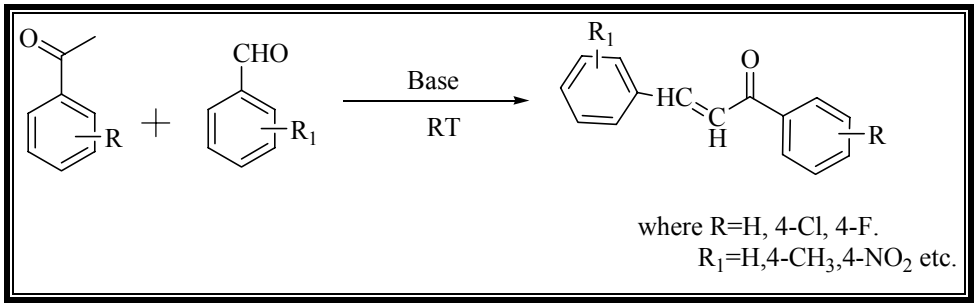
2.3 AIM OF CURRENT WORK

The literature survey revealed that some extensive work has been done on pyrozole and pyrazolone type of the compounds. Also, due to the usefulness of traditional medicines like Coumestrol, Dehydrotremetone and Cicerfuran, the benzofuran moiety has been selected for the research criteria.

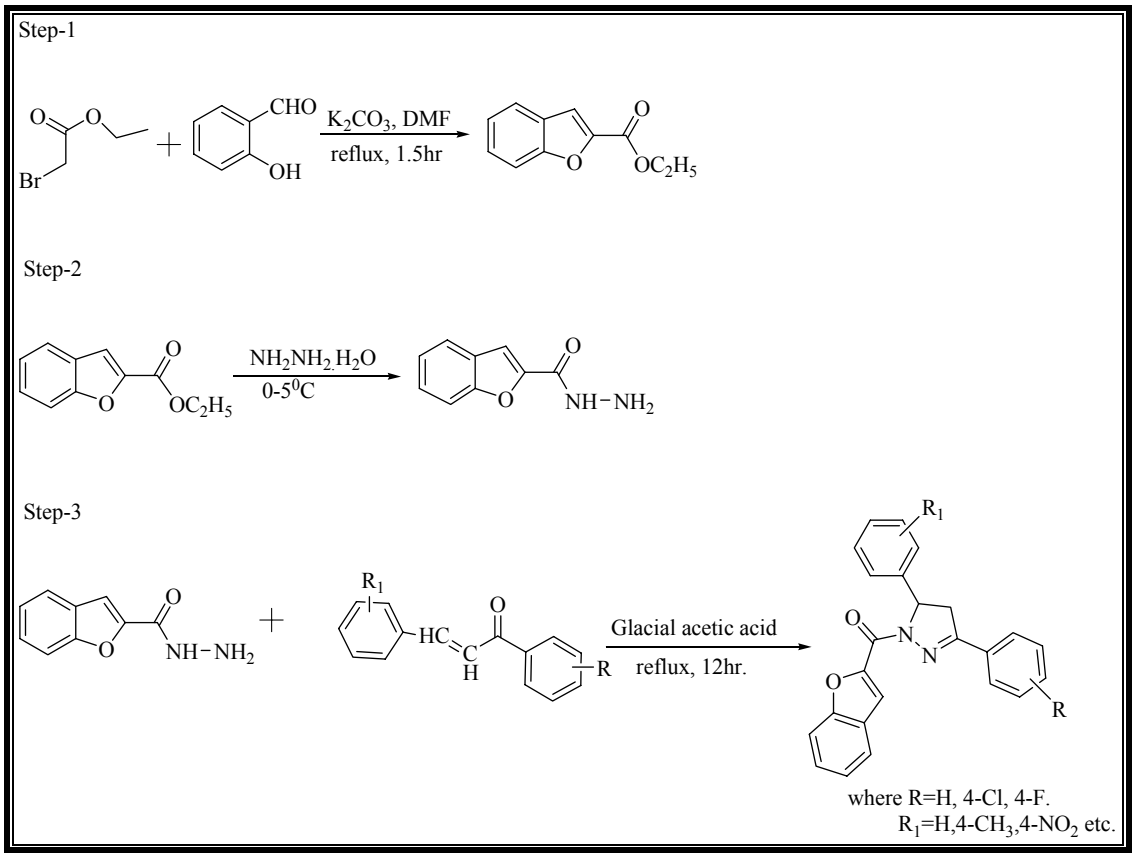
Browsing through the literature of organomedicinal chemistry the most useful moiety found was substituted 4, 5-dihydro pyrazolonone derivatives. Because of the less toxicological properties and good to moderate activities, several compounds have been synthesized by our team in the laboratory. The work encompassed in this chapter is an extension of the aforesaid research activity.

2.4 REACTION SCHEMES

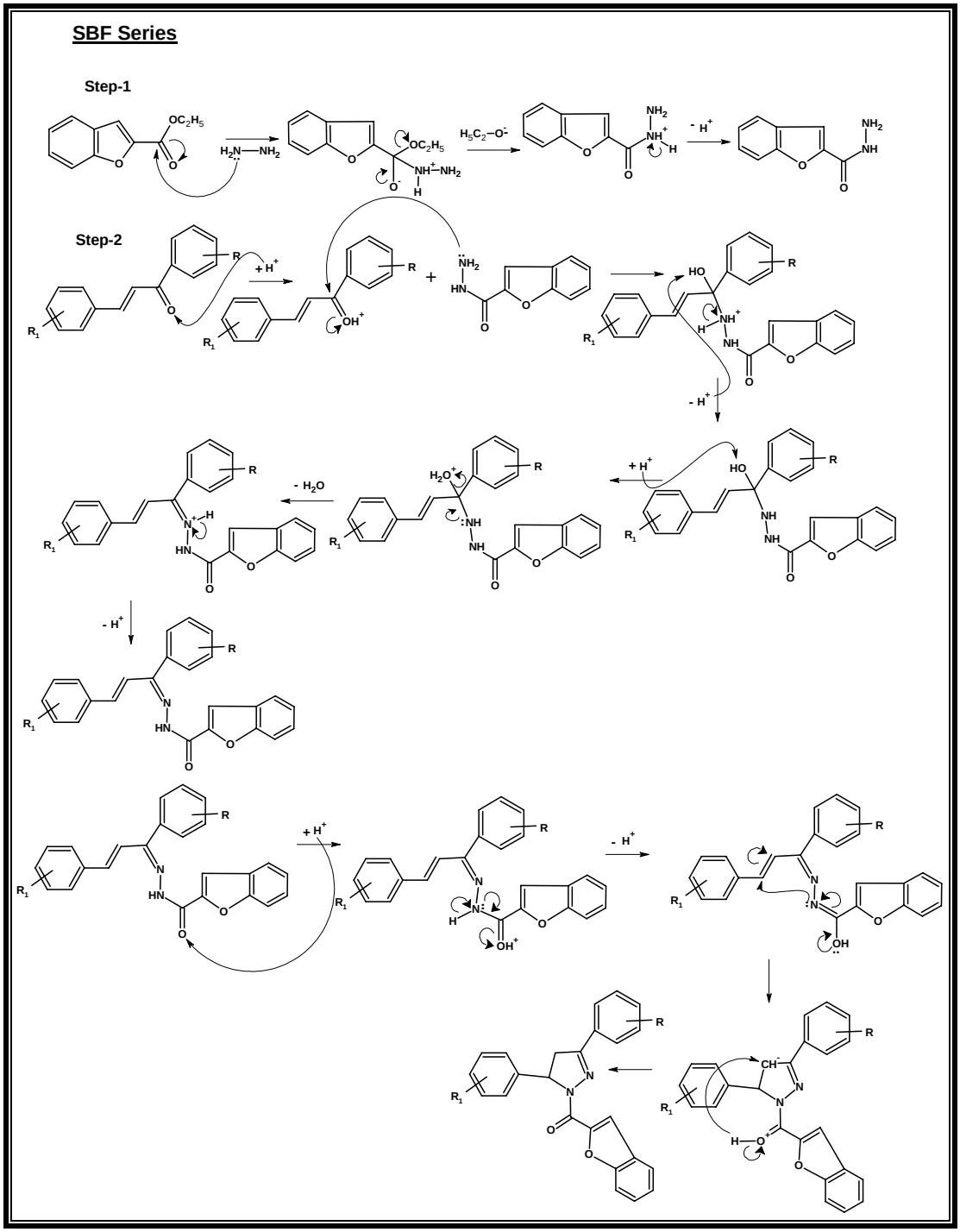
2.4.1 PREPARATION OF BENZOFURAN-2-CARBOHYDRAZIDE



2.4.2 PREPARATION OF BENZOFURAN-2-YL (4, 5- DIHYDRO-3, 5-SUBSTITUTED DIPHENYLPYRAZOL-1- YL) METHANONE



2.5 PLAUSIBLE REACTION MECHANISM



2.6 EXPERIMENTAL

2.6.1. MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. Formation of all compounds was purified by using **flash chromatography**. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6/$CDCl_3$ solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

2.6.2. GENERAL PROCEDURE FOR PREPARATION OF SUBSTITUTED CHALCONES

Substituted aldehydes (0.01 mole) was charged into 250 ml flat bottom flask. 15 ml of methanol was added into above flask to dissolve it under stirring. And add into Substituted acetophenones (0.01mole) to stirr it for RT. 3-4 drops of saturated sodium hydroxide solution was added as a catalyst. The reaction mixture was stirred for 24 hours at RT. As a solid product comes. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed, filtered under reduced pressure.

Similarly other compounds are also prepared.

2.6.2a. PHYSICAL DATA OF SUBSTITUTED CHALCONES[140]:

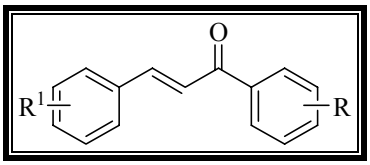


TABLE-2.1

Sr. No.	Code Name & No.	Substitutions		Molecular Formula	Molecular Weight	M.P. ^{0}C	R_f Value
		R	R^1				
1	SC-01	H	H	$C_{15}H_{12}O$	208	55-57	0.46
2	SC-02	H	4-CH_3	$C_{16}H_{14}O$	222	110-112	0.48
3	SC-03	H	4-NO_2	$C_{15}H_{11}NO_3$	253	158-164	0.52
4	SC-04	H	3,4-di-OCH_3	$C_{17}H_{16}O_3$	268	87-89	0.46
5	SC-05	H	4-F	$C_{15}H_{11}FO$	226	85-87	0.50
6	SC-06	H	4-Cl	$C_{15}H_{11}ClO$	243	114-116	0.44
7	SC-07	H	2-Cl	$C_{15}H_{11}ClO$	243	50-52	0.46
8	SC-08	H	4-OCH_3	$C_{16}H_{14}O_2$	238	75-77	0.50
9	SC-09	4-Cl	H	$C_{15}H_{11}ClO$	243	97-101	0.48
10	SC-10	4-Cl	4-Cl	$C_{15}H_{10}Cl_2O$	277	157-159	0.52
11	SC-11	4-Cl	4-OCH_3	$C_{16}H_{13}ClO_2$	273	126-128	0.44
12	SC-12	4-Cl	4-NO_2	$C_{15}H_{10}ClNO_3$	288	162-164	0.48
13	SC-13	4-Cl	2-Cl	$C_{15}H_{10}Cl_2O$	277	82-84	0.38
14	SC-14	4-Cl	3,4-di-OCH_3	$C_{17}H_{15}ClO_3$	303	106-107	0.42
15	SC-15	4-Cl	4-CH_3	$C_{16}H_{13}ClO$	257	162-164	0.48
16	SC-16	4-Cl	4-F	$C_{15}H_{10}ClFO$	261	112-114	0.52
17	SC-17	4-F	H	$C_{15}H_{11}FO$	226	79-80	0.40
18	SC-18	4-F	4-Cl	$C_{15}H_{10}ClFO$	261	131-134	0.44
19	SC-19	4-F	4-OCH_3	$C_{16}H_{13}FO_2$	256	107-108	0.48
20	SC-20	4-F	4-$N0_2$	$C_{15}H_{10}FNO_3$	271	135-137	0.42
21	SC-21	4-F	2-Cl	$C_{15}H_{10}ClFO$	261	88-90	0.40
22	SC-22	4-F	3,4-di-OCH_3	$C_{17}H_{15}FO_3$	286	91-93	0.48
23	SC-23	4-F	4-CH_3	$C_{16}H_{13}FO$	240	122-124	0.44
24	SC-24	4-F	4-F	$C_{15}H_{10}F_2O$	244	117-119	0.46

TLC solvent system R_f, Hexane: Ethyl acetate - 4:6

2.6.3. GENERAL PROCEDURE OF BENZOFURAN-2-YL (4, 5- DIHYDRO-3, 5-SUBSTITUTED DIPHENYLPYRAZOL-1- YL) METHANONES

Step-1 PREPARATION OF ETHYL BENZOFURAN-2-CARBOXYLATE

Salisaldehyde (0.01 mole) was charged into 250 ml round bottom flask. 30 ml of dimethyl formamide was added into above flask. Then added 0.01 mole of ethylbromo acetate and K_2CO_3 (0.03 mole) was added. The reaction mixture was refluxed for 1.5 hours at 100^0C on oil bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (9 : 1) as a mobile phase. After the reaction to be completed, reaction mixture was poured into ice. Then product was extracted using ethyl acetate (50 ml × 3), the combined organic layer was washed using brine solution (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield –77 %, B. P. – 276°C.[a]

Step-2 PREPARATION OF BENZOFURAN-2-CARBOHYDRAZIDES :

Ethyl benzofuran-2-carboxylate (0.01 mole) was charged into 250 ml round bottom flask. Add drop wise into 15 ml of hydrazine hydrate at 0-5^0C above flask. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed, the mixture was stirred at room temperature to give benzofuran-2-carbohydrazide as a white colored shining product. M.P. – 190–194^0C.[b]

[a] Bioorganic & Medicinal Chemistry Letters 18 (2008) 5591–5593.
[b] Halli, M.B.; Oriental Journal of Chemistry 2001, V17(3), P441-444.

Step-3 SYNTHESIS OF BENZOFURAN-2-YL (4, 5- DIHYDRO-3, 5-SUBSTITUTED DIPHENYLPYRAZOL-1- YL) METHANONE

Benzofuran hydrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of Glacial acetic acid was added to dissolve it. Then add into substituted chalcones (0.01 mole). The reaction mixture was refluxed on oil bath for 12hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the complete reaction the mixture was poured into crushed ice to give solid product then filtered it under reduced pressure. Finally, it was purified by flash chromatography using hexane and ethyl acetate as eluents.

Similarly other compounds are also prepared.

2.7 PHYSICAL DATA

PHYSICAL DATA OF BENZOFURAN-2-YL (4, 5- DIHYDRO-3, 5-SUBSTITUTED DIPHENYLPYRAZOL-1- YL) METHANONES (SBF-01 TO SBF-28)

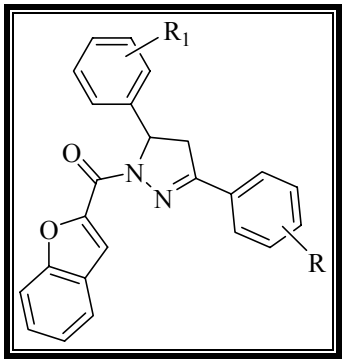


TABLE-2.2

Sr. No.	Code Name & No.	Substitutions		Molecular Formula	Molecular Weight	M.P. ^{0}C	R_f Value
		R	R^1				
1	SBF-01	H	H	$C_{24}H_{18}N_2O_2$	366	210-212	0.44
2	SBF-02	H	4-CH_3	$C_{25}H_{20}N_2O_2$	380	180-182	0.51
3	SBF-03	H	4-NO_2	$C_{24}H_{17}N_3O_4$	411	170-172	0.42
4	SBF-04	H	3,4-di-OCH_3	$C_{26}H_{22}N_2O_4$	426	150-154	0.46
5	SBF-05	H	4-F	$C_{24}H_{17}FN_2O_2$	384	220-224	0.41
6	SBF-06	H	4-Cl	$C_{24}H_{17}ClN_2O_2$	401	240-242	0.44
7	SBF-07	H	2-Cl	$C_{24}H_{17}ClN_2O_2$	401	178-182	0.52
8	SBF-08	H	4-OCH_3	$C_{25}H_{20}N_2O_3$	396	168-170	0.42
9	SBF-11	4-Cl	H	$C_{24}H_{17}ClN_2O_2$	401	154-156	0.45
10	SBF-12	4-Cl	4-Cl	$C_{24}H_{16}Cl_2N_2O_2$	435	176-178	0.48
11	SBF-13	4-Cl	4-OCH_3	$C_{25}H_{19}ClN_2O_3$	431	188-190	0.51
12	SBF-14	4-Cl	4-NO_2	$C_{24}H_{16}ClN_3O_4$	446	192-194	0.40
13	SBF-15	4-Cl	2-Cl	$C_{24}H_{16}Cl_2N_2O_2$	435	160-162	0.44
14	SBF-16	4-Cl	3,4-di-OCH_3	$C_{26}H_{21}ClN_2O_4$	461	188-190	0.40
15	SBF-17	4-Cl	4-CH_3	$C_{25}H_{19}ClN_2O_2$	415	178-182	0.50
16	SBF-18	4-Cl	4-F	$C_{24}H_{16}ClFN_2O_2$	419	220-222	0.44
17	SBF-21	4-F	H	$C_{24}H_{17}FN_2O_2$	384	244-246	0.48
18	SBF-22	4-F	4-Cl	$C_{24}H_{16}ClFN_2O_2$	419	210-212	0.41
19	SBF-23	4-F	4-OCH_3	$C_{25}H_{19}FN_2O_3$	414	220-222	0.48
20	SBF-24	4-F	4-$N0_2$	$C_{24}H_{16}FN_3O_4$	429	240-244	0.44
21	SBF-25	4-F	2-Cl	$C_{24}H_{16}ClFN_2O_2$	419	176-178	0.46
22	SBF-26	4-F	3,4-di-OCH_3	$C_{26}H_{21}FN_2O_4$	444	164-166	0.44
23	SBF-27	4-F	4-CH_3	$C_{25}H_{19}FN_2O_2$	398	174-176	0.42
24	SBF-28	4-F	4-F	$C_{24}H_{16}F_2N_2O_2$	402	184-186	0.48

TLC solvent system for R_f, Hexane : Ethyl acetate - 4:6

2.8 SPECTRAL DISCUSSION

2.8.1. MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. SBF-01 and SBF-02 of Mass spectra are given on page no. 49 and 52.

2.8.2. IR SPECTRA

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

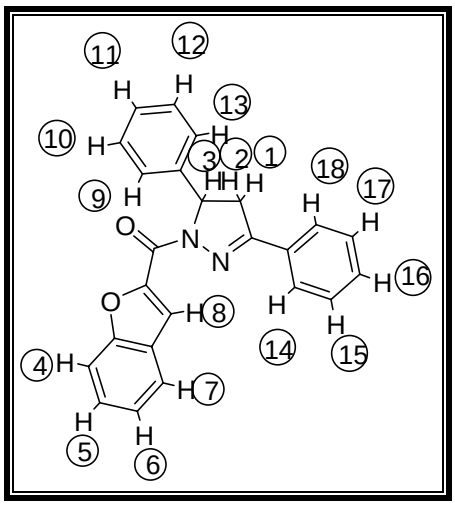
In case of SBF-01 to SBF-24 aromatic C-H stretching frequencies were found near 3050 cm^{-1} C-H stretching frequencies for methylene group were obtained near 2950 cm^{-1}. –C=O Stretching vibrations were found near 1690 cm^{-1}. –C=N vibrations were found in the region of 1640 cm^{-1}. -C-O-C- furan ring vibration was found 1040 cm^{-1}. Along with all the frequencies obtained in above compounds, additionally -C-Cl frequency was found between 780 cm^{-1} to 700 cm^{-1} in SBF-11 to SBF-18. and –C-F frequency was found between 1100 cm^{-1} to 1000 cm^{-1} in SBF-21 to SBF-28 Compounds. SBF-01 and SBF-02 of IR spectra are given on page no. 49 and 52.

2.8.3. ^{1}H NMR SPECTRA

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO/$CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated

to identify *o*, *m* and *p* coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H NMR spectral interpretation can be discussed as under.

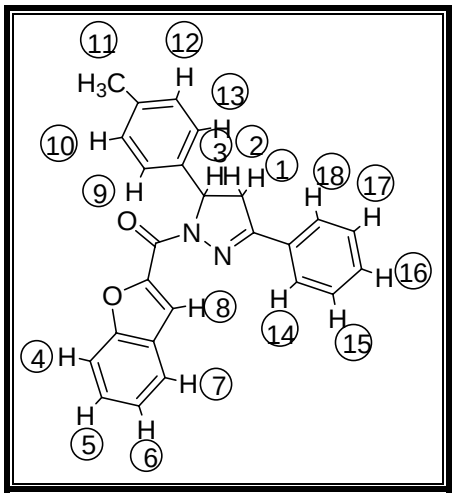
^{1}H NMR OF (BENZOFURAN-2-YL)(4,5-DIHYDRO-3,5-DIPHENYLPYRAZOL-1-YL)METHANONE (SBF-01):



1. The one proton no. 1 and proton no. 2 of the methylene group gave a multiplet at 3.11 δ ppm-3.88 δ ppm. It bit a down field due to the nitrogen atom. And its two protons are divided in 1H and 1H it shows in spectra.
2. The deshielded proton no. 3 of pyrazole ring it gave a multiplet and it shows on down field at 5.80 δ ppm-5.84 δ ppm due to the effect of nitrogen atom of pyrazole ring.
3. The aromatic ring of the two phenyl ring attached with pyrazole, in two phenyl ring proton no. 10, 11, 12, 15, 16 and 17 are on same atmosphere so these six protons gave a multiplet at 7.24 δ ppm-7.31 δ ppm it shows in spectra.
4. The proton no. 8 of Benzofuran ring gave a characteristic singlet at 8.12 δ ppm.
5. The aromatic ring of proton no. 6, 9 and 13 of three protons gave a multiplet at 7.49 δ ppm.
6. The aromatic ring of proton no. 7, 14 and 18 of three proton gave a multiplet at 7.78 δ ppm-7.84 δ ppm.
7. The aromatic ring of proton no. 4 gave a multiplet at 7.54 δ ppm -7.59 δ ppm.
8. The aromatic ring of proton no. 5 gave a multiplet at 7.39 δ ppm-7.43 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound SBF-01 has been confirmed. Spectrum is given on page no. 50.

^{1}H NMR OF (BENZOFURAN-2-YL) (4,5-DIHYDRO-3-PHENYL-5-P-TOLYLPYRAZOL-1-YL) METHANONE (SBF-02):



1. The proton no. 11 of methyl group gave a characteristic singlet at 2.28 δ ppm.
2. The proton no. 1 and 2 of the methylene group gave a multiplate at 3.20 δ ppm-3.82 δ ppm. It bit a down field due to the nitrogen atom. And its 2 protons are divided in 1H and 1H it shows in spectra.
3. The deshielded proton no. 3 of pyrazole it gave a multiplet and it shows on down field at 5.79 δ ppm-5.83 δ ppm due to the effect of nitrogen atom of pyrazole ring.
4. The proton no. 8 of Benzofuran ring gave a characteristic singlet at 8.03 δ ppm.
5. The proton no. 4 gave a doublet at 7.57 δ ppm. - 7.59 δ ppm. And *J* value for this proton calculated and found to be 8.0 Hz, suggesting it is ortho coupled.
6. The proton no. 7 gave a doublet at 7.74 δ ppm. - 7.76 δ ppm. And *J* value for this proton calculated and found to be 8.0 Hz, suggesting it is ortho coupled.
7. The proton no. 14 and 18 of two proton gave a triplet at 7.27 δ ppm-7.42 δ ppm it shown on expanded spectra.

8. The proton no. 9 and 13 of two proton gave a doublet at 7.10 δ ppm-7.12 δ ppm and *J* value is 8.0 Hz, it suggests an ortho coupling.
9. The proton no. 10 and 12 of two proton gave a doublet at 7.19 δ ppm-7.21 δ ppm and *J* value is 8.0 Hz, it suggests an ortho coupling.
10. The proton no. 5 and 6 gave a double doublet at 7.82 δ ppm - 7.84 δ ppm it shown on expanded spectra. and *J* value is 8.0 Hz, it suggests ortho coupling.
11. The proton no. 15, 16 and 17 of three protons gave a multiplet at 7.46 δ ppm - 7.51 δ ppm shown on expanded region.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound SBF-02 has been confirmed. Spectrum is given on page no. 53.

2.8.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

2.9 ANALYTICAL DATA

***(Benzofuran-2-yl)(4,5-dihydro-3,5-diphenylpyrazol-1-yl)methanone (SBF-01)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3059 (Ar. C-H stretching), 2920 (Aliphatic–CH_2 stretching), 1691 (C=O stretching), 1641 (-C=N of pyrazole ring), 1545 (Ar. -C-C- stretching), 1186 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 962 (Ar. -C-H i.p. deformation); ^{1}H NMR (400Hz-DMSO-d_6) δ (ppm) : 3.25 (1H, m), 3.88 (1H, m), 5.84 (1H, m), 7.31 (6H, m), 7.43 (1H, m), 7.49 (3H, m), 7.59 (1H, m), 7.84 (3H, m), 8.12 (1H, s); MS *m/z* = 366 (M^+); Anal. Calcd. for $C_{24}H_{18}N_2O_2$: C, 78.67; H, 4.95; N, 7.65; O, 8.73. Found: C, 78.64; H, 4.92; N, 7.61; O, 8.70%.

***(Benzofuran-2-yl)(4,5-dihydro-3-phenyl-5-p-tolylpyrazol-1-yl)methanone(SBF-02)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3059 (Ar. -C-H stretching), 2941 (Aliphatic -CH_2 stretching), 1695 (-C=O stretching), 1637 (-C=N of pyrazole ring), 1546 (Ar. -C-C- stretching), 1184 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 960 (Ar. -C-H i.p. deformation); ^{1}H NMR (400Hz-$CDCl_3$) δ (ppm): 2.28 (3H, s), 3.26 (1H, m), 3.82 (1H, m), 5.83 (1H, m), 7.12 (2H, d, *J*=8.0 Hz), 7.21 (2H, d, *J*=8.0 Hz), 7.30 (1H, t), 7.42 (1H, t), 7.51 (3H, m), 7.59 (1H, d, *J*=8.0 Hz), 7.76 (1H, d, *J*=8.0 Hz), 7.84 (2H, m), 8.03 (1H, s); MS *m/z* = 380 (M^+); Anal. Calcd. for $C_{25}H_{20}N_2O_2$: C, 78.93; H, 5.30; N, 7.36; O, 8.41. Found: C, 78.91; H, 5.26; N, 7.30; O, 8.40%.

***(Benzofuran-2-yl)(4,5-dihydro-5-(4-nitrophenyl)-3-phenylpyrazol-1yl)methanone(SBF-03)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3032 (Ar. -C-H stretching), 2935 (Aliphatic -CH_2 stretching), 1697 (-C=O stretching), 1600 (-C=N of pyrazole ring), 1518 (Ar. -C-C-

stretching), 1184 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 964 (Ar. -C-H i.p. deformation); MS *m/z* = 411 (M^+); Anal. Calcd. for $C_{24}H_{17}N_3O_4$: C, 70.07; H, 4.16; N, 10.21; O, 15.56. Found: C, 70.04; H, 4.12; N, 10.20; O, 15.51%.

***(Benzofuran-2-yl)(4,5-dihydro-5-(3,4-dimethoxyphenyl)-3-phenylpyrazol-1-yl) methanone (SBF-04)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3044 (Ar. -C-H stretching), 2922 (Aliphatic -CH_2 stretching), 1690 (-C=O stretching), 1638 (-C=N of pyrazole ring), 1543 (Ar. -C-C- stretching), 1180 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 962 (Ar. -C-H i.p. deformation); MS *m/z* = 426 (M^+); Anal. Calcd. for $C_{26}H_{22}N_2O_4$: C, 73.23; H, 5.20; N, 6.57; O, 15.01. Found: C, 73.22; H, 5.20; N, 6.51; O, 15.00%.

***(Benzofuran-2-yl)(5-(4-fluorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)methanone (SBF-05)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3055 (Ar. -C-H stretching), 2922 (Aliphatic -CH_2 stretching), 1694 (-C=O stretching), 1646 (-C=N of pyrazole ring), 1540 (Ar. -C-C- stretching), 1188 (-C-N of pyrazole ring), 1114 (-C-O-C- furan ring), 966 (Ar. -C-H i.p. deformation), 1018 (-C-F stretching); MS *m/z* = 384 (M^+), 386 (M^{+2}); Anal. Calcd. for $C_{24}H_{17}FN_2O_2$: C, 74.99; H, 4.46; N, 7.29; O, 8.32. Found: C, 74.96; H, 4.42; N, 7.22; O, 8.31%.

***(Benzofuran-2-yl)(5-(4-chlorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)methanone (SBF-06)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3062 (Ar. -C-H stretching), 2926 (Aliphatic -CH_2 str.), 1688 (-C=O stretching), 1641 (-C=N of pyrazole ring), 1547 (Ar. -C-C- stretching), 1182 (-C-N of pyrazole ring), 1104 (-C-O-C- furan ring), 966 (Ar. -C-H i.p. deformation), 743 (-C-Cl stretching); MS *m/z* = 401 (M^+), 403 (M^{+2}); Anal. Calcd. for $C_{24}H_{17}ClN_2O_2$: C, 71.91; H, 4.27; N, 6.99; O, 7.98. Found: C, 71.88; H, 4.24; N, 6.92; O, 7.91%.

***(Benzofuran-2-yl)(5-(2-chlorophenyl)-4,5-dihydro-3-phenylpyrazol-1-yl)methanone (SBF-07)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3044 (Ar. -C-H stretching), 2930 (Aliphatic -CH_2 stretching), 1694 (-C=O stretching), 1643 (-C=N of pyrazole ring), 1548 (Ar. -C-C-

stretching), 1183 (-C-N of pyrazole ring), 1110 (-C-O-C- furan ring), 961 (Ar. -C-H i.p. deformation), 745 (-C-Cl stretching); MS *m/z* = 401 (M^+), 403 (M^{+2}); Anal. Calcd. for $C_{24}H_{17}ClN_2O_2$: C, 71.91; H, 4.27; N, 6.99; O, 7.98. Found: C, 71.90; H, 4.22; N, 6.92; O, 7.96%.

***(Benzofuran-2-yl)(4,5-dihydro-5-(4-methoxyphenyl)-3-phenylpyrazol-1-yl)methanone (SBF-08)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3052 (Ar. -C-H stretching), 2928 (Aliphatic -CH_2 stretching), 1692 (-C=O stretching), 1648 (-C=N of pyrazole ring), 1539 (Ar. -C-C- stretching), 1184 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 961 (Ar. -C-H i.p. deformation); MS *m/z* = 396 (M^+); Anal. Calcd. for $C_{25}H_{20}N_2O_3$: C, 75.74; H, 5.08; N, 7.07; O, 12.11. Found: C, 75.71; H, 5.02; N, 7.01; O, 12.10%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-4,5-dihydro-5-phenylpyrazol-1-yl)methanone (SBF-11)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3064 (Ar. -C-H stretching), 2962 (Aliphatic -CH_2 stretching), 1695 (-C=O stretching), 1641 (-C=N of pyrazole ring), 1548 (Ar. -C-C- stretching), 1186 (-C-N of pyrazole ring), 1114 (-C-O-C- furan ring), 964 (Ar. -C-H i.p. deformation); 756 (-C-Cl stretching); MS *m/z* = 401 (M^+), 403 (M^{+2}); Anal. Calcd. for $C_{24}H_{17}ClN_2O_2$: C, 71.91; H, 4.27; N, 6.99; O, 7.98. Found: C, 71.87; H, 4.24; N, 6.96; O, 7.97%.

***(3,5-bis(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)(Benzofuran-2-yl)methanone (SBF-12)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3061 (Ar. -C-H stretching), 2924 (Aliphatic -CH_2 stretching), 1691 (-C=O stretching), 1643 (-C=N of pyrazole ring), 1550 (Ar. -C-C- stretching), 1180 (-C-N of pyrazole ring), 1116 (-C-O-C- furan ring), 963 (Ar. -C-H i.p. deformation), 744 (-C-Cl stretching); MS *m/z* = 435 (M^+), 437 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}Cl_2N_2O_2$: C, 66.22; H, 3.70; N, 6.44; O, 7.35. Found: C, 66.21; H, 3.66; N, 6.44; O, 7.31%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-4,5-dihydro-5-(4-methoxyphenyl)pyrazol-1-yl)methanone (SBF-13)*:**

Yield: 68%, IR (KBr, cm^{-1}): 3052 (Ar. -C-H stretching), 2921 (Aliphatic -CH_2-

stretching), 1691 (-C=O stretching), 1646 (-C=N of pyrazole ring), 1547 (Ar. -C-C- stretching), 1181 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 968 (Ar. -C-H i.p. deformation), 748 (-C-Cl stretching); MS *m/z* = 431 (M^+), 433 (M^{+2}); Anal. Calcd. for $C_{25}H_{19}ClN_2O_3$: C, 69.69; H, 4.44; N, 6.50; O, 11.14. Found: C, 69.65; H, 4.46; N, 6.44; O, 11.12%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl)methanone (SBF-14)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3050 (Ar. -C-H stretching), 2930 (Aliphatic -CH_2 stretching), 1688 (-C=O stretching), 1640 (-C=N of pyrazole ring), 1546 (Ar. -C-C- stretching), 1181 (-C-N of pyrazole ring), 1115 (-C-O-C- furan ring), 963 (Ar. -C-H i.p. deformation), 745 (-C-Cl stretching); MS *m/z* = 446 (M^+), 448 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}ClN_3O_4$: C, 64.65; H, 3.62; N, 9.42; O, 14.35. Found: C, 64.62; H, 3.60; N, 9.40; O, 14.31%.

***(Benzofuran-2-yl)(5-(2-chlorophenyl)-3-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl)methanone (SBF-15)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3064 (Ar. -C-H stretching), 2926 (Aliphatic -CH_2 str.), 1696 (-C=O stretching), 1641 (-C=N of pyrazole ring), 1540 (Ar. -C-C- stretching), 1186 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 957 (Ar. -C-H i.p. deformation), 748 (-C-Cl stretching); MS *m/z* = 435 (M^+), 437 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}Cl_2N_2O_2$: C, 66.22; H, 3.70; N, 6.44; O, 7.35. Found: C, 66.20; H, 3.71; N, 6.43; O, 7.30%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-4,5-dihydro-5-(3,4-dimethoxyphenyl)pyrazol-1-yl) methanone (SBF-16)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3063 (Ar. -C-H stretching), 2928 (Aliphatic -CH_2 stretching), 1694 (-C=O stretching), 1638 (-C=N of pyrazole ring), 1541 (Ar. -C-C- stretching), 1182 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 958 (Ar. -C-H i.p. deformation), 742 (-C-Cl stretching); MS *m/z* = 461 (M^+), 463 (M^{+2}); Anal. Calcd. for $C_{26}H_{21}ClN_2O_4$: C, 67.75; H, 4.59; N, 6.08; O, 13.89. Found: C, 67.71; H, 4.55; N, 6.09; O, 13.80%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-4,5-dihydro-5-p-tolylpyrazol-1-yl)methanone (SBF-17)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3061 (Ar. -C-H stretching), 2925 (Aliphatic -CH_2 stretching), 1696 (-C=O stretching), 1648 (-C=N of pyrazole ring), 1541 (Ar. -C-C- stretching), 1180 (-C-N of pyrazole ring), 1111 (-C-O-C- furan ring), 967 (Ar. -C-H i.p. deformation), 750 (-C-Cl stretching); MS *m/z* = 415 (M^+), 417 (M^{+2}); Anal. Calcd. for $C_{25}H_{19}ClN_2O_2$: C, 72.37; H, 4.62; N, 6.75; O, 7.71. Found: C, 72.32; H, 4.60; N, 6.70; O, 7.70%.

***(Benzofuran-2-yl)(3-(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydropyrazol-1-yl) methanone (SBF-18)*:**

Yield: 68%, IR (KBr, cm^{-1}): 3048 (Ar. -C-H stretching), 2927 (Aliphatic -CH_2 stretching), 1695 (-C=O stretching), 1650 (-C=N of pyrazole ring), 1541 (Ar. -C-C- stretching), 1190 (-C-N of pyrazole ring), 1115 (-C-O-C- furan ring), 968 (Ar. -C-H i.p. deformation), 746 (-C-Cl stretching), 1020 (-C-F stretching); MS *m/z* = 419 (M^+), 421 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}ClFN_2O_2$: C, 68.82; H, 3.85; N, 6.69; O, 7.64. Found: C, 68.80; H, 3.81; N, 6.62; O, 7.61%.

***(Benzofuran-2-yl)(3-(4-fluorophenyl)-4,5-dihydro-5-phenylpyrazol-1-yl)methanone (SBF-21)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3051 (Ar. -C-H stretching), 2927 (Aliphatic -CH_2 stretching), 1694 (-C=O stretching), 1648 (-C=N of pyrazole ring), 1536 (Ar. -C-C- stretching), 1186 (-C-N of pyrazole ring), 1109 (-C-O-C- furan ring), 955 (Ar. -C-H i.p. deformation), 1018 (-C-F stretching); MS *m/z* = 384 (M^+), 386 (M^{+2}); Anal. Calcd. for $C_{24}H_{17}FN_2O_2$: C, 74.99; H, 4.46; N, 7.29; O, 8.32. Found: C, 74.96; H, 4.40; N, 7.25; O, 8.30%.

***(Benzofuran-2-yl)(5-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydropyrazol-1-yl) methanone (SBF-22)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3036 (Ar. -C-H stretching), 2922 (Aliphatic -CH_2 stretching), 1686 (-C=O stretching), 1643 (-C=N of pyrazole ring), 1548 (Ar. -C-C- stretching), 1182 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 954 (Ar. -C-H i.p. deformation), 740 (-C-Cl stretching), 1020 (-C-F stretching); MS *m/z* = 419 (M^+), 421(M^{+2}); Anal. Calcd. for $C_{24}H_{16}ClFN_2O_2$: C, 68.82; H, 3.85; N, 6.69; O, 7.64.

Found: C, 68.80; H, 3.81; N, 6.65; O, 7.60%.

***(Benzofuran-2-yl)(3-(4-fluorophenyl)-4,5-dihydro-5-(4-methoxyphenyl)pyrazol-1-yl) methanone (SBF-23)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3050 (Ar. -C-H stretching), 2918 (Aliphatic -CH_2 stretching), 1696 (-C=O stretching), 1644 (-C=N of pyrazole ring), 1546 (Ar. -C-C- stretching), 1196 (-C-N of pyrazole ring), 1115 (-C-O-C- furan ring), 964 (Ar. -C-H i.p. deformation), 1020 (-C-F stretching); MS *m/z* = 414 (M^+), 416 (M^{+2}); Anal. Calcd. for $C_{25}H_{19}FN_2O_3$: C, 72.45; H, 4.62; N, 6.76; O, 11.58. Found: C, 72.42; H, 4.60; N, 6.70; O, 11.51%.

***(Benzofuran-2-yl)(3-(4-fluorophenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl) methanone (SBF-24)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3063 (Ar. -C-H stretching), 2921 (Aliphatic -CH_2 stretching), 1694 (-C=O stretching), 1641 (-C=N of pyrazole ring), 1547 (Ar. -C-C- stretching), 1190 (-C-N of pyrazole ring), 1114 (-C-O-C- furan ring), 964 (Ar. -C-H i.p. deformation), 1028 (-C-F stretching); MS *m/z* = 429 (M^+), 431 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}FN_3O_4$: C, 67.13; H, 3.76; N, 9.79; O, 14.90. Found: C, 67.10; H, 3.71; N, 9.77; O, 14.88%.

***(Benzofuran-2-yl)(5-(2-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydropyrazol-1-yl) methanone (SBF-25)*:**

Yield: 66%, IR (KBr, cm^{-1}): 3048 (Ar. -C-H stretching), 2926 (Aliphatic -CH_2 str.), 1697 (-C=O stretching), 1641 (-C=N of pyrazole ring), 1541 (Ar. -C-C- stretching), 1188 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 960 (Ar. -C-H i.p. def.), 744 (-C-Cl stretching), 1022 (-C-F stretching); MS *m/z* = 419 (M^+), 421 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}ClFN_2O_2$: C, 68.82; H, 3.85; N, 6.69; O, 7.64. Found: C, 68.80; H, 3.81; N, 6.66; O, 7.61%.

***(Benzofuran-2-yl)(3-(4-fluorophenyl)-4,5-dihydro-5-(3,4-dimethoxyphenyl)pyrazol-1-yl) methanone (SBF-26)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3038 (Ar -C-H stretching), 2938 (Aliphatic -CH_2 - stretching), 1690 (-C=O stretching), 1655 (-C=N of pyrazole ring), 1544(Ar. -C-C- stretching), 1184 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 960 (Ar. -C-H

i.p. deformation); 1021 (-C-F stretching); MS *m/z* = 444 (M^+), 446 (M^{+2}); Anal. Calcd. for $C_{26}H_{21}FN_2O_4$: C, 70.26; H, 4.76; N, 6.30; O, 14.40. Found: C, 70.22; H, 4.70; N, 6.31; O, 14.38%.

***(Benzofuran-2-yl)(3-(4-fluorophenyl)-4,5-dihydro-5-p-tolylpyrazol-1-yl)methanone (SBF-27)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3044 (Ar -C-H stretching), 2936 (Aliphatic -CH_2 stretching), 1689 (-C=O stretching), 1651 (-C=N of pyrazole ring), 1548 (Ar. -C-C- stretching), 1180 (-C-N of pyrazole ring), 1112 (-C-O-C- furan ring), 966 (Ar. -C-H i.p. deformation), 1024(-C-F stretching); MS *m/z* = 398 (M^+), 400 (M^{+2}); Anal. Calcd. for $C_{25}H_{19}FN_2O_2$: C, 75.36; H, 4.81; N, 7.03; O, 8.03. Found: C, 75.30; H, 4.81; N, 7.00; O, 8.01%.

***(3,5-bis(4-fluorophenyl)-4,5-dihydropyrazol-1-yl)(Benzofuran-2-yl)methanone (SBF-28)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3042 (Ar -C-H stretching), 2935 (Aliphatic -CH_2 str.), 1696 (-C=O stretching), 1656 (-C=N of pyrazole ring), 1542(Ar. -C-C- stretching), 1180 (-C-N of pyrazole ring), 1115 (-C-O-C- furan ring), 966 (Ar. -C-H i.p. deformation), 1022 (-C-F stretching); MS *m/z* = 402 (M^+), 404 (M^{+2}); Anal. Calcd. for $C_{24}H_{16}F_2N_2O_2$: C, 71.64; H, 4.01; N, 6.96; O, 7.95. Found: C, 71.60; H, 4.01; N, 6.92; O, 7.90%.

2.10 RESULTS AND DISCUSSION

Most of the methods for the synthesis of pyrazoles involve approaches based on either cyclocondensation of 1,3-dicarbonyl compounds and their equivalent 1,3-dienophilic synthons such as propargylic ketones, dialkylamino/alkoxy/chloro ketones with arylhydrazines or intermolecular cycloadditions of 1,3-dipoles to alkynes.

In this chapter, known methods were adopted. for the preparation of benzofuran-2-yl (4, 5- dihydro-3, 5-substituted diphenylpyrazol-1- yl) methanone from Benzofuran hydrazide and substituted chalcones by using glacial acetic acid.

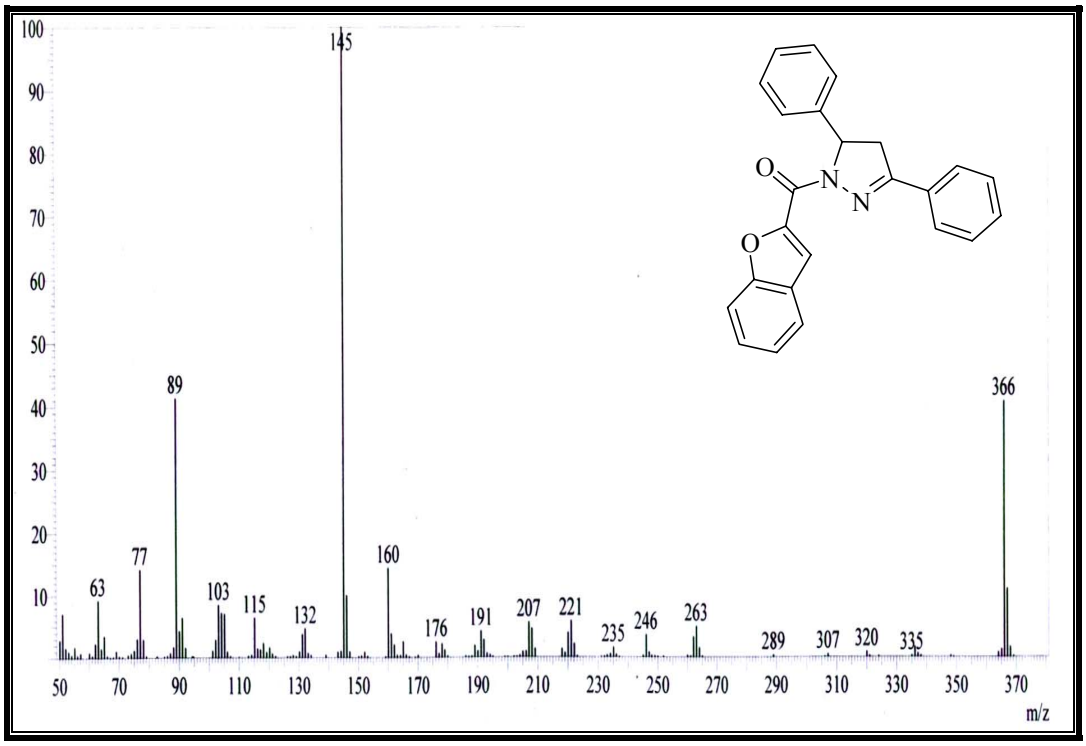
2.11 CONCLUSION

Pyrazoles belong to an important class of heterocycles due to their biological and pharmacological activities[49,50] such as anti-inflammatory,[51] herbicidal,[52] fungicidal,[53] bactericidal,[53] plant growth inhibitory,[52] antipyretic,[54] and protein kinase inhibitory activities.[55] Also, they are the key starting materials for the synthesis of commercial aryl/ hetero-arylazopyrazolone dyes.[56,57]

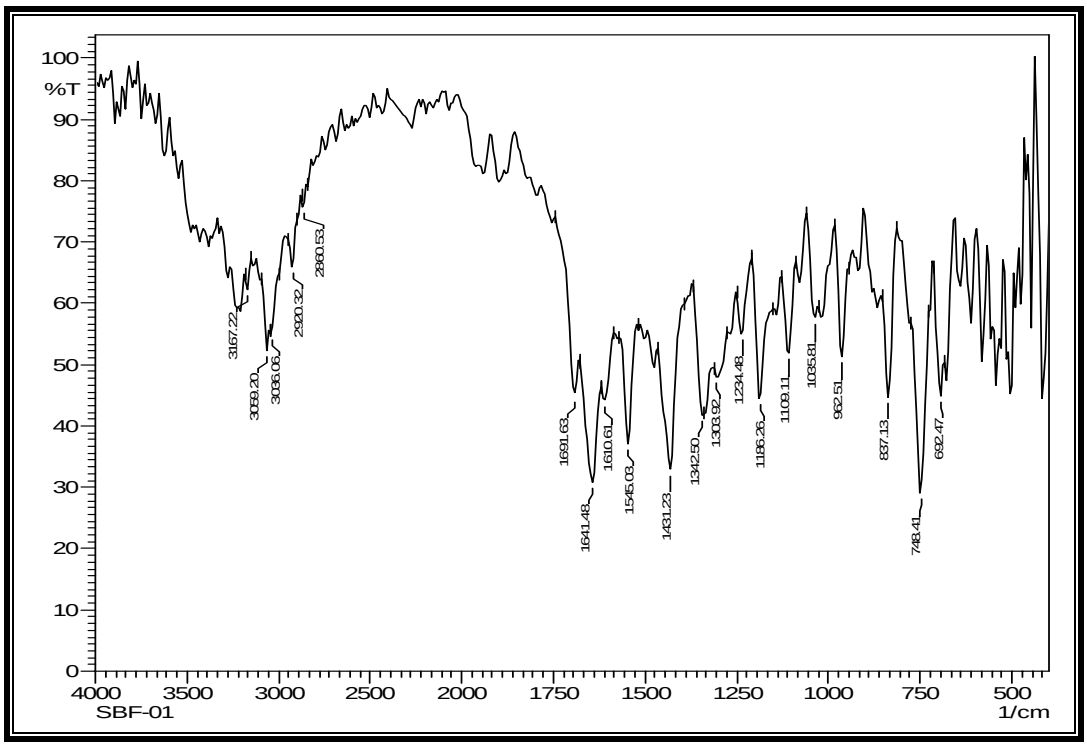
This chapter involves some interesting chemical aspects as far as organic chemical synthesis is concerned. The New chemical entities synthesized in this chapter were screened for MDR (Multidrug Resistance) study. The results of which are discussed separately in Chapter-7.

2.12 REPRESENTATIVE SPECTRA

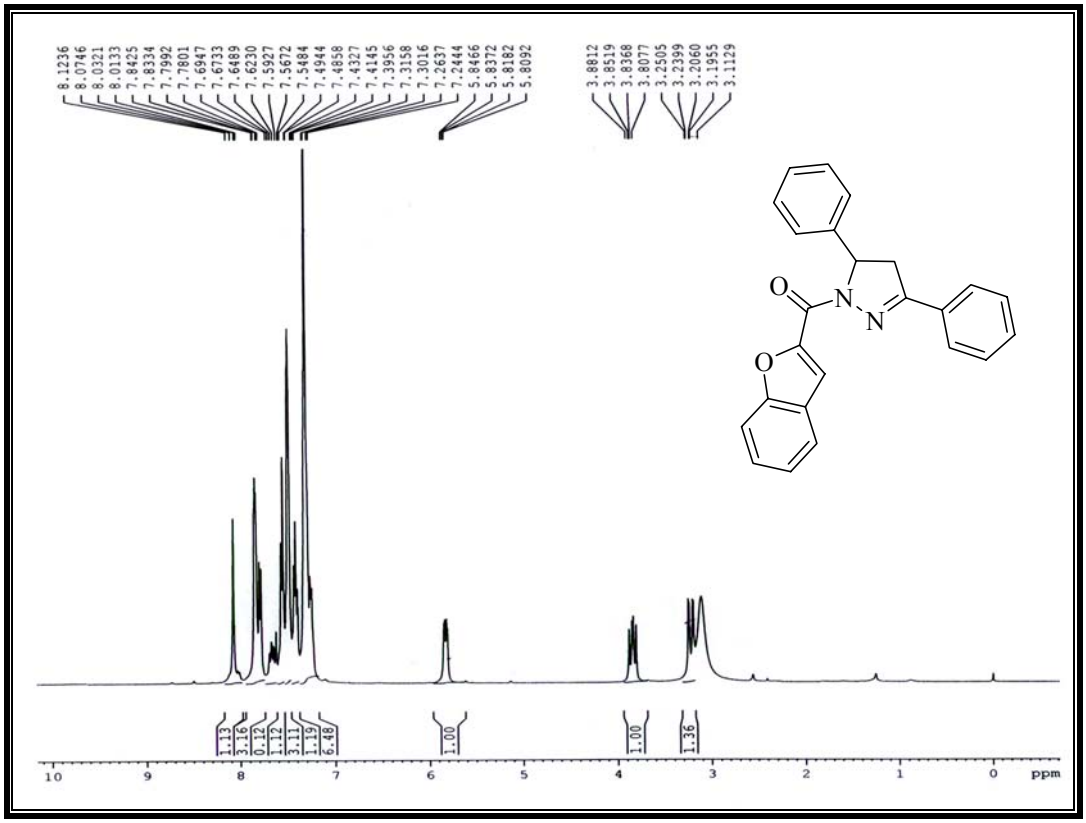
2.12.1 Mass Spectrum of SBF-01



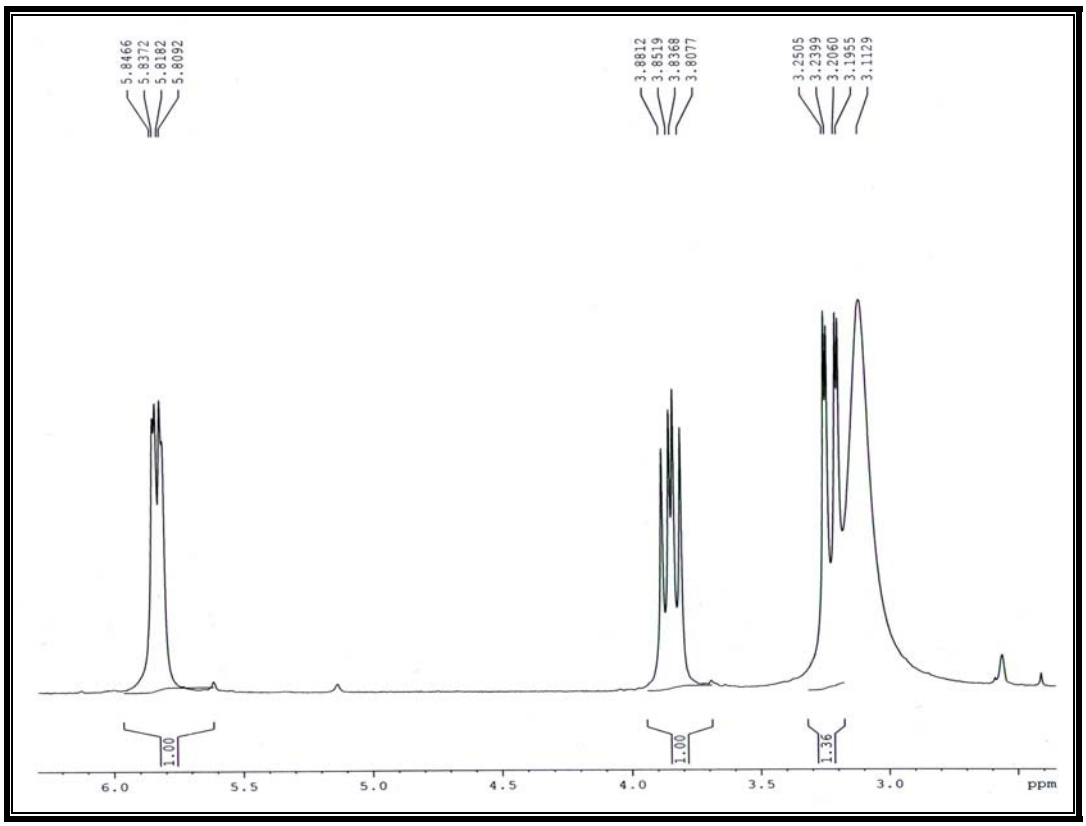
2.12.2 IR Spectrum of SBF-01



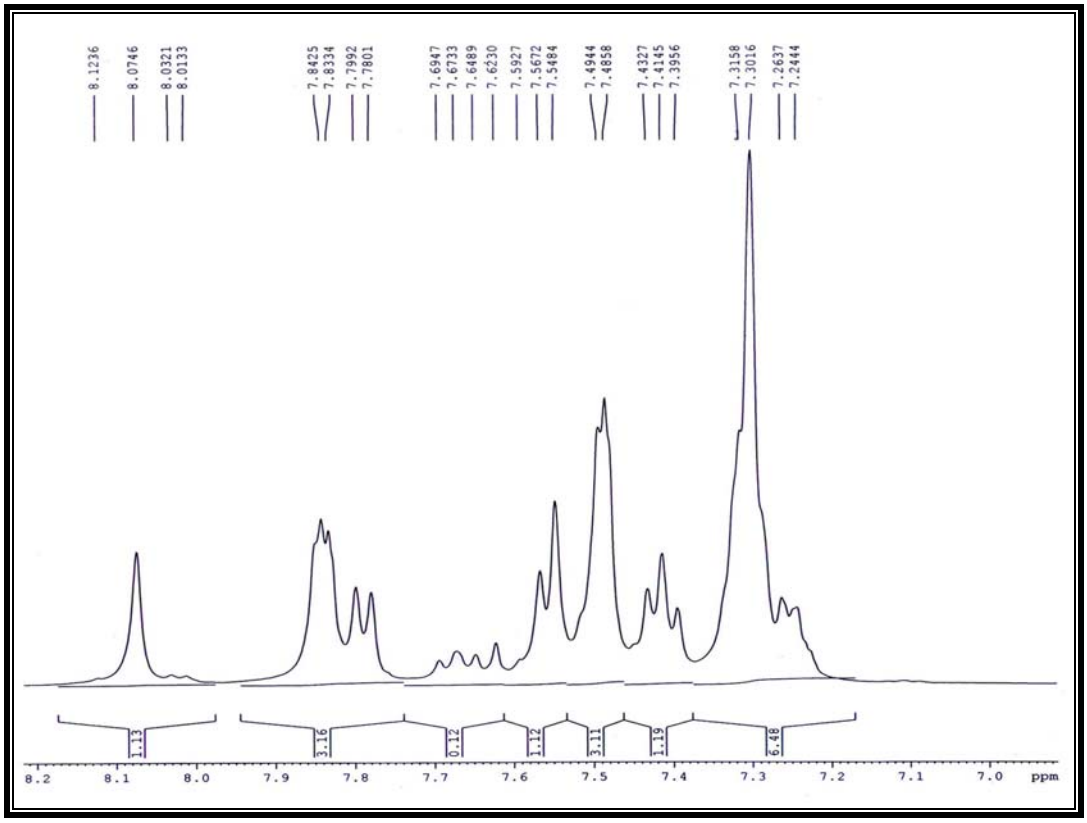
2.12.3 ^{1}H NMR Spectrum of SBF-01



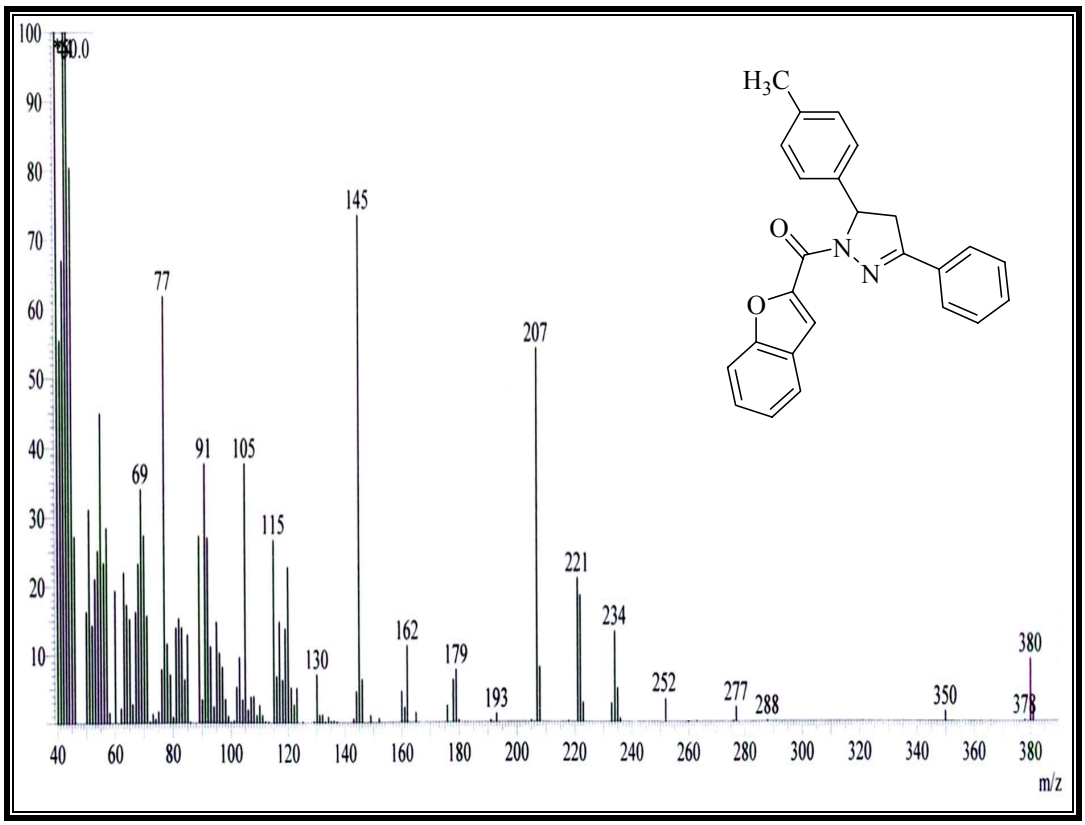
Expanded ^{1}H-NMR Spectrum of SBF-01



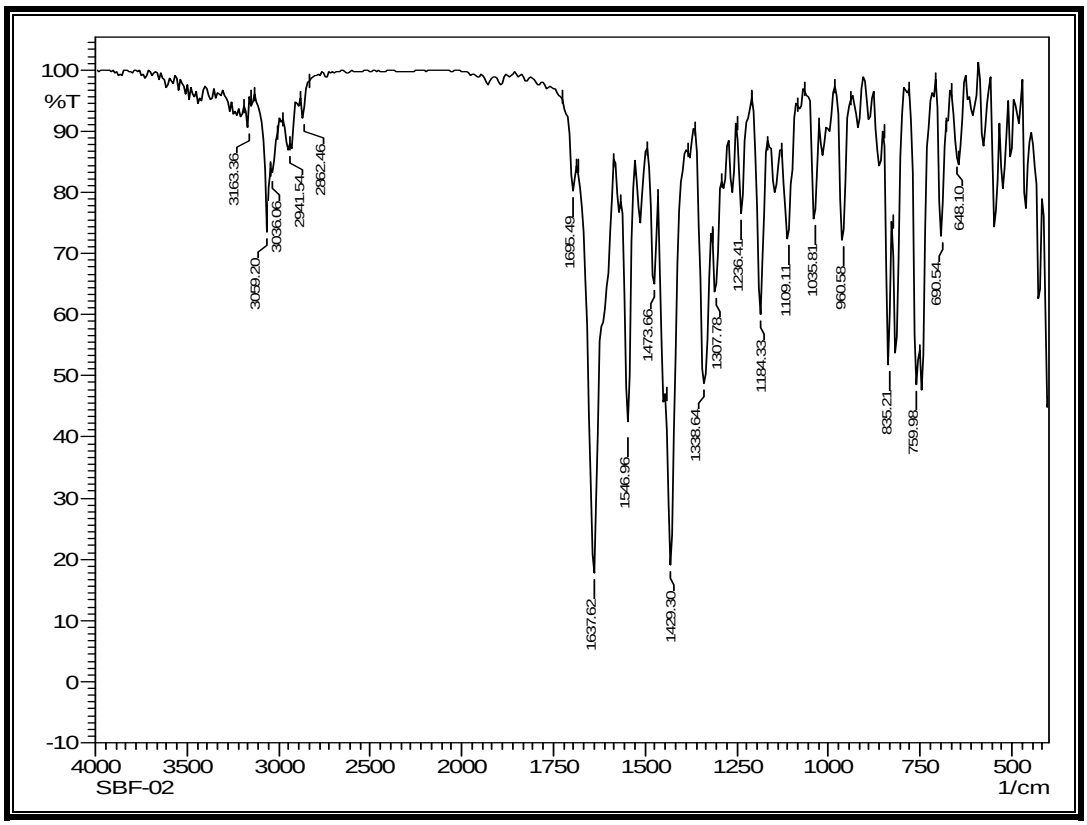
Expanded ^{1}H-NMR Spectrum of SBF-01



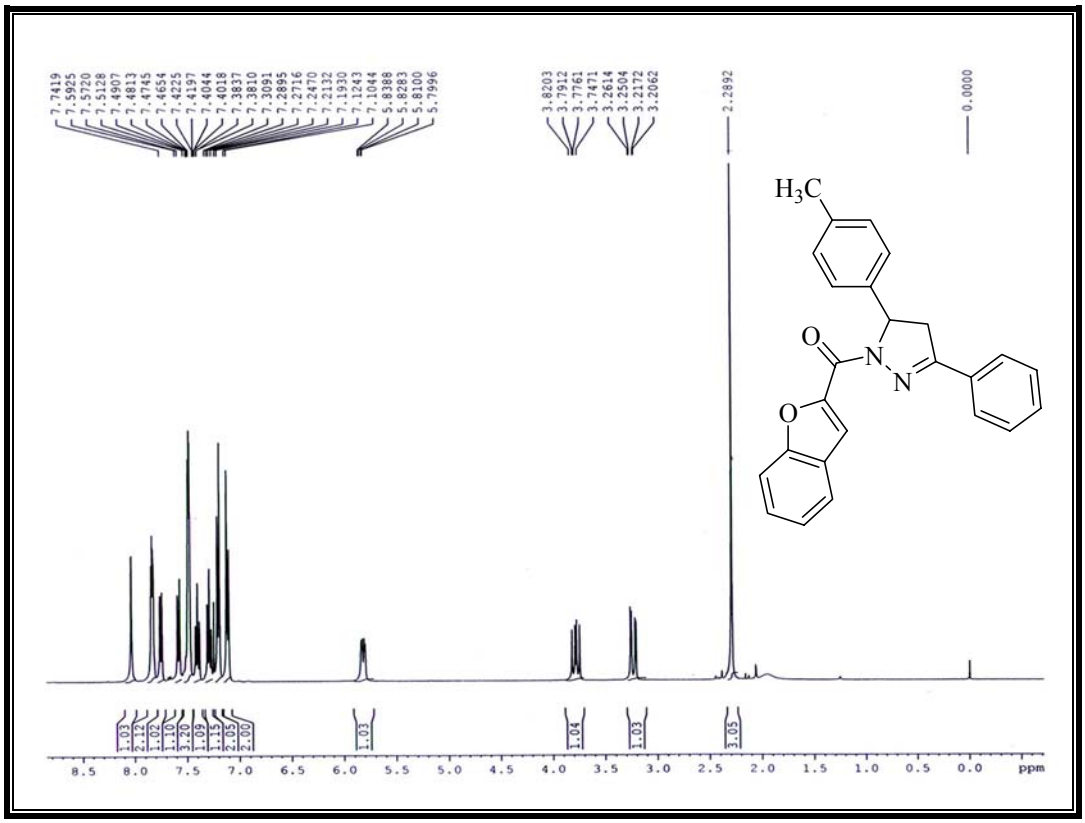
2.12.4 Mass Spectrum of SBF-02



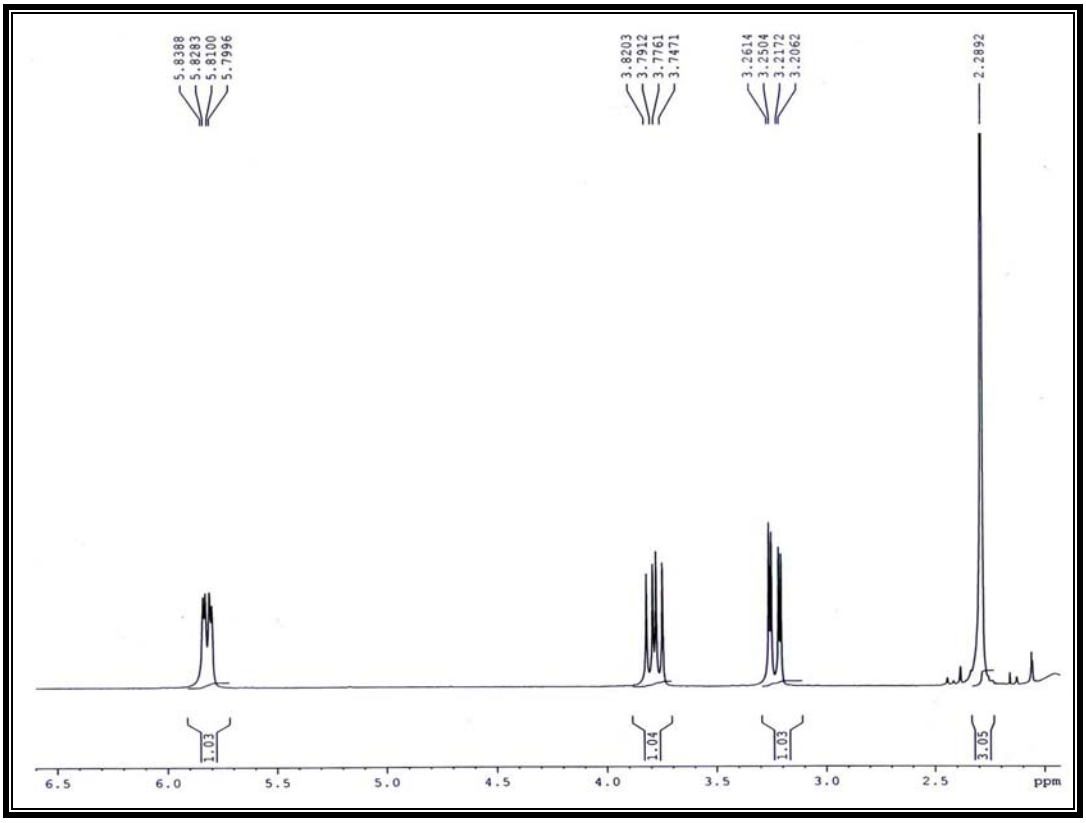
2.12.5 IR Spectrum of SBF-02



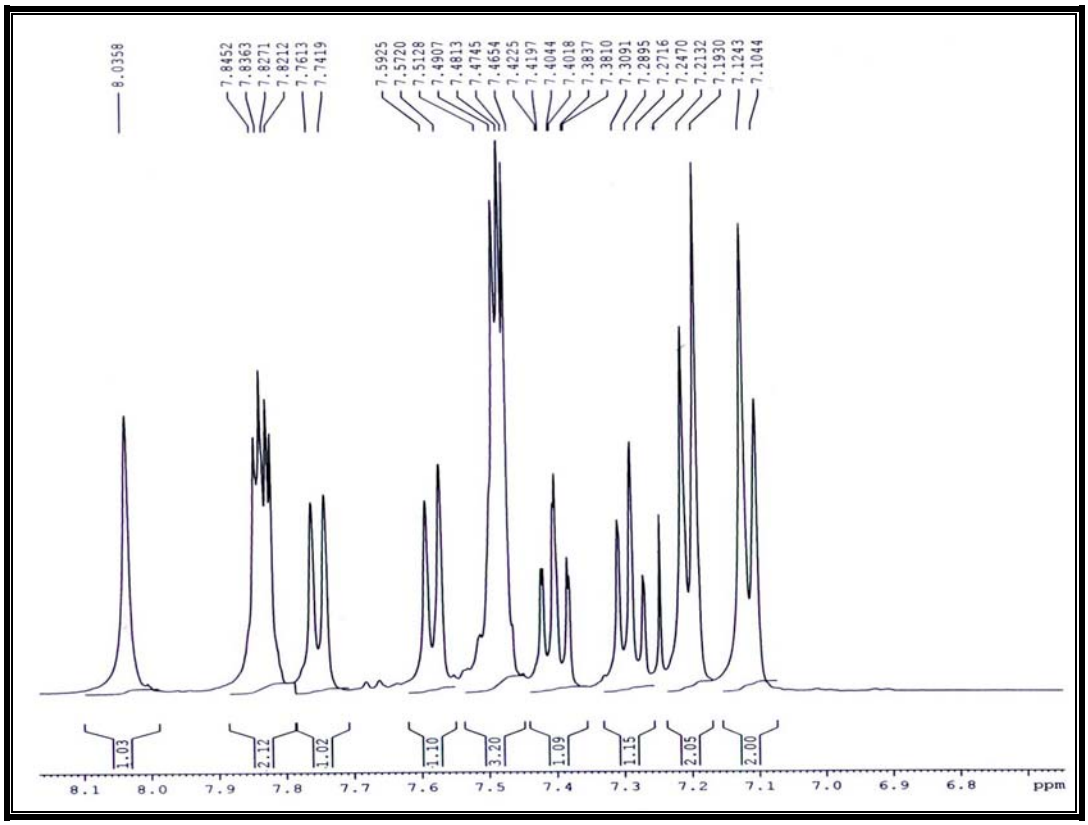
2.12.6 ^{1}H NMR Spectrum of SBF-02



Expanded ^{1}H-NMR Spectrum of SBF-02



Expanded ^{1}H-NMR Spectrum of SBF-02



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(o) Mehra, H. S.; Journal of the Indian Chemical Society **1968**, V45(2), P178-81.

(p) Qiu, Xiao Yang; Acta Crystallographica, Section E: Structure Reports Online **2006**, VE62(8), Po3525-o3526.

(q) Lyle, Robert E.; Journal of the American Chemical Society **1955**, V77, P6667-8.

(r) Kozlov, N. S.; Katalit. Sintez Organ. Soedinenii 1976, P8-12.

(s) Kochetkov, N. K.; Zhurnal Obshchei Khimii **1960**, V30, P1495-7.

(t) Bansal, R. K.; Journal of the Indian Chemical Society **1988**, V65(2), P134-6.

(u) Joshi, Krishna C.; Journal of the Indian Chemical Society **1988**, V65(11), P773-7.

(v) Bansal, R. K.; Journal fuer Praktische Chemie (Leipzig) **1981**, V323(1), P49-54.

(w) Cacchi, Sandro; Synthesis **1978**, 11, P848-9.

(x) Comisar, C. M. and Savage, P. E. Green Chem., **2004**, 6, 227 - 231.

CHAPTER - 3

INTRODUCTION
OF
VALPROIC ACID

3.1 INTRODUCTION

Valproic acid is simple branched chain carboxylic acid used in epilepsy. Valproic acid increases GABA (γ-amino butyric acid) synthesis and release and potentiates by this mechanism GABA ergic transmission in specific brain regions. Valproic acid also reduces the release of excitatory amino acid β-hydroxy butyric acid and to attenuate neuronal excitation mediated by activation of N-methyl-D-aspartames glutamate receptors. Valproic acid is a broad-spectrum antiepileptic drug effective against all seizure types.

Valproic acid (by its official name 2-propylvaleric acid) was first synthesized in 1882 by Burton as an analogue of valeric acid, found naturally in valerian. A clear liquid fatty acid at room temperature, for many decades its only use was in laboratories as a "metabolically inert" solvent for organic compounds. In 1962, the French researcher Pierre Eymard serendipitously discovered the anticonvulsant properties of Valproic acid while using it as a vehicle for a number of other compounds that were being screened for anti-seizure activity. He found that it prevented pentylenetetrazol-induced convulsions in rodents. Since then it has also been used for migraine and bipolar disorder.

3.2 VALPROATE: PAST, PRESENT, AND FUTURE

Preclinical studies have been carried out during the past four decades to investigate the different mechanisms of action of valproate (VPA). The mechanisms of VPA which seem to be of clinical importance include increased GABA ergic activity, reduction in excitatory neurotransmission, and modification of monoamines. These mechanisms are discussed in relation to the various clinical uses of the drug. VPA is widely used as an antiepileptic drug with a broad spectrum of activity. In patients, VPA possesses efficacy in the treatment of various epileptic seizures such as absence, myoclonic, and generalized tonic-clonic seizures. It is also effective in the treatment of partial seizures with or without secondary generalization and acutely in status epileptics. The pharmacokinetic aspects of VPA and the frequent drug interactions between VPA and other drugs are discussed. The available methods for

the determination of VPA in body fluids are briefly evaluated. At present, investigations and clinical trials are carried out and evaluated to explore the new indications for VPA in other conditions such as in psychiatric disorders, migraine and neuropathic pain. Furthermore, the toxicity of VPA, both regarding commonly occurring side effects and potential idiosyncratic reactions are described. Derivatives of VPA with improved efficacy and tolerability are in development.

3.3 CHEMICAL STRUCTURE OF VALPROIC ACID THEIR DERIVATIVES

Valproic acid Molecular formula is $C_8H_{16}O_2$ and molecular weight is 144.2 and other derivatives of Valproic acid are Sodium Valproate molecular formula is $C_8H_{15}NaO_2$ and molecular weight is 166.2, Semi sodium Valproate molecular formula is $C_{16}H_{31}NaO_4$ and molecular weight is 310.4, Valproate Pivoxil molecular formula is $C_{14}H_{26}O_4$ and molecular weight is 258.4, Valpromide molecular formula is $C_8H_{17}NO$ and molecular weight is 143.2.

Chemical names of Valproic acid:

2-Propylpentanoic acid, 2-Propylvaleric acid, Di-n-dipropylacetic acid

Chemical names of Sodium Valproate:

Sodium 2-propylvalerate, Sodium 2-propylpentanoate

Chemical names of Semi sodium Valproate:

2-Propylvaleric acid-sodium 2-propylvalerate, Sodium hydrogen bis(2-propylvalerate)

Chemical names of Valproate Pivoxil:

Hydroxymethyl 2-propylvalerate pivalate

Chemical names of Valpromide:

Dipropylacetamide, 2-propylvaleramide

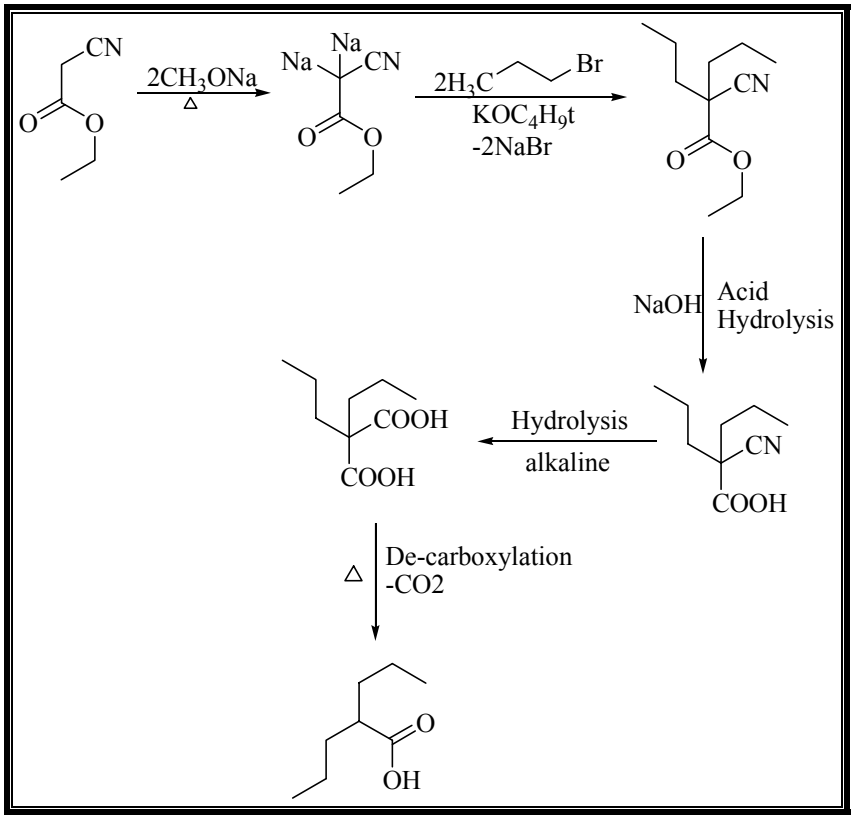
3.4 PHYSICAL PROPERTIES OF VALPROIC ACID

Valproic acid is Colorless to pale yellow viscous liquid. It is It is slightly soluble in water (1.2 mg/mL); fully soluble in acetone, chloroform, ether and methyl alcohol. Valproic acid is Store in airtight containers and protect from light. Valproic acid capsules should be stored at 15 to 30°C and freezing should be avoided.

3.5 USES OF VALPROIC ACID

Valproic acid is used solely or in combination with other anticonvulsants in the treatment of simple (petit mal) and complex absence seizures. Valproate may be effective against myoclonic and atonics seizures in young children and considered.

3.6 SYNTHESIS OF VALPROIC ACID



3.7 ACTION ON MECHANISM OF VALPROIC ACID

VALPROIC ACID (VPA) is indicated for the treatment of epilepsy and bipolar disorder and in the prevention of migraine headaches. VPA has also become more widely prescribed due to several off-label indications such as in the treatment of neuropathic pain and cancer.[1,2] Despite VPA being well tolerated and having a low incidence of serious side effects, one concern with VPA therapy is weight gain. A prospective study identified that 37% of female patients with epilepsy developed obesity, as defined as a body mass index (BMI) greater than 25, after 1 yr of treatment with VPA.[3] Numerous retrospective and cross-sectional analyses also report that treatment with VPA is associated with a significant increase in weight ranging from 5 to 49 kg.[4, 5, 6, 7] Studies examining VPA-induced weight gain have been conducted predominantly in adult women because VPA can induce a number of reproductive endocrine abnormalities that include hyperandrogenism, menstrual disturbances, weight gain, and/or polycystic ovaries.[8, 9,10] Fifty-two percent of males treated with VPA, however, also have BMI scores within the obesity category,[11] and youth and adolescents treated with VPA are reported to have BMI scores over expected age norms.[12,13,14] Similarly, a prospective double-blind comparison of the incidence and magnitude of weight gain in patients receiving VPA *vs.* lamotrigine monotherapy demonstrated that weight gain was greater for those patients treated with VPA and was significant within 10 wk of treatment onset.[15,16] Weight gain associated with VPA treatment is of great concern due to its physical and psychological consequences.[17] Notably, obesity leads to increase risk for numerous other diseases, such as diabetes mellitus, coronary heart disease,[18] and increased noncompliance with pharmacotherapy in psychiatric patients.[19]

The mechanism underlying VPA-induced weight gain has not been elucidated. Age, gender, medical condition, dose and serum concentrations of VPA and family history of body weight problems are not significantly correlated with the gain in weight associated with VPA treatment.[20, 21] In attempts to generate animal models of VPA-induced weight gain, VPA has been shown to induce a significant increase in body weight in female rhesus monkeys [22]; however, we and others have demonstrated that VPA does not cause weight gain in rodents.[23, 24, 25] The etiology of VPA-induced

weight gain is most likely multifactorial because weight is the output of energy homeostasis controlled by many organs that produce and secrete a variety of appetite-regulating peptides and cytokines that act within the hypothalamus.[26] VPA treatment in humans increases the serum level of two hormones, leptin and insulin, which are produced by the adipose tissue and pancreatic ß-cells, respectively. After VPA treatment for 1 yr, 37% of female patients with epilepsy who developed obesity had a 1.8-fold increase in fasting serum insulin and 3.4-fold increase in serum leptin levels. Similarly, in women receiving VPA for treatment of bipolar disorder, insulin and leptin levels were significantly elevated when compared with women receiving lithium.[27] High levels of serum leptin are commonly associated with obesity and could represent a state of leptin resistance.[26, 28] the increase in serum leptin associated with weight gain after VPA treatment may be a consequence of the increase in adipose tissue; however, it is also possible that VPA may have a direct effect on leptin secretion from adiposities or may alter leptin signaling and decrease negative feedback. VPA has been shown to have direct effects on hormone secretion from other endocrine cells. For example, an *ex vivo* study using human pancreatic islet cells has shown that VPA can directly increase insulin release.[29] Moreover, VPA can also potentiate androgen production from ovarian theca cells.[30] We previously demonstrated that VPA inhibited mouse 3T3-L1 and human preadipocyte differentiation.[31] Treatment with VPA during adiposeness reduced the protein levels for several key adiposity-specific transcription factors, including CCAAT/enhancer binding protein (C/EBP)-α, peroxisome proliferators-activated receptor (PPAR)-γ, and steroid regulatory element binding protein (SREBP) 1a.[32] The present work demonstrates that treatment with VPA in mature adiposities significantly reduces leptin mRNA levels and secretion of the leptin protein in a dose- and time-dependent manner. These findings were paradoxical because treatment of patients with VPA is associated with increased serum leptin levels. The reduction in leptin secretion from adiposities was not accompanied by alterations in glucose uptake or altered intracellular free fatty acid levels, which are known regulators of leptin secretion. In addition, C/EBPα, PPARγ, or SREBP1a protein levels did not change with VPA treatment, suggesting the levels of these transcription factors are not responsible for the effect of VPA on leptin expression. Evidence from experiments using actinomycin D (Act D) or cyclohexamide (CHX) show that VPA does not promote degradation of leptin mRNA; however, VPA can alter leptin transcription through an unknown

mechanism independent of new protein synthesis. These results show that VPA can have direct effects on adiposities that may contribute to altered energy balance in patients treated with VPA.

3.8 ANTI-CANCER ACTIVITY OF VALPROIC ACID

The short chain fatty acid valproic acid (VPA, 2-propylpetanoic acid) is approved for the treatment of epilepsia, bipolar disorders and migraine and clinically used for schizophrenia. In 1999, the first clinical anti-cancer trial using VPA was initiated. Currently, VPA is examined in numerous clinical trials for different leukemia's and solid tumor entities. In addition to clinical assessment, the experimental examination of VPA as anti-cancer drug is ongoing and many questions remain unanswered. Although other mechanisms may also contribute to VPA-induced anti-cancer effects, inhibition of histone deacetylase appears to play a central role. This review focuses on recent developments regarding the anti-cancer activity of VPA.

CHAPTER - 3

SECTION - A

SYNTHESIS OF
N-(2-(SUBSTITUTED AMINO)
-ACETYL)-2-
PROPYL PENTANEHYDRAZIDE

3.A.1 AIM OF CURRENT WORK

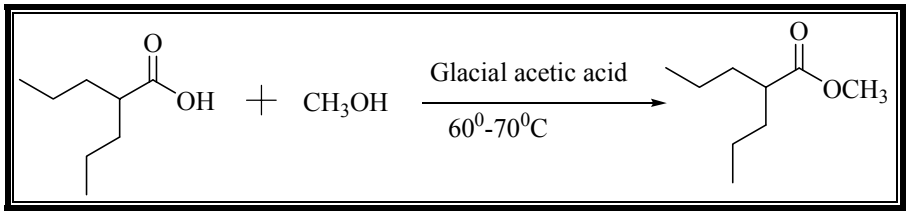
Over and above, the known antiepileptic properties of Valproic acids and its salt, renewed interest of these molecules in anticancer[a] and antiviral[b] therapy has led wide interest in newer derivatives of these molecules.

In the current chapter, the Valproic 'Core' structure was used as a starting material to arrive at many molecules with different pharmacophores which obeys Lipinski's rule.

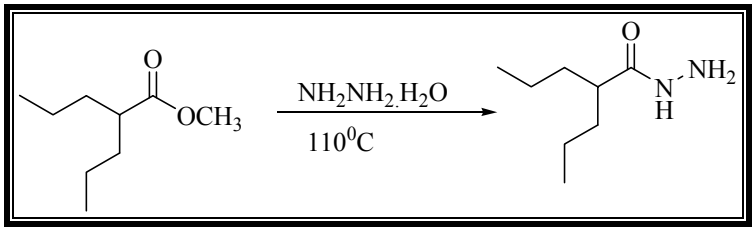
[a] Current Pharmaceutical Design, Volume 13, Number 33, November 2007, pp. 3378-3393(16).
[b] Clin Pharmacokinet. 1996 May;30(5):385-401.

3.A.2 REACTION SCHEMES

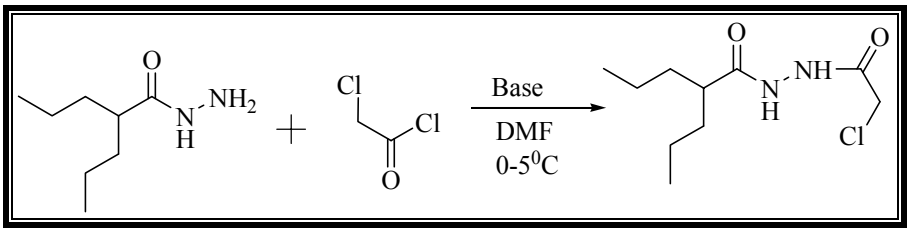
3.A.2.1 PREPARATION OF METHYL-2-PROPYL PENTANOATE



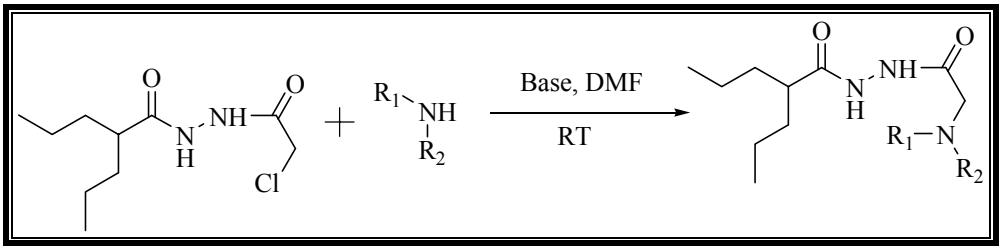
3.A.2.2 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE



3.A.2.3 PREPARATION OF *N'*-(2-CHLOROACETYL)-2-PROPYL PENTANEHYDRAZIDE

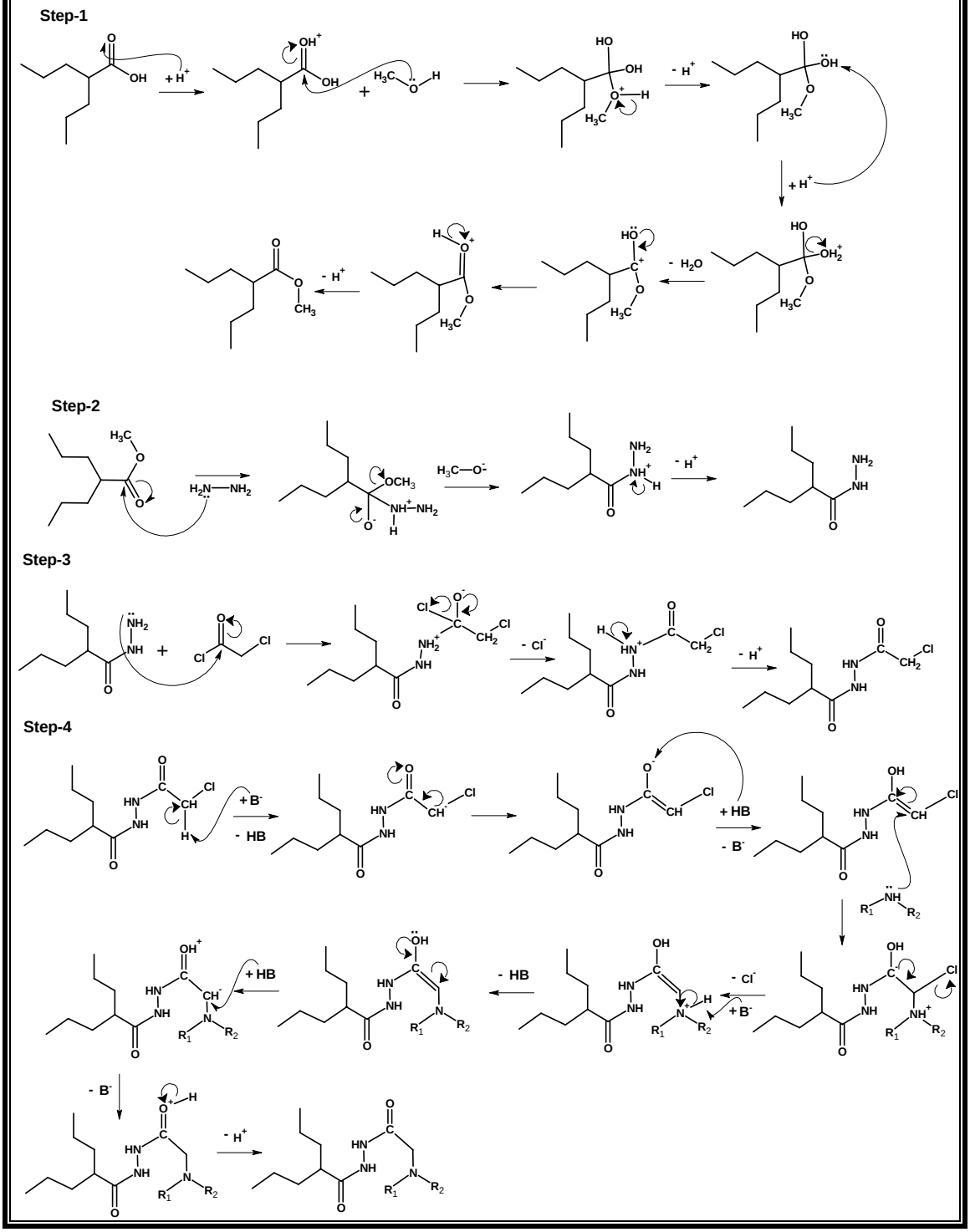


3.A.2.4 PREPARATION OF *N'*-(2-(SUBSTITUTED AMINO)-ACETYL)-2-PROPYL PENTANEHYDRAZIDE



3.A.3 PLAUSIBLE REACTION MECHANISM

SPSC Series



3.A.4 EXPERIMENTAL

3.A.4.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. Formation of all compounds was purified by using **flash chromatography**. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in $CDCl_3$ solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

3.A.4.2 PREPARATION OF METHYL 2-PROPYL PENTANOATE

2-propyl pentanoic acid (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. Sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 hours on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml × 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml × 2) followed by water (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 90 %, B. P. – 170-172°C.[c]

[c] Dostovalova, V. I. ; Organic Magnetic Resonance 1983, V21(1), P111-19.

3.A.4.3 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE

Methyl 2-propylpentanoate (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white colored shining fluffy product. Yield - 60 %, M. P. – 124–126°C[d].

3.A.4.4 PREPARATION OF *N*'-(2-CHLOROACETYL)-2-PROPYL PENTANEHYDRAZIDE

2-propylpentanohydrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of tetrahydrofuran was added to dissolve it. Then add 0.015 mole of triethylamine as a catalyst. And add into 0.01 mole of chloroacetyl chloride at 0-5^0 C. the reaction mixture was stirred at RT overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (4: 6) as a mobile phase. After the reaction is completed, mixture poured into crushed ice. *N*'-(2-chloroacetyl)-2-propylpentanehydrazide a brown colored solid product.

Similarly other compounds are also prepared.

3.A.4.5 PREPARATION OF *N*'-(2-(SUBSTITUTED AMINO)-ACETYL)-2-PROPYL PENTANE HYDRAZIDES

N'-(2-chloroacetyl)-2-propylpentanehydrazide (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of dimethylformamide was added to dissolve it. Then add in to 0.03 mole of potassium carbonate as a catalyst. And add into 0.01 mole of different secondary amines at RT. the reaction mixture was stirred at RT overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (3: 7) as a mobile phase.

[d] Benoit-Guyod, Jean L. ; Chemica Therapeutica 1968, V3(5), P336-42.

After the reaction to completed mixture poured into crushed ice, a solid product-comes. Finally, it was purified by Flash chromatography using hexane and ethyl-acetate as eluents.

Similarly other compounds are also prepared.

3.A.5 PHYSICAL DATA

PHYSICAL DATA OF *N*'-(2-(SUBSTITUTED AMINO)-ACETYL)-2-PROPYLPENTANE HYDRAZIDE (SPSC-01 TO SPSC-12)

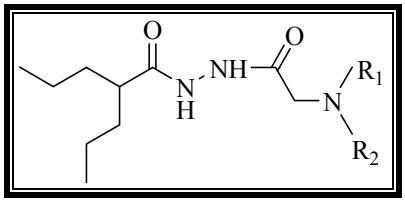
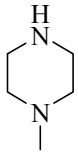
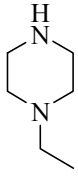
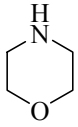
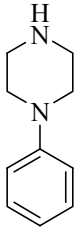
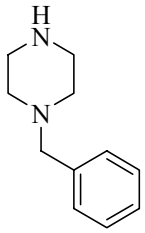
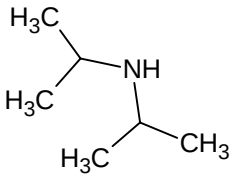
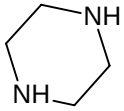
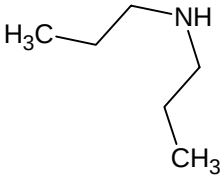
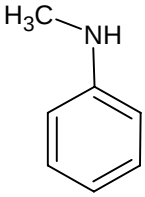
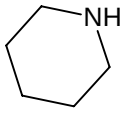
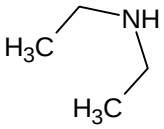
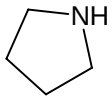


TABLE-3.A.1

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	M.W.	MP ^{0}C	R_f Value
01	SPSC-01		$C_{15}H_{30}N_4O_2$	298	160-162	0.52
02	SPSC-02		$C_{16}H_{32}N_4O_2$	312	178-180	0.48
03	SPSC-03		$C_{14}H_{27}N_3O_3$	285	204-206	0.42
04	SPSC-04		$C_{20}H_{32}N_4O_2$	360	168-170	0.50

05	SPSC-05		$C_{21}H_{34}N_4O_2$	375	172-174	0.44
06	SPSC-06		$C_{16}H_{33}N_3O_2$	299	174-176	0.42
07	SPSC-07		$C_{14}H_{28}N_4O_2$	284	150-152	0.46
08	SPSC-08		$C_{16}H_{33}N_3O_2$	299	166-168	0.52
09	SPSC-09		$C_{17}H_{27}N_3O_2$	305	170-172	0.38
10	SPSC-10		$C_{15}H_{29}N_3O_2$	283	168-170	0.42
11	SPSC-11		$C_{14}H_{29}N_3O_2$	271	178-180	0.44
12	SPSC-12		$C_{14}H_{27}N_3O_2$	269	184-186	0.46

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (3: 7)

3.A.6 SPECTRAL DISCUSSION

3.A.6.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. SPSC-01 and SPSC-02 of Mass spectra are given on page no. 83 and 85.

3.A.6.2 IR SPECTRA

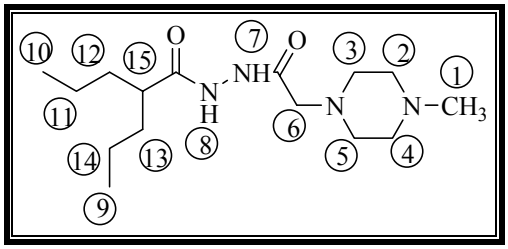
IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

-N-H stretching frequency was observed near 3400 cm^{-1} in case of SPSC-01 to SPSC-12. There are two carbonyl groups present in all the compounds but due to the same environment, the peaks are merged in the region of 1680 cm^{-1}. C-H stretching frequencies were observed between 2810 cm^{-1} and 3000 cm^{-1}, N-H bending frequency were observed near 1600 cm^{-1}. SPSC-01 and SPSC-02 of IR spectra are given on page no. 83 and 85.

3.A.6.3 ^{1}H NMR SPECTRA

^{1}H spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO-d_6/$CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from proton NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify *o, m* and *p* coupling. ^{1}H NMR spectral interpretation can be discussed as under.

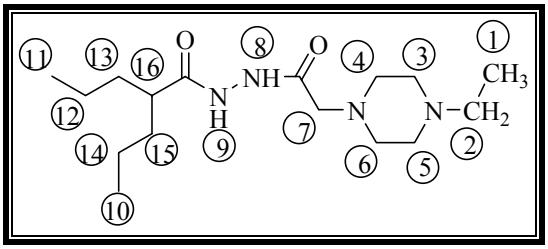
^{1}H NMR OF *N*'-(2-(4-METHYLPIPERAZIN-1-YL) ACETYL)-2-PROPYLPENTANEHYDRAZIDE (SPSC-01)



1. The Proton no.1 of methyl group gave a characteristic singlet at 2.30 δ ppm.
2. Another four protons of piprazine methylene groups of proton no. 2 and 3 gave multiplet at 2.47 δ ppm. Due to the nitrogen atmosphere, four protons of methylene groups flipped individually and peaks got merged.
3. Another four protons of piprazine methylene groups of proton no. 4 and 5 gave multiplet at 2.63 δ ppm. Due to the nitrogen atmosphere, four protons of methylene groups flipped individually and peaks got merged.
4. Two protons of methylene group of proton no.6 attached with N atom of piprazine ring gave a characteristic singlet at 3.14 δ ppm.
5. Proton no. 9 and 10 of proppyl chain of two methyl group of six protons gave a multiplet at 0.87 δ ppm - 0.91 δ ppm.
6. Proton no. 11, 12, 13 and 14 of dipropyl chain gave a multiplet at 1.25 δ ppm - 1.68 δ ppm. It showed expanded spectra.
7. Proton no. 15 of dipropyl chain gave a multiplet at 2.21 δ ppm-2.27 δ ppm.
8. Two most deshielded proton no.7 and 8 of secondary amine in hydrazide linkage of two (-NH) groups gave two separable singlet in the down field at 9.07 δ ppm and 9.53 δ ppm respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound SPSC-01 has been confirmed. Spectrum is given on page no. 84.

^{1}H NMR OF *N*'-(2-(4-ETHYLPIPERAZIN-1-YL) ACETYL)-2-PROPYLPENTANEHYDRAZIDE (SPSC-02)



1. The Proton no.1 of methyl group gave a Triplet at 1.05 δ ppm -1.08 δ ppm.
2. The Proton no. 2 of two protons gave a quartet at 2.32 δ ppm -2.44 δ ppm.
3. Another four protons of piprazine methylene groups of proton no. 3 and 4 gave multiplet at 2.59 δ ppm. Due to the nitrogen atmosphere, four protons of methylene groups flipped individually and peaks got merged.
4. Another four protons of piprazine methylene groups of proton no. 5 and 6 gave multiplet at 2.62 δ ppm. Due to the nitrogen atmosphere, four protons of methylene groups flipped individually and peaks got merged.
5. Two protons of methylene group of proton no.7 attached with N atom of piprazine ring gave a characteristic singlet at 3.08 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
6. Proton no. 10 and 11 of proppyl chain of two methyl group of six proton gave a multiplet at 0.85 δ ppm-0.90 δ ppm.
7. Proton no. 12, 13, 14 and 15 of dipropyl chain gave a multiplet at 1.25 δ ppm-1.61 δ ppm. it shown an expanded spectra.
8. Proton no. 16 of dipropyl chain of a proton gave a quartet at 2.27 δ ppm-2.30 δ ppm.
9. Two most deshielded proton no.7 and 8 of secondary amine in hydrazide linkage of two (-NH) group gave two separable singlet in the down field at 9.30 δ ppm and 9.99 δ ppm respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the Calculation of the *J* values for each of the above proton, the proposed structure for

Compound SPSC-02 has been confirmed. Spectrum is given on page no. 86.

3.A.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

3.A.7 ANALYTICAL DATA

***N'-(2-(4-methylpiperazin-1-yl) acetyl)-2-propylpentanehydrazide (SPSC-01)*:**
Yield: 76%, IR (KBr, cm^{-1}): 3446 (N-H stretching), 2956 (-CH_3 asym. str.), 2931 (-CH_2 asym. str.), 2874 (-CH_3 sym. Str.), 2841 (-CH_2 sym. Str.), 1672 (-C=O str.), 1608 (N-H bending), 1452 (-CH_3 asym. bending deformation), 1367 (-CH_3 sym bending deformation),; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.91 (6H, m), 1.46 (6H, m), 1.68 (2H, m), 2.27 (1H, m), 2.30 (3H, s), 2.52 (4H, m), 2.63 (4H, m), 3.14 (2H, s), 9.07 (1H, s), 9.53 (1H, s), MS *m/z* = 298 (M^+); Anal. Calcd. for $C_{15}H_{30}N_4O_2$: C, 60.37; H, 10.13; N, 18.77; O, 10.72. Found: C, 60.35; H, 10.11; N, 18.71; O, 10.70%.

***N'-(2-(4-ethylpiperazin-1-ylacetyl)-2-propylpentanehydrazide (SPSC-02)*:**
Yield: 78%, IR (KBr, cm^{-1}): 3448 (N-H stretching), 2956 (-CH_3 asym. str.), 2928 (-CH_2 asym. str.), 2812 (-CH_2 sym. Str.), 1676 (-C=O str.), 1614 (N-H bending), 1456 (-CH_3 asym. bending deformation), 1375 (-CH_3 sym bending deformation); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.90 (6H, m), 1.08 (3H, t), 1.39 (6H, m), 1.61 (2H, m), 2.30 (1H, m), 2.44 (2H, q), 2.59 (4H, m), 2.62 (4H, m), 3.08 (2H, s), 9.30 (1H, s), 9.99 (1H, s) MS *m/z* = 312 (M^+); Anal. Calcd. for $C_{16}H_{32}N_4O_2$: C, 61.50; H, 10.32; N, 17.93; O, 10.24. Found: C, 61.48; H, 10.30; N, 17.91; O, 10.24%.

***N'-(2-morpholinoacetyl)-2-propylpentanehydrazide (SPSC-03)*:**
Yield: 74%, IR (KBr, cm^{-1}): 3444 (N-H stretching), 2954 (-CH_3 asym. str.), 2926 (-CH_2 asym. str.), 2878 (-CH_3 sym. Str.), 2838 (-CH_2 sym. Str.), 1670 (-C=O str.), 1610

(N-H bending), 1450 (-CH_3 asym. bending deformation), 1366 (-CH_3 sym bending deformation); MS *m/z* = 285 (M^+); Anal. Calcd. for $C_{14}H_{27}N_3O_3$: C, 58.92; H, 9.54; N, 14.72; O, 16.82. Found: C, 58.88; H, 9.50; N, 14.70; O, 16.81%.

***N'-(2-(4-phenylpiperazin-1-yl)acetyl)-2-propylpentanehydrazide (SPSC-04)*:**
Yield: 72%, IR (KBr, cm^{-1}): 3452 (N-H stretching), 2955 (-CH_3 asym. str.), 2929 (-CH_2 asym. str.), 2874 (-CH_3 sym. Str.), 2823 (-CH_2 sym. Str.), 1683 (-C=O str.), 1608 (N-H bending), 1485 (-CH_3 asym. bending deformation), 1377 (-CH_3 sym bending deformation); MS *m/z* = 360 (M^+); Anal. Calcd. for $C_{20}H_{32}N_4O_2$: C, 66.63; H, 8.95; N, 15.54; O, 8.88. Found: C, 66.60; H, 8.91; N, 15.51; O, 8.84%.

***N'-(2-(4-benzylpiperazin-1-yl)acetyl)-2-propylpentanehydrazide (SPSC-05)*:**
Yield: 78%, IR (KBr, cm^{-1}): 3454 (N-H stretching), 2944 (-CH_3 asym. str.), 2938 (-CH_2 asym. str.), 2870 (-CH_3 sym. Str.), 2833 (-CH_2 sym. Str.), 1672 (-C=O str.), 1612 (N-H bending), 1450 (-CH_3 asym. bending deformation), 1378 (-CH_3 sym bending deformation); MS *m/z* = 375 (M^+); Anal. Calcd. for $C_{21}H_{34}N_4O_2$: C, 67.35; H, 9.15; N, 14.96; O, 8.54. Found: C, 67.32; H, 9.10; N, 14.91; O, 8.52%.

***N'-(2-(diisopropylamino)acetyl)-2-propylpentanehydrazide (SPSC-06)*:**
Yield: 72%, IR (KBr, cm^{-1}): 3440 (N-H stretching), 2950 (-CH_3 asym. str.), 2931 (-CH_2 asym. str.), 2879 (-CH_3 sym. Str.), 2840 (-CH_2 sym. Str.), 1680 (-C=O str.), 1610 (N-H bending), 1456 (-CH_3 asym. bending deformation), 1361 (-CH_3 sym bending deformation); MS *m/z* = 299 (M^+); Anal. Calcd. for $C_{16}H_{33}N_3O_2$: C, 64.17; H, 11.11; N, 14.03; O, 10.69. Found: C, 64.12; H, 11.10; N, 14.01; O, 10.66%.

***N'-(2-(piperazin-1-yl)acetyl)-2-propylpentanehydrazide (SPSC-07)*:**
Yield: 82%, IR (KBr, cm^{-1}): 3438 (N-H stretching), 2953 (-CH_3 asym. str.), 293 (-CH_2 asym. str.), 2874 (CH_3 sym. Str.), 2836 (CH_2 sym. Str.), 1668 (-C=O str.), 1616 (N-H bending), 1450 (-CH_3 asym. bending deformation), 1362 (-CH_3 sym bending deformation); MS *m/z* = 284 (M^+); Anal. Calcd. for $C_{14}H_{28}N_4O_2$: C, 59.12; H, 9.92; N, 19.70; O, 11.25. Found: C, 59.10; H, 9.90; N, 19.68; O, 11.22%.

***N'-(2-(dipropylamino)acetyl)-2-propylpentanehydrazide (SPSC-08)*:**
Yield: 78%, IR (KBr, cm^{-1}): 3446 (N-H stretching), 2951 (-CH_3 asym. str.), 2941 (-

CH_2 asym. str.), 2870 (-CH_3 sym. Str.), 2840 (-CH_2 sym. Str.), 1678 (-C=O str.), 1608 (N-H bending), 1444 (-CH_3 asym. bending deformation), 1361 (-CH_3 sym bending deformation); MS *m/z* = 299 (M^+); Anal. Calcd. for $C_{16}H_{33}N_3O_2$: C, 64.17; H, 11.11; N, 14.03; O, 10.69. Found: C, 64.17; H, 11.12; N, 14.02; O, 10.70%.

***N'-(2-(N-methyl-N-phenylamino)acetyl)-2-propylpentanehydrazide (SPSC-09)*:**
Yield: 72%, IR (KBr, cm^{-1}): 3440 (N-H stretching), 2952 (-CH_3 asym. str.), 2930 (-CH_2 asym. str.), 2868 (-CH_3 sym. Str.), 2838 (-CH_2 sym. Str.), 1670 (-C=O str.), 1608 (N-H bending), 1450 (-CH_3 asym. bending deformation), 1378 (-CH_3 sym bending deformation); MS *m/z* = 305 (M^+); Anal. Calcd. for $C_{17}H_{27}N_3O_2$: C, 66.85; H, 8.91; N, 13.76; O, 10.48. Found: C, 66.81; H, 8.92; N, 13.70; O, 10.46%.

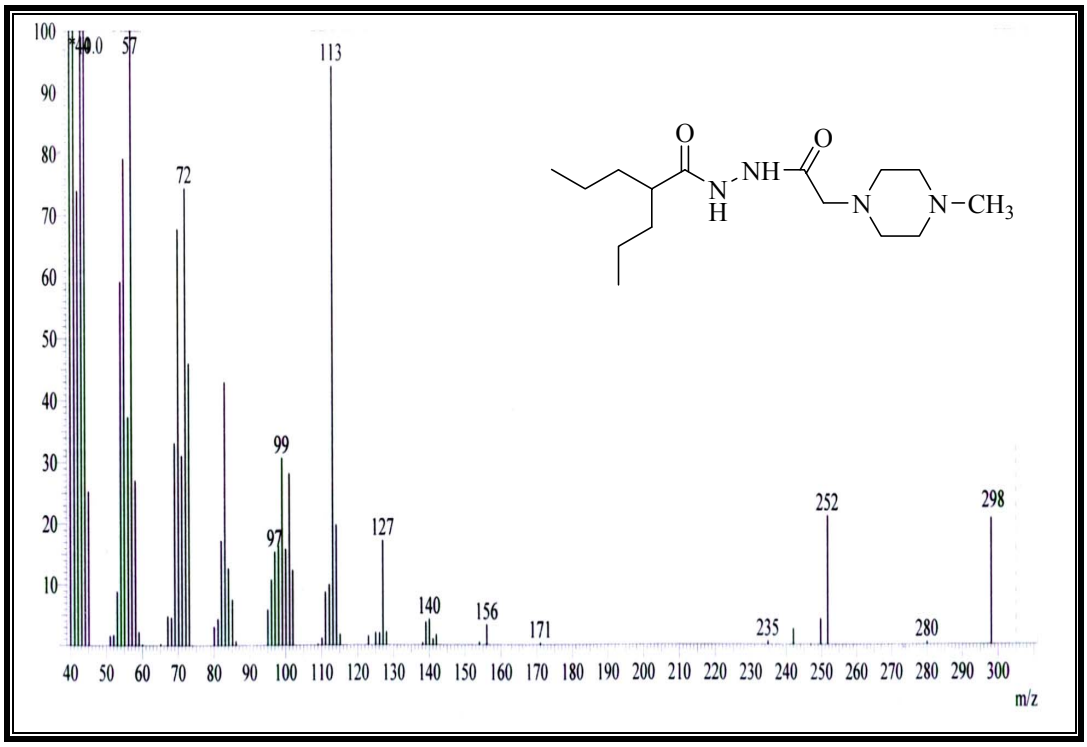
***N'-(2-(piperidin-1-yl)acetyl)-2-propylpentanehydrazide (SPSC-10)*:**
Yield:76%, IR (KBr, cm^{-1}): 3440 (N-H stretching), 2952 (-CH_3 asym. str.), 2930 (-CH_2 asym. str.), 2868 (-CH_3 sym. Str.), 2846 (-CH_2 sym. Str.), 1670 (-C=O str.), 1614 (N-H bending), 1458 (-CH_3 asym. bending deformation), 1361 (-CH_3 sym bending deformation); MS *m/z* = 283 (M^+); Anal. Calcd. for $C_{15}H_{29}N_3O_2$: C, 63.57; H, 10.31; N, 14.83; O, 11.29. Found: C, 63.51; H, 10.22; N, 14.77; O, 11.23%.

***N'-(2-(diethylamino)acetyl)-2-propylpentanehydrazide (SPSC-11)*:**
Yield: 72%, IR (KBr, cm^{-1}): 3452 (N-H stretching), 2958 (-CH_3 asym. str.), 2928 (-CH_2 asym. str.), 2872 (-CH_3 sym. Str.), 2838 (-CH_2 sym. Str.), 1678 (-C=O str.), 1611 (N-H bending), 1450 (-CH_3 asym. bending deformation), 1361 (-CH_3 sym bending deformation); MS *m/z* = 271 (M^+); Anal. Calcd. for $C_{14}H_{29}N_3O_2$: C, 61.96; H, 10.77; N, 15.48; O, 11.79. Found: C, 61.95; H, 10.76; N, 15.47; O, 11.78%.

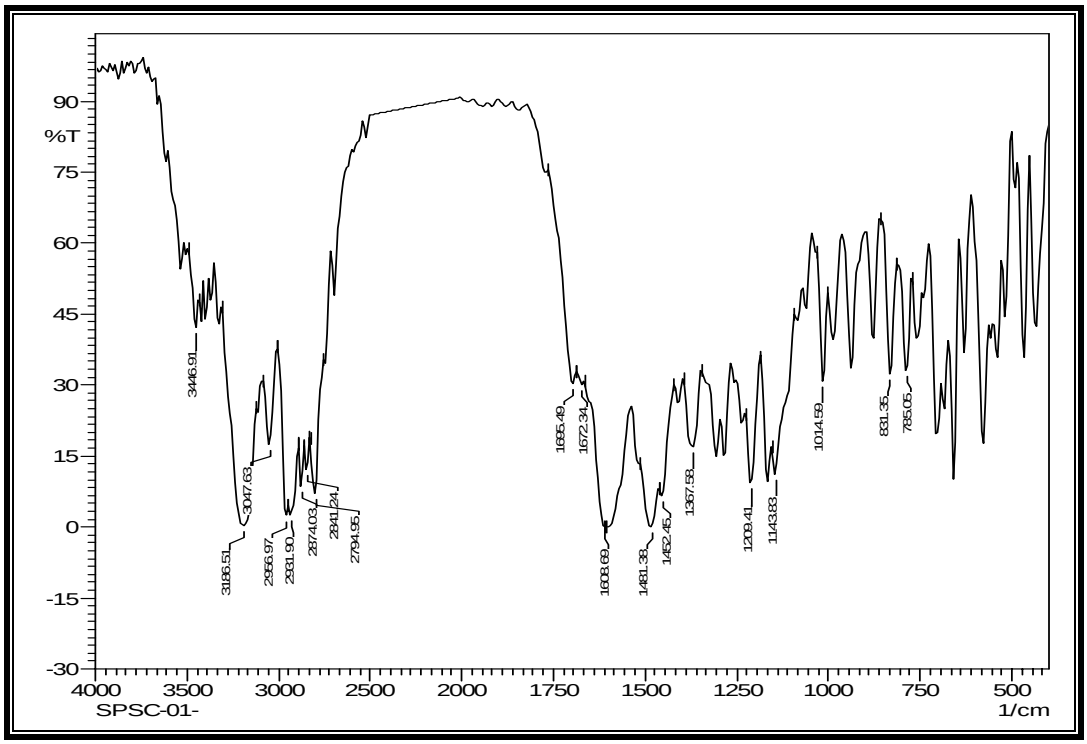
***2-propyl-N'-(2-(pyrrolidin-1-yl)acetyl)pentanehydrazide (SPSC-12)*:**
Yield: 76%, IR (KBr, cm^{-1}): 3444 (N-H stretching), 2951 (-CH_3 asym. str.), 2928 (-CH_2 asym. str.), 2875 (-CH_3 sym. Str.), 2834 (-CH_2 sym. Str.), 1670 (-C=O str.), 1608 (N-H bending), 1450 (-CH_3 asym. bending deformation), 1361 (-CH_3 sym bending deformation); MS *m/z* = 269 (M^+); Anal. Calcd. for $C_{14}H_{27}N_3O_2$: C, 62.42; H, 10.10; N, 15.60; O, 11.88. Found: C, 62.41; H, 10.10; N, 15.61; O, 11.86%.

3.A.8 REPRESENTATIVE SPECTRA

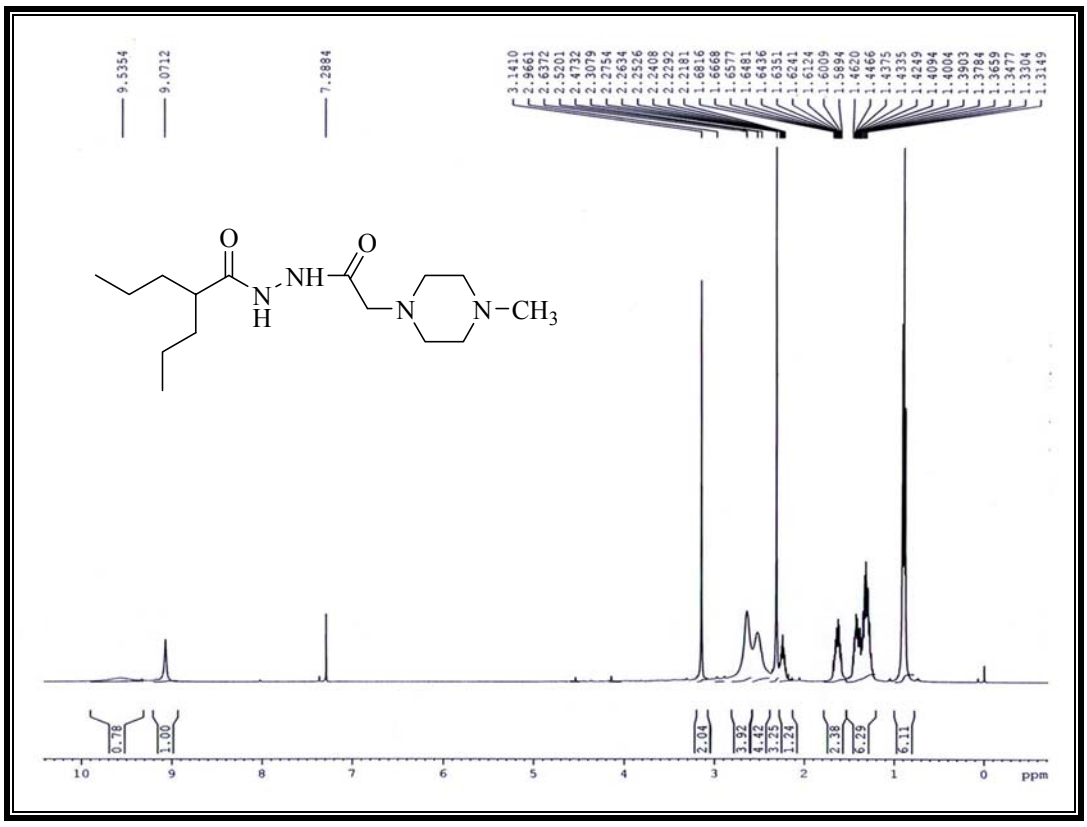
3.A.8.1 Mass Spectrum of SPSC-01



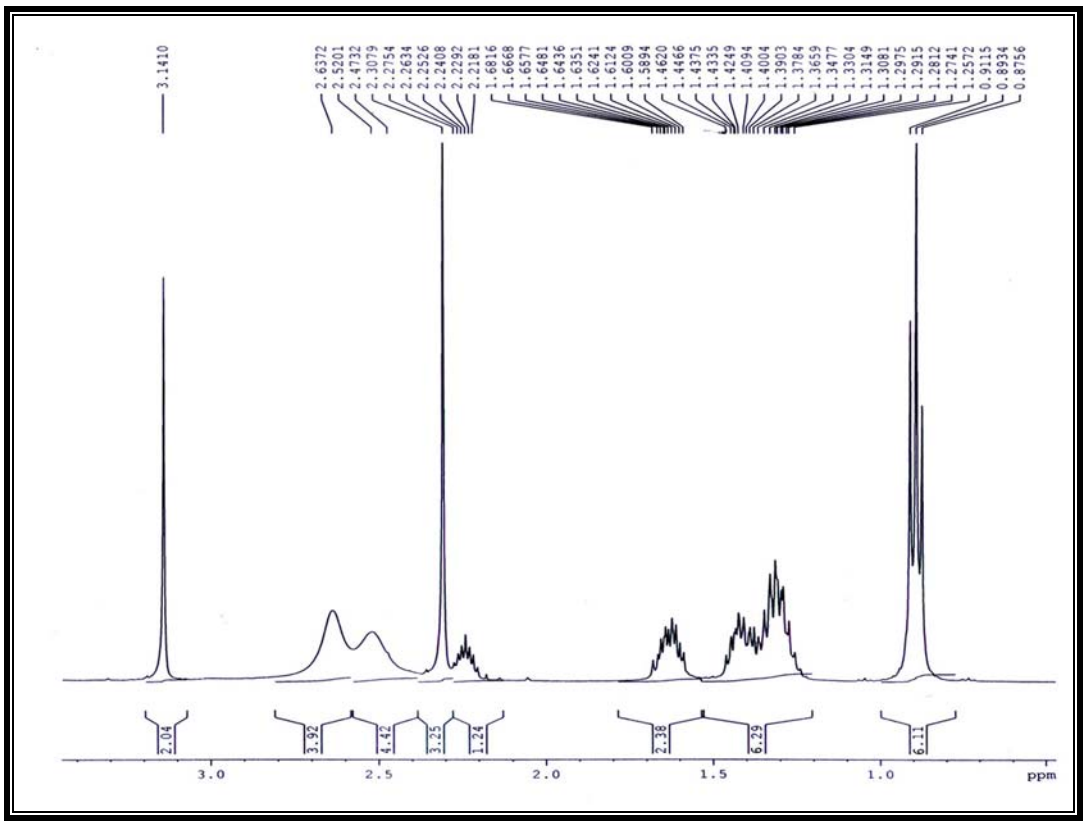
3.A.8.2 IR Spectrum of SPSC-01



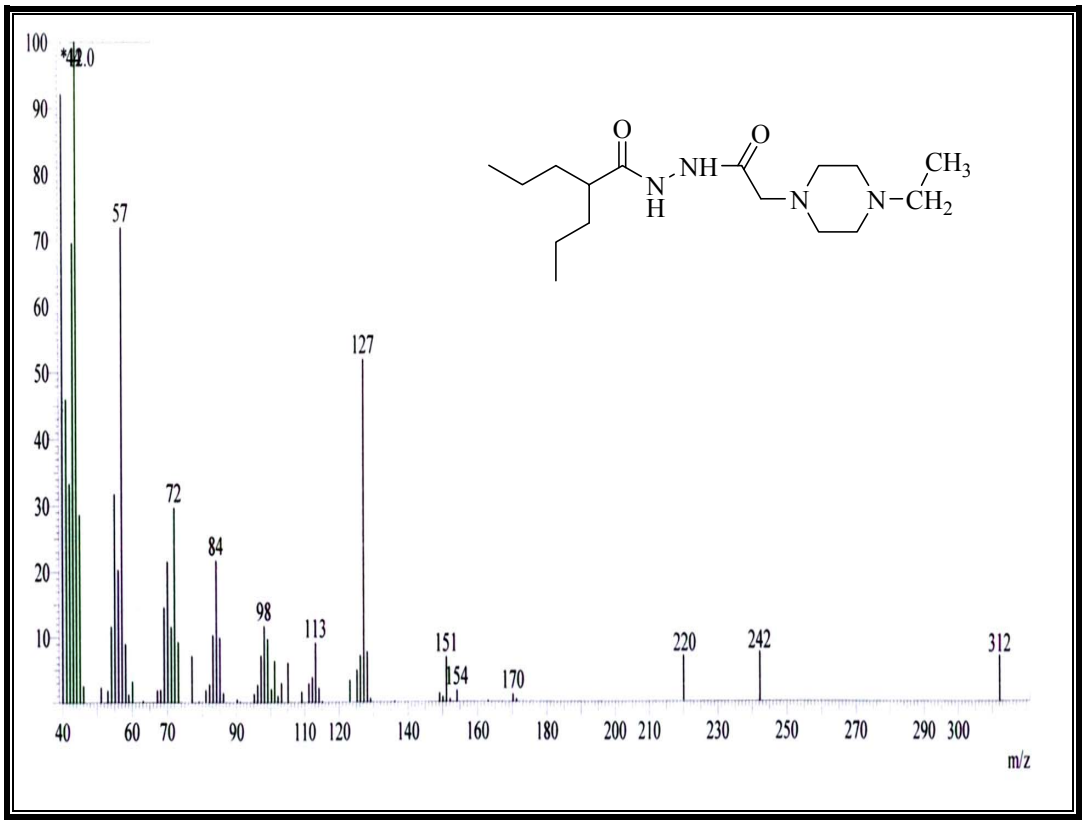
3.A.8.3 ^{1}H NMR Spectrum of SPSC-01



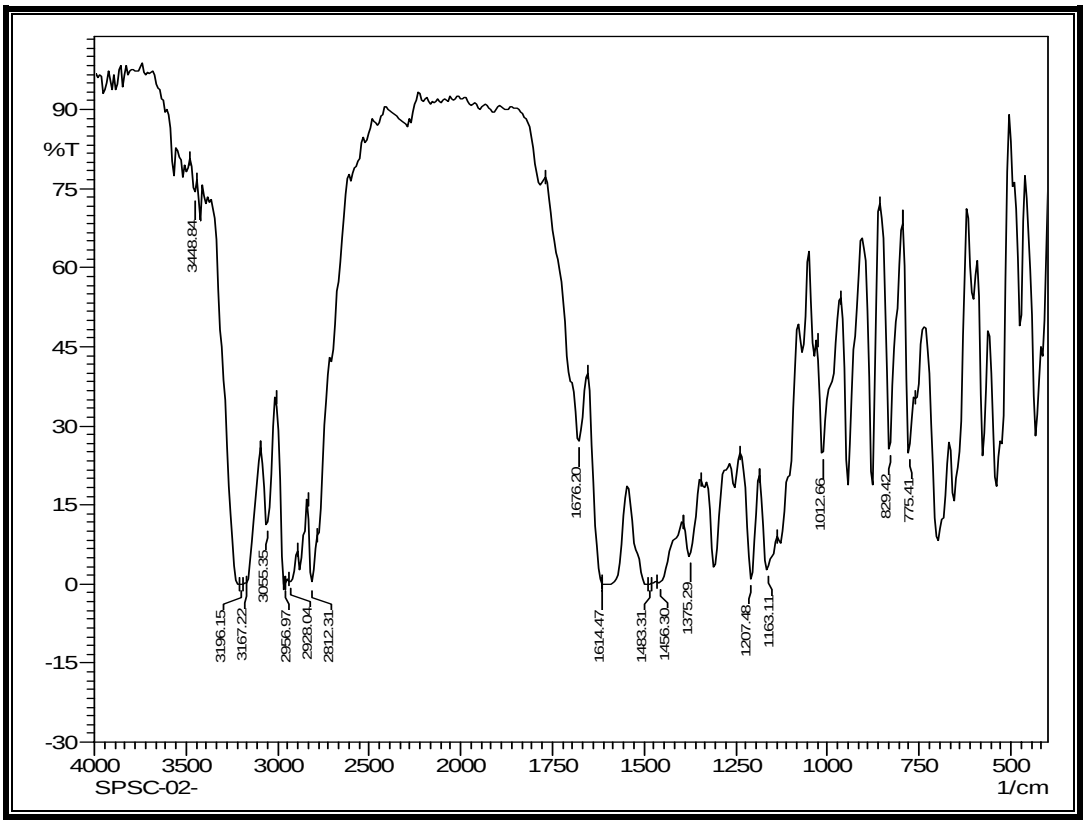
EXPANDED ^{1}H-NMR SPECTRUM OF SPSC-01



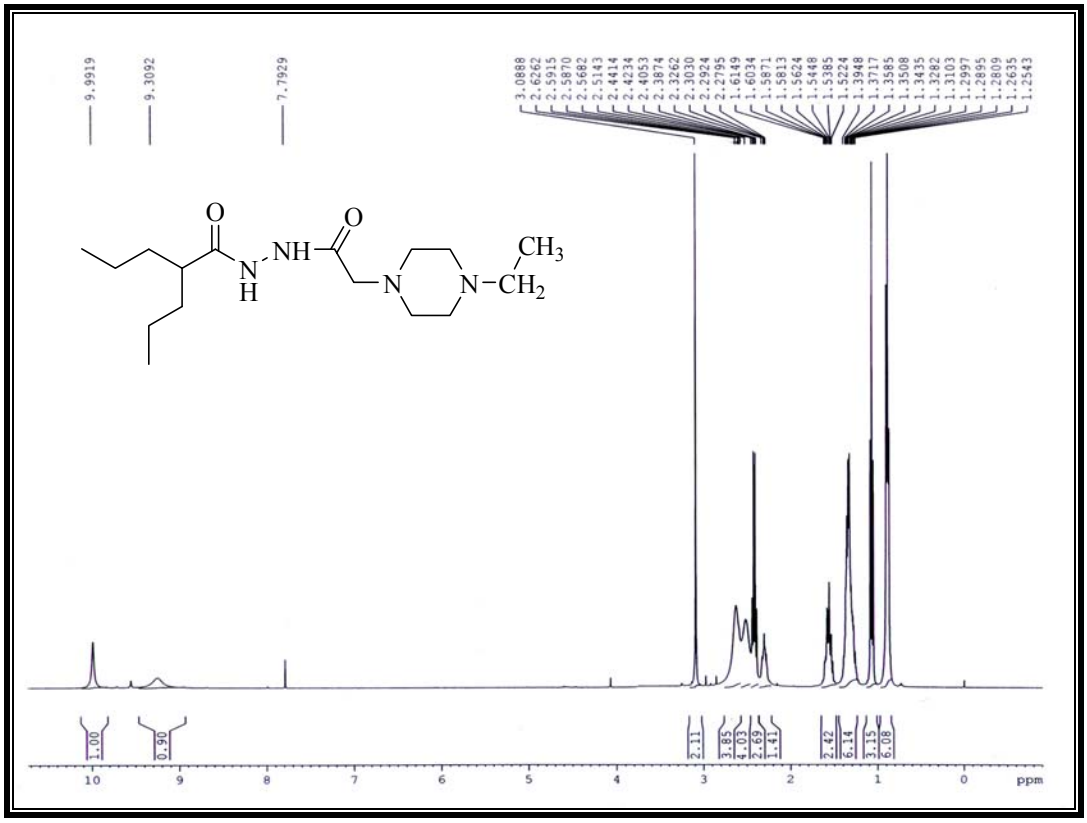
3.A.8.4 Mass Spectrum of SPSC-02



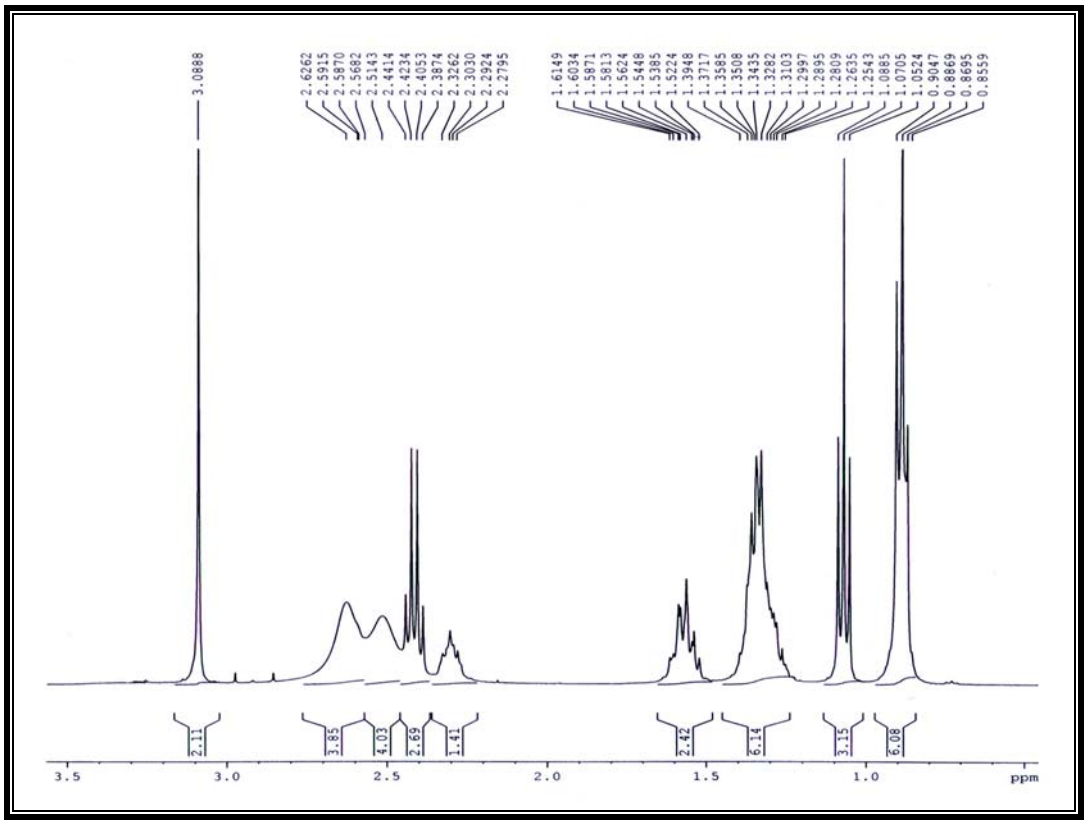
3.A.8.5 IR Spectrum of SPSC-02



3.A.8.6 ^{1}H NMR Spectrum of SPSC-02



EXPANDED ^{1}H-NMR SPECTRUM OF SPSC-02



CHAPTER - 3

SECTION - B

A RAPID MICROWAVE ASSISTED SYNTHESIS OF SCHIFF BASES LIKE *N*'-(1-(SUBSTITUTED PHENYL) ETHYLIDENE) 2-PROPYL PENTANEHYDRAZIDE

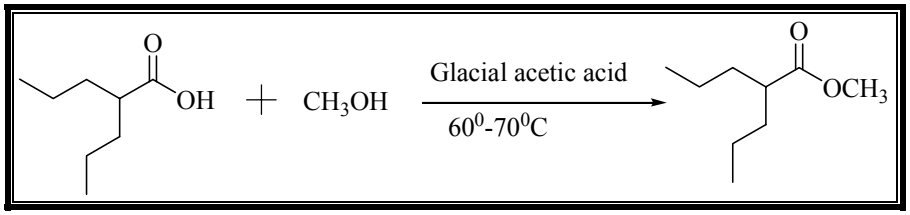
3.B.1 AIM OF CURRENT WORK

In recent years, environmentally benign synthetic methods have received considerable attention and some solvent-free protocols have been developed. In present work is a multistep synthesis in which the final step is performed by using microwave synthesizer for rapid synthesis.

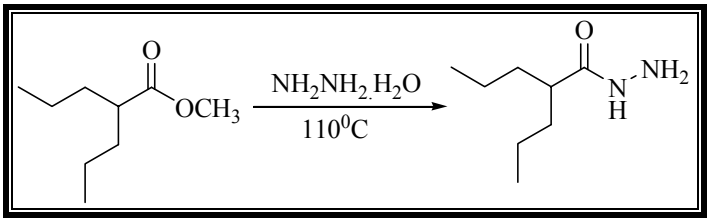
In this sub chapter, Valproic acid is used as a starting product and converted into respective ester to obtain a hydrazide by using hydrazine hydrate which further treated with substituted acetophenone to obtain a series of novel compounds having structural moiety of Valproic acid and hydrazide linker with extended systems, using microwave synthesizer.

3.B.2 REACTION SCHEMES

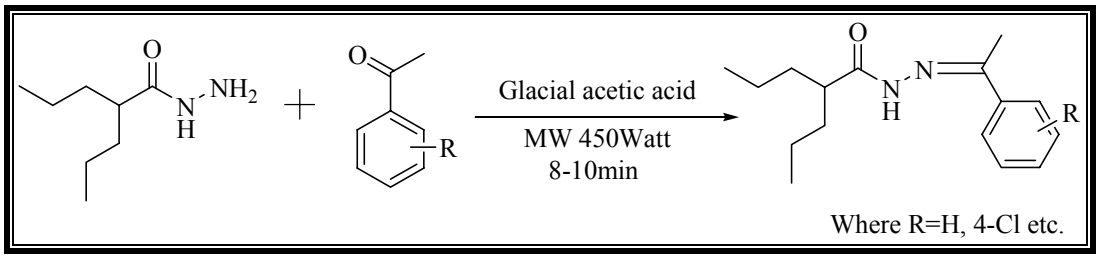
3.B.2.1 PREPARATION OF METHYL-2-PROPYL PENTANOATE



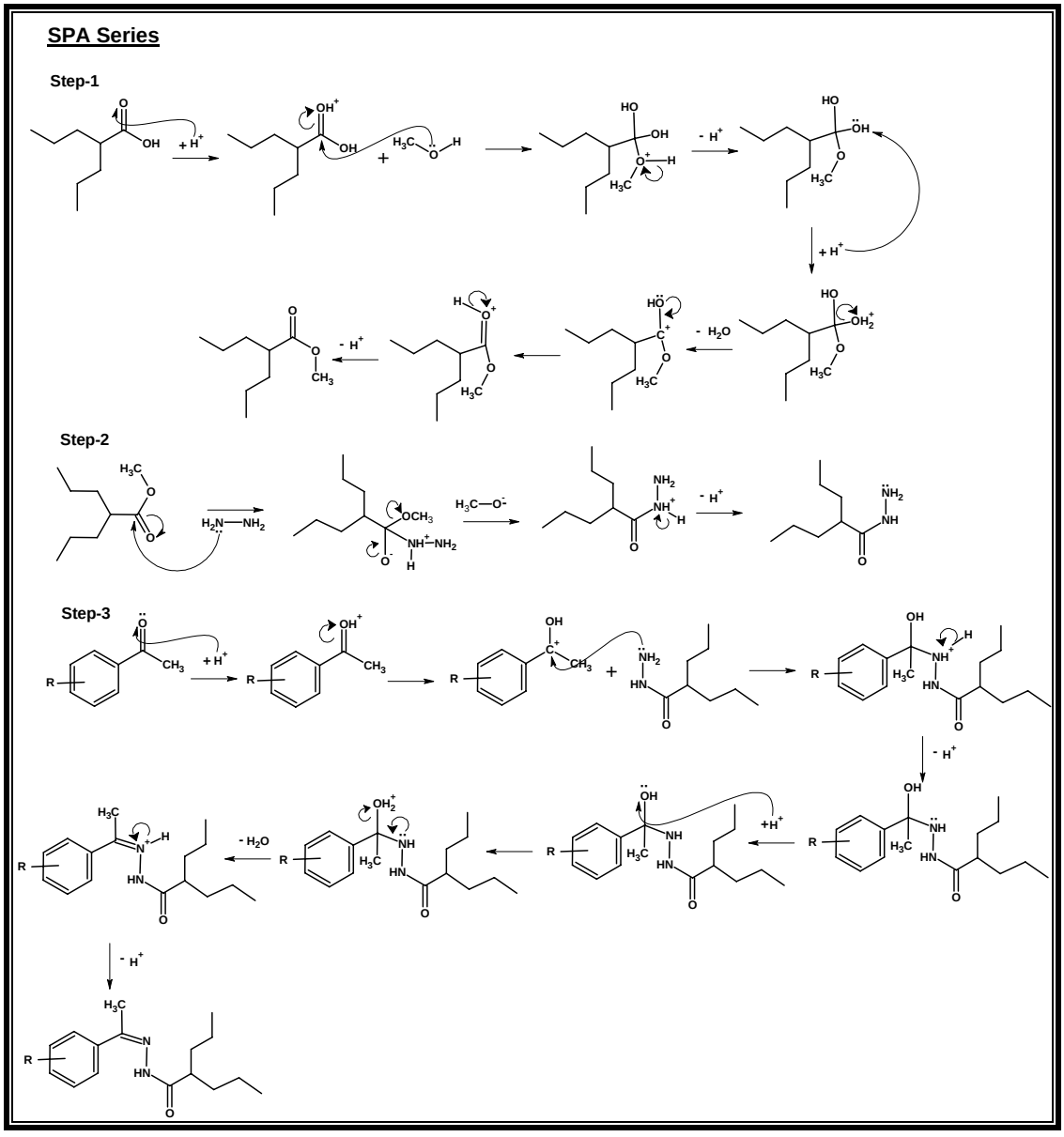
3.B.2.2 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE



3.B.2.3 PREPARATION OF (*Z*) – *N*'-(1-(SUBSTITUTED PHENYL) ETHYLIDENE) 2-PROPYL PENTANEHYDRAZIDE



3.B.3 PLAUSIBLE REACTION MECHANISM



3.B.4 EXPERIMENTAL

3.B.4.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in **Samsung MW83Y Microwave Oven** which was locally modified for carrying out chemical reactions. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in $CDCl_3$ solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

3.B.4.2 PREPARATION OF METHYL 2-PROPYL PENTANOATE

2-propyl pentanoic acid (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. Sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 hours on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml × 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml × 2) followed by water (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. Yield - 90 %, B. P. – 170–172°C.[a]

[a] Dostovalova, V. I. ; Organic Magnetic Resonance 1983, V21(1), P111-19.

3.B.4.3 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE

Methyl 2-propylpentanoate (0.01 mole) was taken into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white colored shining fluffy product. Yield - 60 %, M. P. – 124–126°C.[b]

3.B.4.4 GENERAL PROCEDURE OF (*Z*) – *N*'-(1-(SUBSTITUTED PHENYL) ETHYLIDENE) 2-PROPYL PENTANEHYDRAZIDE

2-propylpentanohydrazide (0.01 mole) was added into 250 ml round bottom flask. 15 ml of Methanol was added into above flask. 0.01 mole of substituted acetophenones were added and add into 2-3 drops of glacial acetic acid as a catalyst. The reaction mixture the reaction mixture carried out under microwave irradiation at 450 Watt. for 8-10 minutes. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene: ethyl acetate (7: 3) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give solid product. Finally it recrystallized from methanol. Similarly other compounds are also prepared.

[b] Benoit-Guyod, Jean L.; Chemica Therapeutica 1968, V3(5), P336-42.

3.B.5 PHYSICAL DATA

PHYSICAL DATA OF (*Z*) – *N*'-(1-(SUBSTITUTED PHENYL) ETHYLIDENE) PROPYL PENTANEHYDRAZIDES (SPA-01 TO SPA-10)

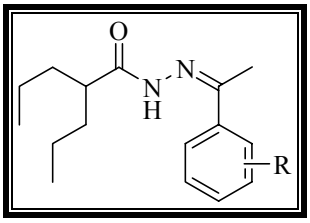


TABLE-3.B.1

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	MP ^{0}C	R_f Value
01	SPA-01	H	$C_{16}H_{24}N_2O$	260	188-190	0.44
02	SPA-02	4-Cl	$C_{16}H_{23}ClN_2O$	295	208-210	0.48
03	SPA-03	4-CH_3	$C_{17}H_{26}N_2O$	274	222-224	0.50
04	SPA-04	2-OH	$C_{16}H_{24}N_2O_2$	276	238-240	0.42
05	SPA-05	4-OCH_3	$C_{17}H_{26}N_2O_2$	290	192-194	0.44
06	SPA-06	4-NO_2	$C_{16}H_{23}N_3O_3$	305	188-190	0.48
07	SPA-07	4-F	$C_{16}H_{23}FN_2O$	278	198-200	0.54
08	SPA-08	2- OCH_3	$C_{17}H_{26}N_2O_2$	290	220-222	0.46
09	SPA-09	3-NO_2	$C_{16}H_{23}N_3O_3$	305	238-240	0.42
10	SPA-10	2-Cl	$C_{16}H_{23}ClN_2O$	295	202-204	0.52

R_f value was calculated using solvent system, Toluene: Ethyl Acetate (7: 3)

3.B.6 SPECTRAL DISCUSSION

3.B.6.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. SPA-01 and SPA-02 of Mass spectra are given on page no. 99 and 101.

3.B.6.2 IR SPECTRA

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

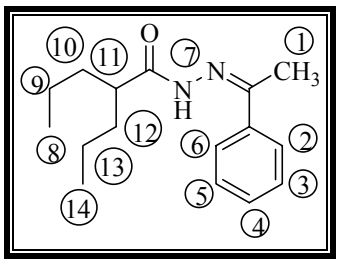
NH stretching vibration observed near 3440 cm^{-1}, C-H stretching frequencies of aromatic were observed between 3100 cm^{-1} and 3000 cm^{-1}, C-H asym. stretching frequencies were observed between 2975 cm^{-1} and 2920 cm^{-1}, C-H sym. stretching frequencies were observed between 2880 cm^{-1} and 2860 cm^{-1}, Azomethine frequency were observed between 1650 cm^{-1} and 1580 cm^{-1}, N-H bending vibration observed near 1550 cm^{-1}, There are two carbonyl groups present in all the compounds but due to the same environment, the peaks merged in the region of 1660 cm^{-1}. CN stretching frequency was observed near 1250 cm^{-1} in all the compounds. SPA-01 and SPA-02 of IR spectra are given on page no. 99 and 101.

3.B.6.3 ^{1}H NMR SPECTRA

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in $CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from proton NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated

to identify *o*, *m* and *p* coupling. ^{1}H NMR spectral interpretation can be discussed as under.

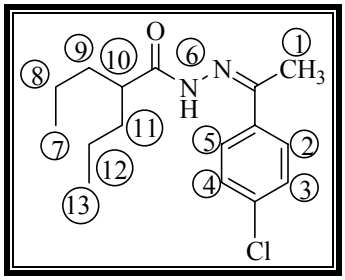
^{1}H NMR OF (*Z*)-*N*'-(1-PHENYLETHYLIDENE)-2-PROPYLPENTANEHYDRAZIDE (SPA-01)



1. The Proton no.1 of methyl group gave a characteristic singlet at 2.25 δ ppm.
2. The Proton no. 2 and 6 of aromatic phenyl ring of two proton gave a multiplet at 7.74 δ ppm-7.77 δ ppm.
3. The Proton no. 3, 4 and 5 of phenyl ring of three proton gave a multiplet at 7.35 δ ppm-7.42 δ ppm.
4. The Proton no. 8 and 9 of propyl chain of five proton gave a multiplet at 0.90 δ ppm-0.94 δ ppm.
5. Proton no. 13 and 14 of propyl chain of five proton gave a multiplet at 1.28 δ ppm-1.41 δ ppm.
6. Proton no. 10 of propyl chain of proton gave a multiplet at 1.45 δ ppm- 1.53 δ ppm.
7. Proton no. 12 of propyl chain of proton gave a multiplet at 1.69 δ ppm-1.78 δ ppm.
8. Proton no. 11 of di propyl chain gave a multiplet at 3.57 δ ppm- 3.62 δ ppm.
9. Most deshielded proton no. 7 of secondary amine in hydrazide (amide linkage CO-NH) gave singlet in the down field at 9.32 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPA-01 has been confirmed. Spectrum is given on page no. 100.

^{1}H NMR OF (*Z*)-*N*'-(1-(4-CHLOROPHENYL) ETHYLIDENE)-2-PROPYLPENTANEHYDRAZIDE (SPA-02)



1. The Proton no.1 of methyl group gave a characteristic singlet at 2.26 δ ppm.
2. The Proton no. 3 and 4 of aromatic phenyl ring of two proton gave a doublet at 7.34 δ ppm-7.36 δ ppm. And *J* value of this proton is 8.0 Hz. that suggests a ortho coupled.
3. The Proton no. 2 and 5 of phenyl ring of two proton gave a doublet at 7.68 δ ppm-7.70 δ ppm. And *J* value of this proton is 8.0 Hz. that suggests a ortho coupled.
4. The Proton no. 7 and 8 of propyl chain of five protons gave a multiplet at 0.93 δ ppm.
5. Proton no. 12 and 13 of propyl chain of five proton gave a multiplet at 1.29 δ ppm-1.39 δ ppm.
6. Proton no. 9 of propyl chain of two protons gave a multiplet at 1.44 δ ppm-1.53 δ ppm.
7. Proton no. 11 of propyl chain of two protons gave a multiplet at 1.68 δ ppm-1.75δ ppm.
8. Proton no. 10 of dipropyl chain gave a multiplet at 3.55 δ ppm - 3.59 δ ppm.
9. Most deshielded proton no. 6 of secondary amine in hydrazide (amide linkage CO-NH) gave singlet in the down field at 9.71 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPA-02 has been confirmed. Spectrum is given on page no. 102.

3.B.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

3.B.7 ANALYTICAL DATA

***(Z)-N'-(1-phenylethylidene)-2-propylpentanehydrazide (SPA-01)*:**

Yield:76%, IR (KBr, cm^{-1}): 3443 (-NH str. Vib.), 3034 (Ar. –C-H str.), 2951 (-C-H asym. str.), 2868 (-C-H sym. Str.), 1658 (-C=O str.), 1600 (-N=C str.), 1546 (-NH bending), 1444 (-C-H deformation asym. str.), 1377 (-C-H deformation sym. Str.), 1303 (CN str. Vib.), 1111 (-C-H i.p. deformation), 877 (-C-H oop bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.94 (5H, m), 1.41 (5H, m), 1.53 (2H, m), 1.78 (2H, m), 2.25 (3H, s), 3.62 (1H, m), 7.42 (3H, m), 7.77 (2H, m), 9.32 (1H, s); MS *m/z* = 260 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O$: C, 73.81; H, 9.29; N, 10.76; O, 6.14. Found: C, 73.80; H, 9.26; N, 10.70; O, 6.12%.

***(Z)-N'-(1-(4-chlorophenyl) ethylidene)-2-propylpentanehydrazide (SPA-02)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3448 (-NH str. Vib.), 3014 (Ar. –C-H str.), 2953 (-C-H asym. str.), 2868 (-C-H sym. Str.), 1660 (-C=O str.), 1604 (-N=C str.), 1543 (-NH bending), 1460 (-C-H deformation asym. str.), 1398 (-C-H deformation sym. Str.), 1307, 1246 (CN str. Vib.), 1095 (-C-H i.p. deformation), 827 (-C-H oop bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.93 (5H, m), 1.39 (5H, m), 1.53 (2H, m), 1.75 (2H, m), 2.26 (3H, s), 3.59 (1H, m), 7.36 (2H, m), 7.70 (2H, m), 9.71 (1H, s); MS *m/z* =295 (M^+), 296 (M^{+1}); Anal. Calcd. for $C_{16}H_{23}ClN_2O$: C, 65.18; H, 7.86; N, 9.50; O, 5.43. Found: C, 65.12; H, 7.81; N, 9.50; O, 5.38%.

***(Z)-2-propyl-N'-(1-p-tolylethylidene)pentanehydrazide (SPA-03)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3450 (-NH str. vib.), 3032 (Ar. -C-H str.), 2953 (-C-H asym. str.), 2866 (-C-H sym. Str.), 1662 (-C=O str.), 1612 (-N=C str.), 1550 (-NH

bending), 1460 (-C-H deformation asym. str.), 1375 (-C-H deformation sym. Str.), 1303, 1244 (CN str. Vib.), 1120 (-C-H i.p. deformation), 815 (-C-H oop bending); MS *m/z* = 274 (M^+); Anal. Calcd. for $C_{17}H_{26}N_2O$: C, 74.41; H, 9.55; N, 10.21; O, 5.83. Found: C, 74.41; H, 9.52; N, 10.21; O, 5.82%.

***(Z)-N'-(1-(2-hydroxyphenyl)ethylidene)-2-propylpentanehydrazide (SPA-04)*:** Yield:78%, IR (KBr, cm^{-1}): 3446 (-NH str. vib.), 3046 (Ar. –C-H str.), 2946 (-C-H asym. str.), 2860 (-C-H sym. Str.), 1672 (-C=O str.), 1612 (-N=C str.), 1580 (-NH bending), 1462 (-C-H deformation asym. str.), 1370 (-C-H deformation sym. Str.), 1304, 1255 (CN str. Vib.), 1116 (-C-H i.p. deformation), 831 (-C-H oop bending); MS *m/z* = 276 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14; O, 11.58. Found: C, 69.51; H, 8.70; N, 10.11; O, 11.52%.

***(Z)-N'-(1-(4-methoxyphenyl)ethylidene)-2-propylpentanehydrazide (SPA-05)*:** Yield:78%, IR (KBr, cm^{-1}): 3440 (-NH str. vib.), 3052 (Ar. –C-H str.), 2951 (-C-H asym. str.), 2862 (-C-H sym. Str.), 1670 (-C=O str.), 1616 (-N=C str.), 1583 (-NH bending), 1458 (-C-H deformation asym. str.), 1372 (-C-H deformation sym. Str.), 1307, 1250 (CN str. Vib.), 1124 (-C-H i.p. deformation), 826 (-C-H oop bending); MS *m/z* = 290 (M^+); Anal. Calcd. for $C_{17}H_{26}N_2O_2$: C, 70.31; H, 9.02; N, 9.65; O, 11.02. Found: C, 70.28; H, 9.00; N, 9.62; O, 11.01%.

***(Z)-N'-(1-(4-nitrophenyl)ethylidene)-2-propylpentanehydrazide (SPA-06)*:** Yield:74%, IR (KBr, cm^{-1}): 3430 (-NH str. vib.), 3048 (Ar. –C-H str.), 2948 (-C-H asym. str.), 2860 (-C-H sym. Str.), 1668 (-C=O str.), 1610 (-N=C str.), 1582 (-NH bending), 1462 (-C-H deformation asym. str.), 1374 (-C-H deformation sym. Str.), 1312, 1250 (CN str. Vib.), 1112 (-C-H i.p. deformation), 824 (-C-H oop bending); MS *m/z* = 305 (M^+); Anal. Calcd. for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76; O, 15.72. Found: C, 62.91; H, 7.52; N, 13.70; O, 15.71%.

***(Z)-N'-(1-(4-fluorophenyl)ethylidene)-2-propylpentanehydrazide (SPA-07)*:** Yield:72%, IR (KBr, cm^{-1}): 3456 (-NH str. vib.), 3050 (Ar. –C-H str.), 2960 (-C-H asym. str.), 2861 (-C-H sym. Str.), 1672 (-C=O str.), 1622 (-N=C str.), 1583 (-NH bending), 1456 (-C-H deformation asym. str.), 1370 (-C-H deformation sym. Str.), 1307, 1248 (CN str. Vib.), 1124 (-C-H i.p. deformation), 820 (-C-H oop bending); MS

m/z = 278 (M^+), 280 (M^{+2}); Anal. Calcd. for $C_{16}H_{23}FN_2O$: C, 69.04; H, 8.33; N, 10.06; O, 5.75. Found: C, 69.01; H, 8.32; N, 10.04; O, 5.71%.

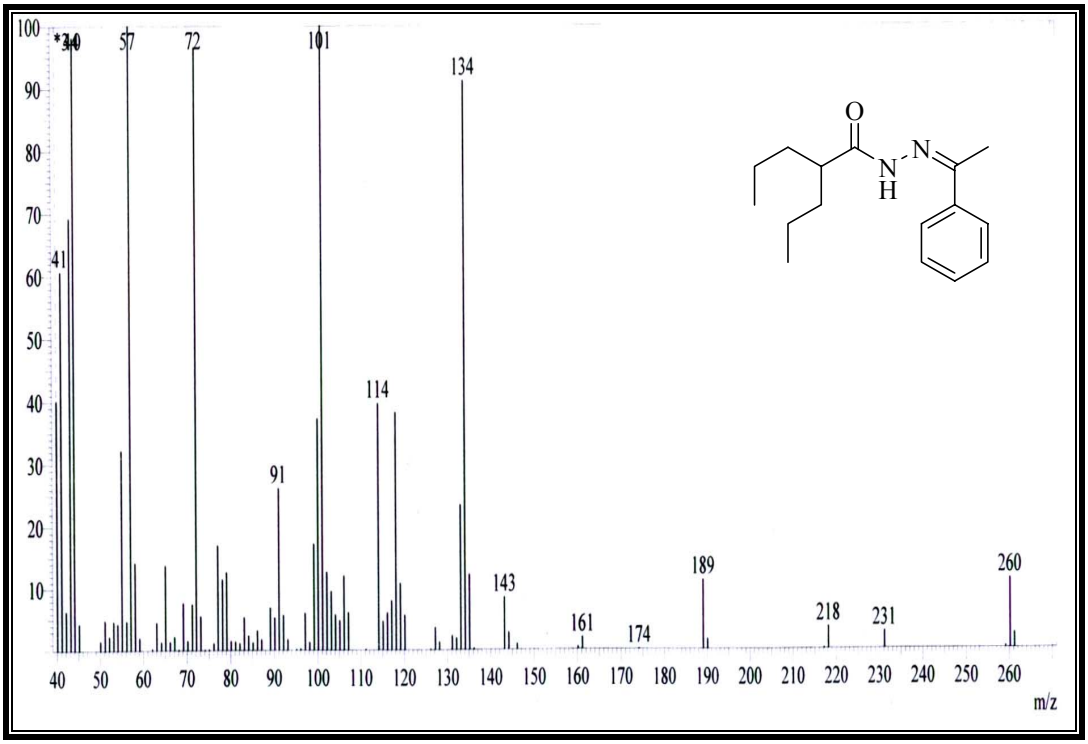
***(Z)-N'-(1-(2-methoxyphenyl)ethylidene)-2-propylpentanehydrazide (SPA-08)*:** Yield:80%, IR (KBr, cm^{-1}): 3448 (-NH str. vib.), 3048 (Ar. –C-H str.), 2956 (-C-H asym. str.), 2860 (-C-H sym. Str.), 1670 (-C=O str.), 1612 (-N=C str.), 1580 (-NH bending), 1458 (-C-H deformation asym. str.), 1372 (-C-H deformation sym. Str.), 1314, 1256 (CN str. vib.), 1130 (-C-H i.p. deformation), 827 (-C-H oop bending),; MS *m/z* = 290 (M^+); Anal. Calcd. for $C_{17}H_{26}N_2O_2$: C, 70.31; H, 9.02; N, 9.65; O, 11.02. Found: C, 70.26; H, 9.02; N, 9.61; O, 11.04%.

***(Z)-N'-(1-(3-nitrophenyl)ethylidene)-2-propylpentanehydrazide (SPA-09)*:** Yield:76%, IR (KBr, cm^{-1}): 3450 (-NH str. vib.), 3054 (Ar. –C-H str.), 2945 (-C-H asym. str.), 2866 (-C-H sym. Str.), 1676 (-C=O str.), 1610 (-N=C str.), 1582 (-NH bending), 1462 (-C-H deformation asym. str.), 1374 (-C-H deformation sym. Str.), 1310, 1254 (CN str. vib.), 1122 (-C-H i.p. deformation), 826 (-C-H oop bending); MS *m/z* = 305 (M^+); Anal. Calcd. for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76; O, 15.72. Found: C, 62.92; H, 7.52; N, 13.71; O, 15.73%.

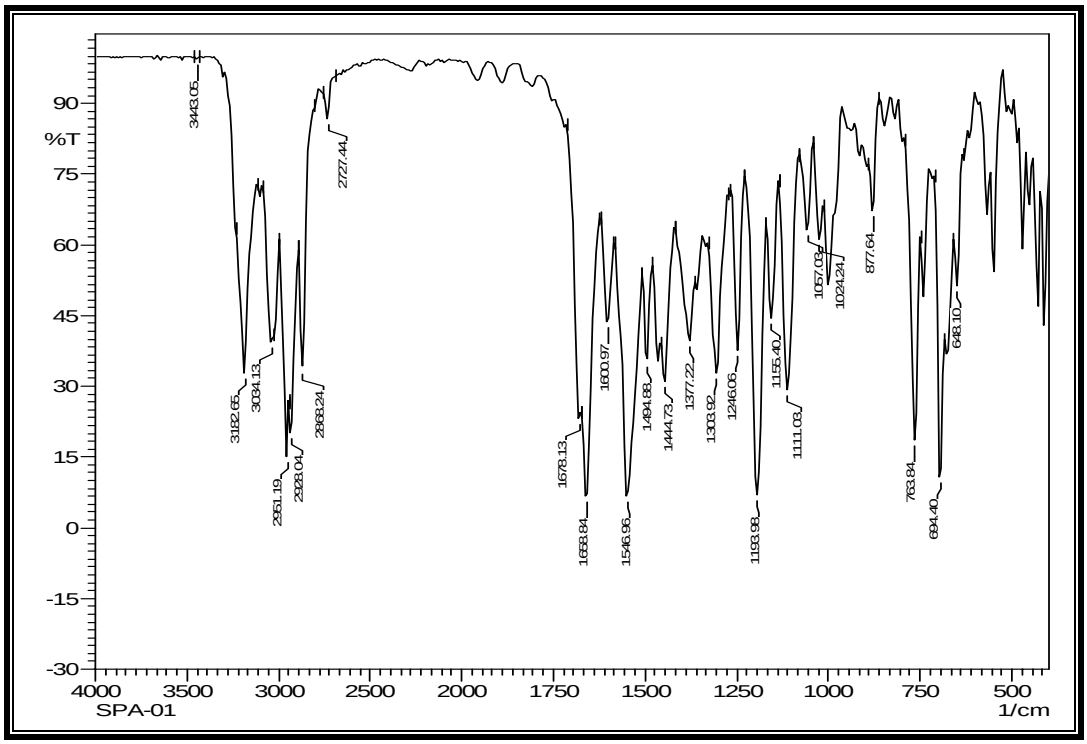
***(Z)-N'-(1-(2-chlorophenyl)ethylidene)-2-propylpentanehydrazide (SPA-10)*:** Yield:80%, IR (KBr, cm^{-1}): 3438 (-NH str. vib.), 3053 (Ar. –C-H str.), 2958 (-C-H asym. str.), 2864 (-C-H sym. Str.), 1674 (-C=O str.), 1612 (-N=C str.), 1583 (-NH bending), 1460 (-C-H deformation asym. str.), 1378 (-C-H deformation sym. Str.), 1310, 1251 (CN str. Vib.), 1124 (-C-H i.p. deformation), 838 (-C-H oop bending); MS *m/z* = 295 (M^+), 296 (M^{+1}); Anal. Calcd. for $C_{16}H_{23}ClN_2O$: C, 65.18; H, 7.86; N, 9.50; O, 5.43. Found: C, 65.11; H, 7.80; N, 9.50; O, 5.41%.

3.B.8 REPRESENTATIVE SPECTRA

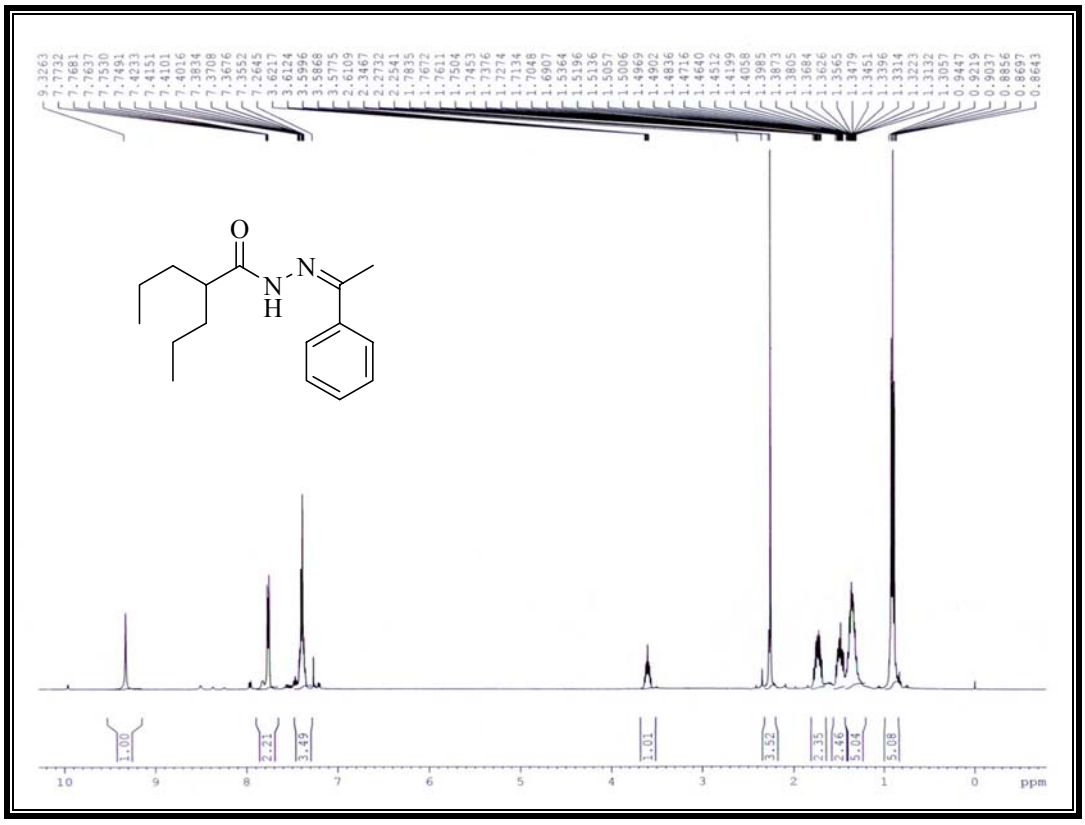
3.B.8.1 Mass Spectrum of SPA-01



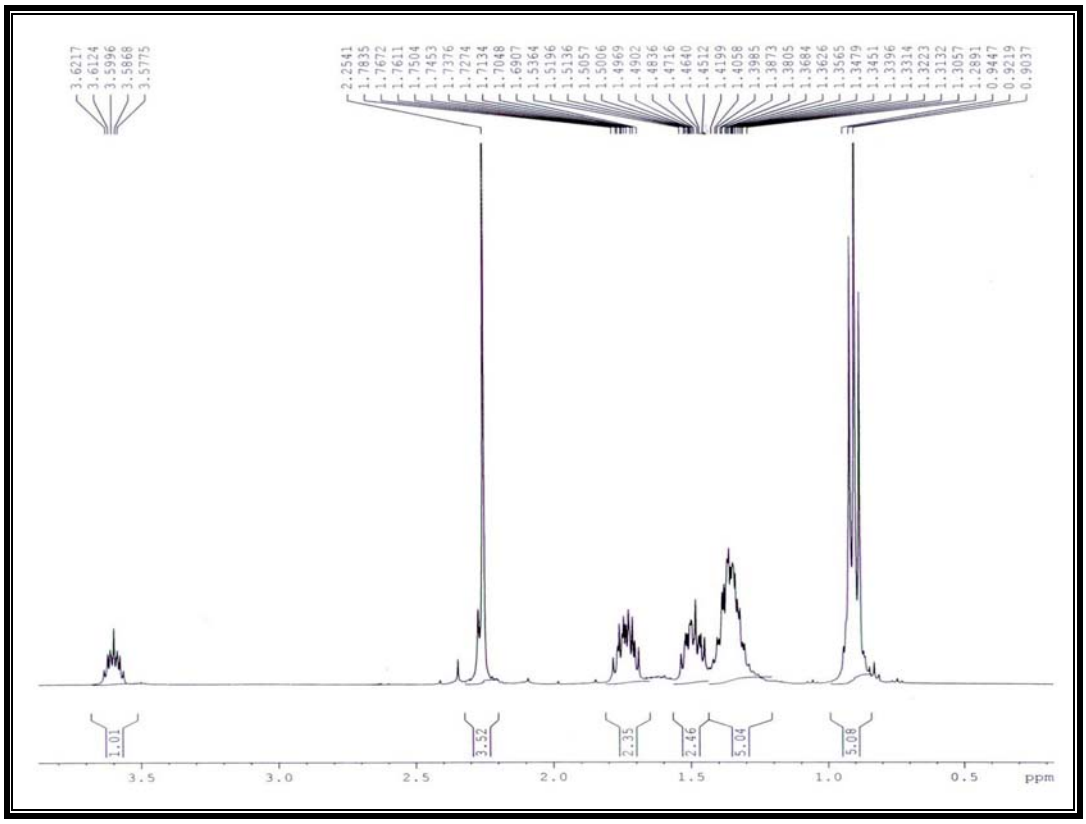
3.B.8.2 IR Spectrum of SPA-01



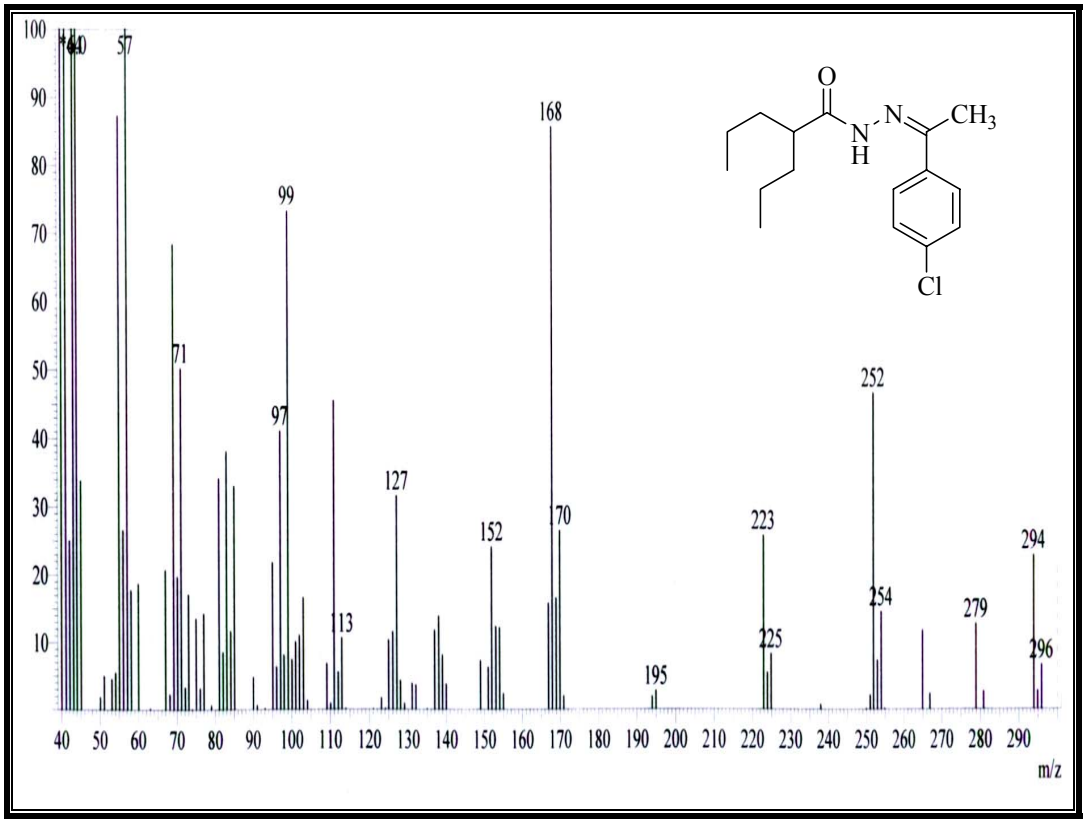
3.B.8.3 ^{1}H NMR Spectrum of SPA-01



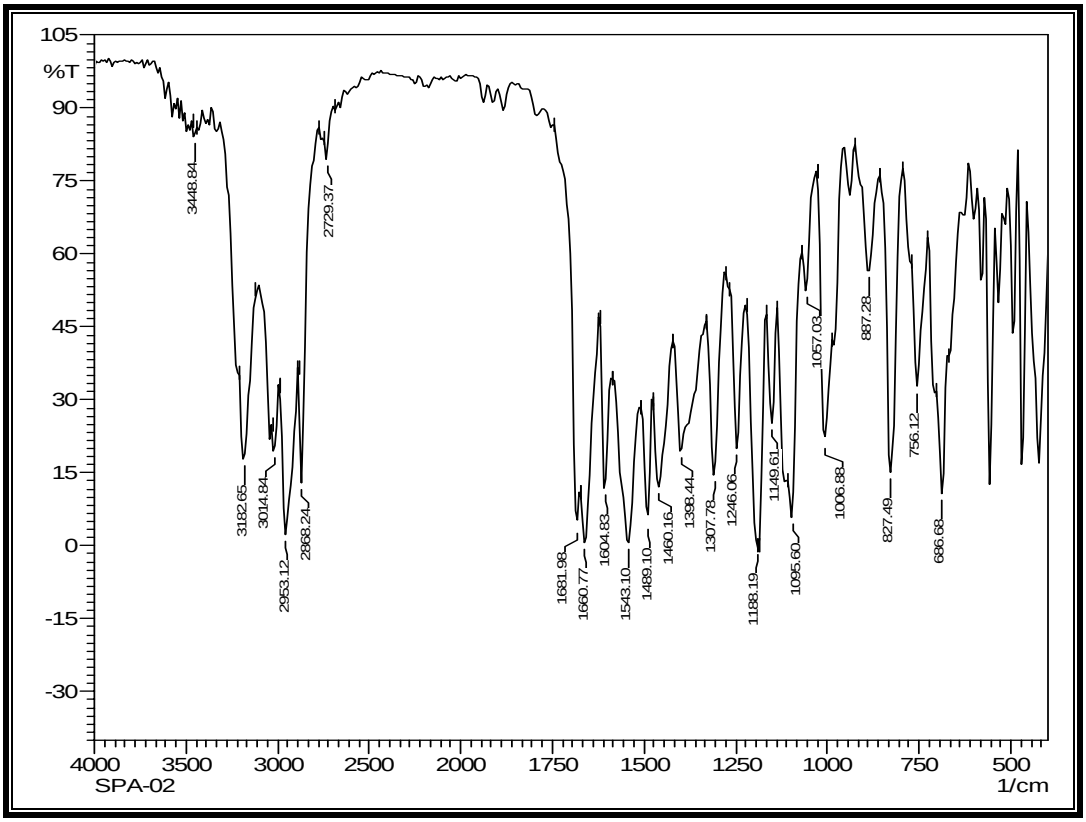
EXPANDED ^{1}H-NMR SPECTRUM OF SPA-01



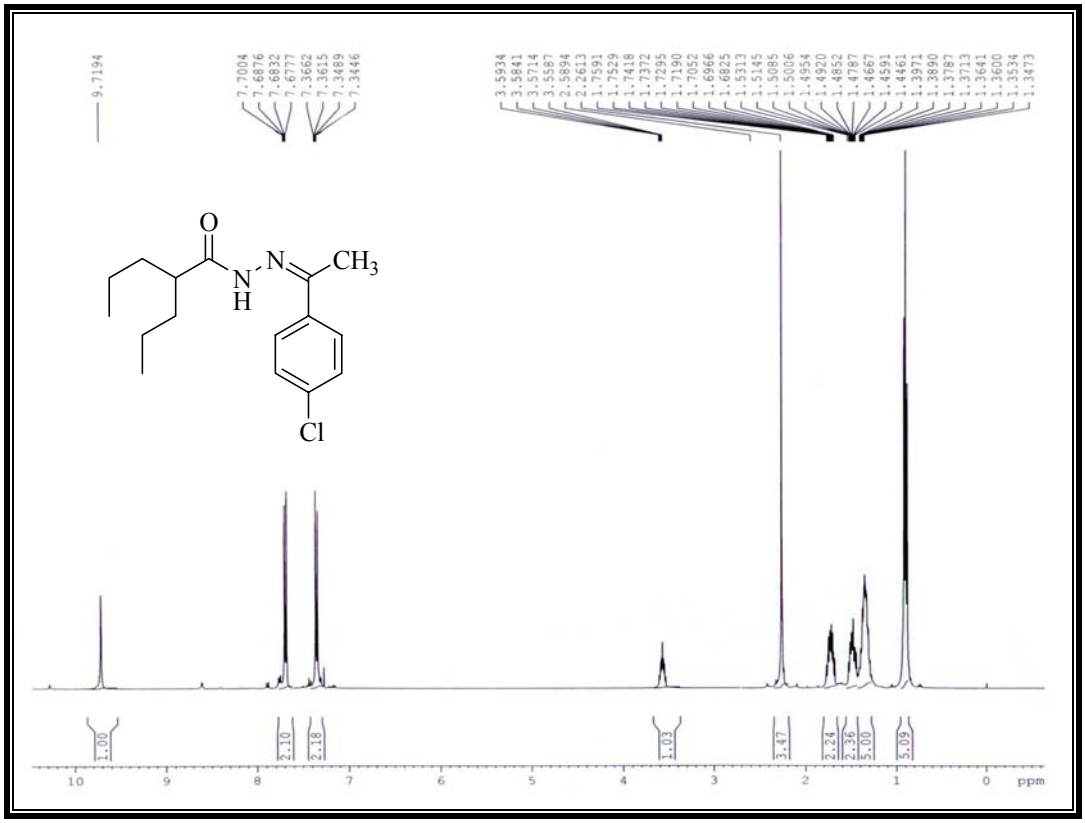
3.B.8.4 Mass Spectrum of SPA-02



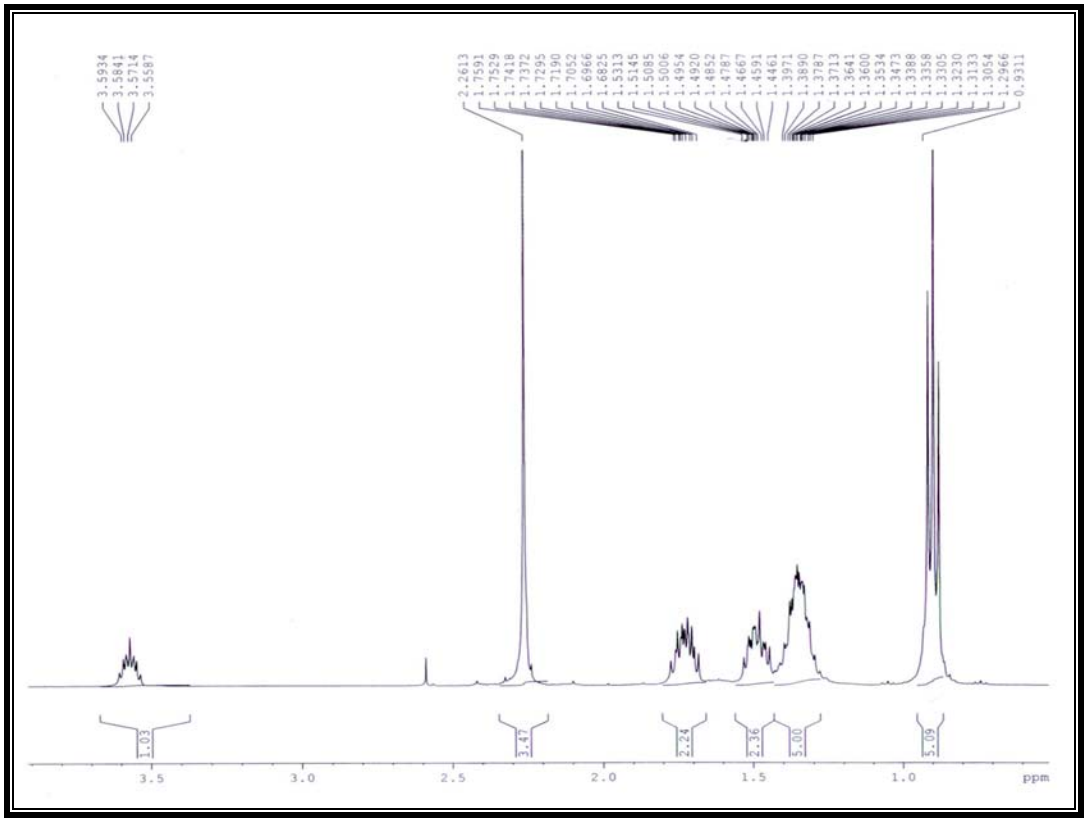
3.B.8.5 IR Spectrum of SPA-02



3.B.8.6 ^{1}H NMR Spectrum of SPA-02



EXPANDED ^{1}H-NMR SPECTRUM OF SPA-02



CHAPTER - 3

SECTION - C

STUDIES ON *N'*-SUBSTITUTED BENZOYL-2-PROPYL PENTANEHYDRAZIDE

3.C.1 AIM OF CURRENT WORK

The XC(CO)NHY linkage, under the assumption Y=H called the amide linkage, or referred to as the peptide linkage, is generally assumed to have a planar structure .

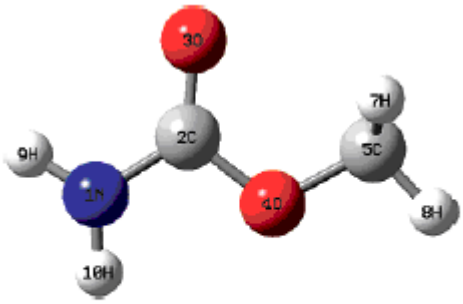
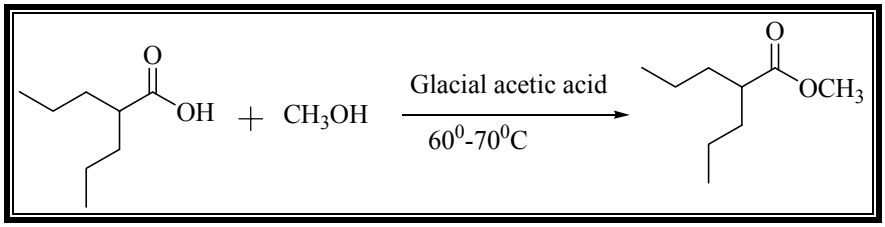


Figure-15: Conformation and atomic numbering of syn-methyl carbamate

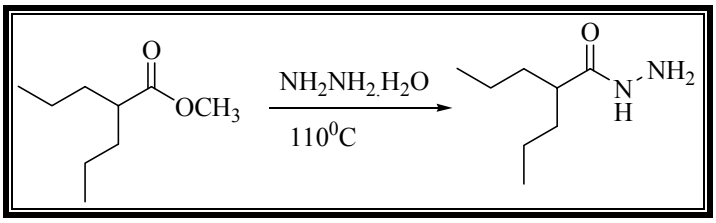
Several molecules containing the CONH linkage seem to have pyramidal nitrogen at equilibrium and a double-minimum inversion potential with a very small inversion barrier allowing for an effective planar ground-state structure. Various studies were conducted on development of various prodrugs and derivatives of Valproic acid. In continuations to the current research work that has been discussed in section-A. and section-B, an aryl amide linkage is created by using Valproic acid hydrazide to prepare several new derivatives.

3.C.2 REACTION SCHEMES

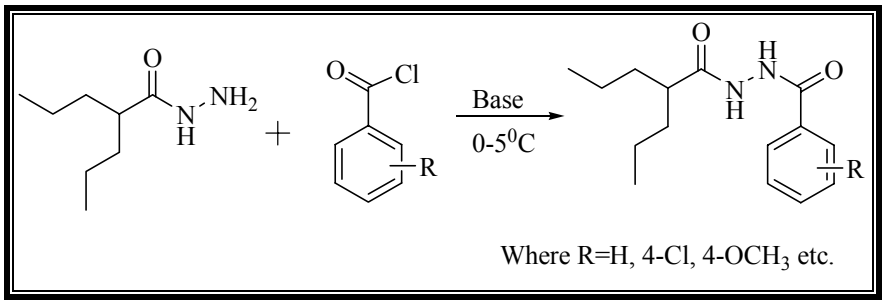
3.C.2.1 PREPARATION OF METHYL-2-PROPYL PENTANOATE



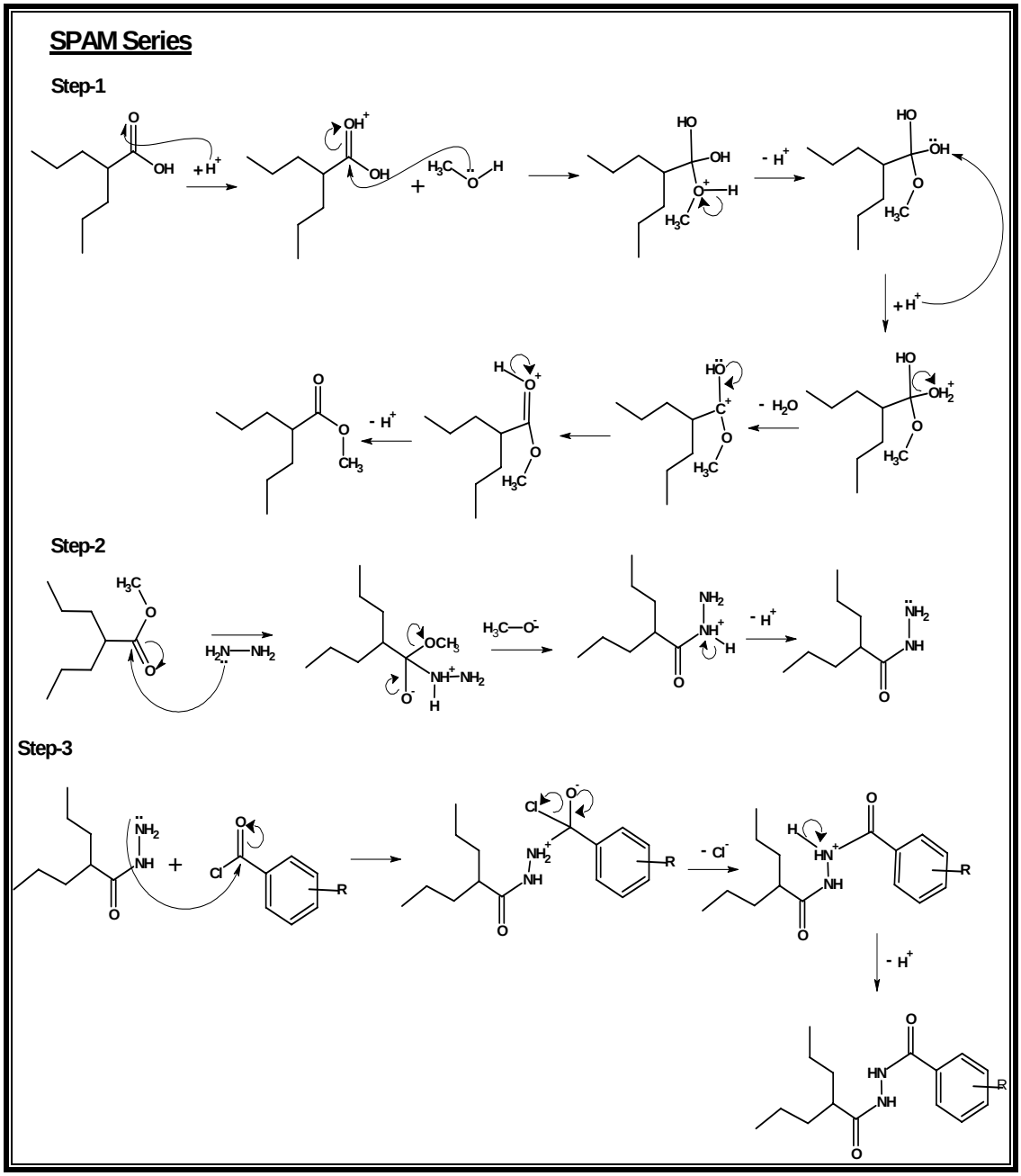
3.C.2.2 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE



3.C.2.3 PREPARATION OF N'-SUBSTITUTED BENZOYL-2-PROPYL PENTANEHYDRAZIDE



3.C.3 PLAUSIBLE REACTION MECHANISM



3.C.4 EXPERIMENTAL

3.C.4.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. Formation of all compounds was purified by using **flash chromatography**. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

3.C.4.2 PREPARATION OF METHYL 2-PROPYL PENTANOATE

2-propyl pentanoic acid (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of methanol was added into above flask. 3-4 drops of Con. Sulphuric acid was added as a catalyst. The reaction mixture was refluxed for 12-14 hours on water bath. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4: 6) as a mobile phase. After the reaction to be completed excess of methanol was removed under reduced pressure. The separated product was extracted using ethyl acetate (30 ml × 3), the combined organic layer was washed using 5% sodium bicarbonate solution (20 ml × 2) followed by water (20 ml × 2). The organic layer was dried on anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product in a viscous liquid form. B. P. - 170-172°C[a].

[a] Dostovalova, V. I. ; Organic Magnetic Resonance 1983, V21(1), P111-19.

3.C.4.3 PREPARATION OF 2-PROPYL PENTANOHYDRAZIDE

Methyl 2-propylpentanoate (0.01 mole) was charged into 250 ml round bottom flask. 15 ml of hydrazine hydrate was added into above flask. The reaction mixture was refluxed on water bath for 12-14 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (4 : 6) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature to give 2-propylpentanohydrazide as a white colored shining fluffy product. Yield - 60 %, M. P. - 124-126°C.[b]

3.C.4.4 GENERAL PROCEDURE OF N'-SUBSTITUTED BENZOYL-2-PROPYL PENTANEHYDRAZIDE

2-Propylpentanehydrazide (0.01 mole) dissolve it in 10-12 ml of Dichloromethane then add into of triethylamine (0.03 mole) as a catalyst and add into Substituted benzoyl chloride (0.01 mole) in MDC. at 0-5^0 C for 4-5 hrs under stirring. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the mixture was cooled to room temperature and poured into crushed ice. A solid product comes. Finally, it was purified by flash chromatography using hexane and ethyl acetate as eluents.

Similarly other compounds are also prepared.

[b] Benoit-Guyod, Jean L. ; Chemica Therapeutica 1968, V3(5), P336-42.

3.C.5 PHYSICAL DATA

PHYSICAL DATA OF N'-SUBSTITUTEDBENZOYL-2-PROPYL PENTANE - HYDRAZIDES (SPAM-01 TO SPAM-10)

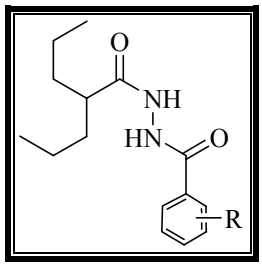


TABLE-3.C.1

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	MP ^{0}C	Rf Value
01	SPAM-01	4-Cl	$C_{15}H_{21}ClN_2O_2$	296	180-182	0.46
02	SPAM-02	H	$C_{15}H_{22}N_2O_2$	262	224-226	0.52
03	SPAM-03	4-OCH_3	$C_{16}H_{24}N_2O_3$	292	192-194	0.44
04	SPAM-04	4-CH_3	$C_{16}H_{24}N_2O_2$	276	244-246	0.48
05	SPAM-05	4-NO_2	$C_{15}H_{21}N_3O_4$	307	198-200	0.40
06	SPAM-06	3-NO_2	$C_{15}H_{21}N_3O_4$	307	202-204	0.54
07	SPAM-07	2-NO_2	$C_{15}H_{21}N_3O_4$	307	188-190	0.50
08	SPAM-08	2-CH_3	$C_{16}H_{24}N_2O_2$	276	224-226	0.52
09	SPAM-09	3-Cl	$C_{15}H_{21}ClN_2O_2$	296	212-214	0.42
10	SPAM-10	2-Cl	$C_{15}H_{21}ClN_2O_2$	296	200-202	0.44

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (7: 3)

3.C.6 SPECTRAL DISCUSSION

3.C.6.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. SPAM-01 and SPAM-02 of Mass spectra are given on page no. 116 and 118.

3.C.6.2 IR SPECTRA

IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

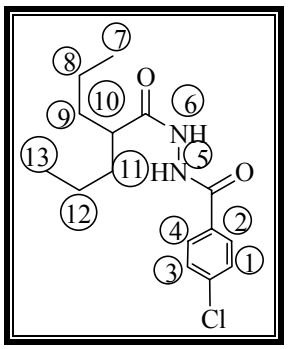
N-H stretching vibrations were observed near 3440 cm^{-1}, There are two carbonyl groups present in all the compounds but due to the same environment, the peaks merged in the region of 1680 cm^{-1}, N-H bending vibrations were observed near 1600 cm^{-1}, C-H stretching frequencies were observed between 2810 cm^{-1} to 3000 cm^{-1}, while ring skeleton frequencies were observed near 1460 cm^{-1} in all the compounds. SPAM-01 and SPAM-02 of IR spectra are given on page no. 116 and 118.

3.C.6.3 ^{1}H NMR SPECTRA

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO-d_6/$CDCl_3$ solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from proton NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify *o, m* and *p* coupling.

^{1}H NMR spectral interpretation can be discussed as under.

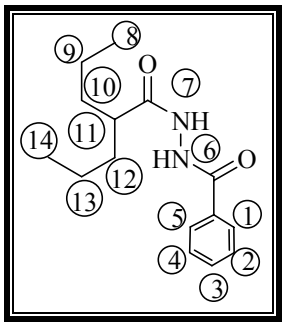
^{1}H NMR OF N'-4-CHLOROBENZOYL-2-PROPYL PENTANEHYDRAZIDE (SPAM-01)



1. The Proton no.1 and 3 of aromatic ring of two proton gave a characteristic doublet at 7.35 δ ppm-7.37 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
2. The Proton no. 2 and 4 of aromatic ring of two proton gave a characteristic doublet at 7.85 δ ppm-7.87 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests a para coupled.
3. The Proton no. 7 and 13 of two propyl chain of six proton gave a multiplet at 0.86 δ ppm -0.92 δ ppm.
4. Proton no. 8, 9, and 11 of two propyl chain gave a multiplet at 1.25 δ ppm - 1.47 δ ppm.
5. Proton no. 12 of propyl chain gave a multiplet at 1.55 δ ppm- 1.66 δ ppm.
6. Proton no. 10 of propyl chain gave a multiplet at 2.33 δ ppm -2.38 δ ppm.
7 Two most deshielded proton no. 5 and 6 of secondary amine in hydrazide linkage (-NH) gave two separable singlet in the down field at 9.72 δ ppm. and 10.37 δ ppm. Respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPAM-01 has been confirmed. Spectrum is given on page no. 117.

^{1}H NMR OF N'-BENZOYL-2-PROPYLPENTANEHYDRAZIDE (SPAM-02)



1. The Proton no. 2 and 4 of aromatic ring gave a triplet at 7.27 δ ppm -7.34 δ ppm.
2. The Proton no. 1 and 5 of aromatic ring gave a doublet at 7.79 δ ppm -7.81 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests a para coupled.
3. Proton no. 3 of aromatic ring gave a multiplet at 7.44 δ ppm -7.48 δ ppm.
4. The Proton no. 8 and 14 of two propyl chain gave a multiplet at 0.80 δ ppm-0.88 δ ppm.
4. Proton no. 9, 10 and 12 of two propyl chain gave a multiplet at 1.25 δ ppm -1.44 δ ppm.
5. Proton no. 13 of propyl chain gave a multiplet at 1.57 δ ppm-1.66 δ ppm.
6. Proton no. 11 of propyl chain gave a multiplet at 2.38 δ ppm -2.42 δ ppm.
7 Two most deshielded proton no. 6 and 7 of secondary amine in hydrazide linkage of two (-NH) group gave two separable singlet in the down field at 9.90 δ ppm. and 10.66 δ ppm. Respectively.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPAM-02 has been confirmed. Spectrum is given on page no. 119.

3.C.6.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

3.C.7 ANALYTICAL DATA

***N'-4-chlorobenzoyl-2-propyl pentanehydrazide (SPAM-01)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3444 (-NH stretching vib.), 3030 (-CH_3), 2868 (-CH_2), 1670 (-C=O), 1604 (-NH bending vib.), 1498, 1467 (aromatic ring skleton); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.92 (6H, m), 1.47 (6H, m), 1.66 (2H, m), 2.38 (1H, m), 7.37 (2H, d, *J*=8.0 Hz), 7.87 (2H, d, *J*=8.0 Hz), 9.72 (1H, d), 10.37 (1H, d), MS *m/z* = 296 (M^+), 298 (M^{+2}); Anal. Calcd. for $C_{15}H_{21}ClN_2O_2$: C, 60.70; H, 7.13; N, 9.44; O, 10.78. Found: C, 60.66; H, 7.11; N, 9.41; O, 10.73%.

***N'-benzoyl-2-propylpentanehydrazide (SPAM-02)*:**

Yield: 76%, IR (KBr, cm^{-1}): 3439 (-NH stretching vib.), 3026, 2953 (-CH_3), 2868 (-CH_2), 1670 (-C=O), 1602 (-NH bending vib.), 1500, 1469 (aromatic ring skleton); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 0.88 (6H, m), 1.44 (6H, m), 1.66 (2H, m), 2.42 (1H, m), 7.34 (2H, m), 7.48 (1H, m), 7.81 (2H, m), 9.90 (1H, d), 10.66 (1H, d); MS *m/z* = 262 (M^+); Anal. Calcd. for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68; O, 12.20. Found: C, 68.61; H, 8.42; N, 10.63; O, 12.17%.

***N'-4-methoxybenzoyl-2-propylpentanehydrazide (SPAM-03)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3448 (-NH stretching vib.), 3046, 2958 (-CH_3), 2846 (-CH_2), 1672 (-C=O), 1602 (-NH bending vib.), 1494, 1456 (aromatic ring skleton); MS *m/z* = 292 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58; O, 16.42. Found: C, 65.70; H, 8.24; N, 9.53; O, 16.41%.

***N'-4-methylbenzoyl-2-propylpentanehydrazide (SPAM-04)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3438 (-NH stretching vib.), 3054, 2958 ($-CH_3$), 2852 ($-CH_2$), 1666 (-C=O), 1600 (-NH bending vib.), 1500, 1454 (aromatic ring skleton); MS *m/z* = 276 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14; O, 11.58. Found: C, 69.51; H, 8.72; N, 10.11; O, 11.52%.

***N'-4-nitrobenzoyl-2-propyl pentanehydrazide (SPAM-05)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3458 (-NH stretching vib.), 3040, 2958 ($-CH_3$), 2838 ($-CH_2$), 1672 (-C=O), 1608 (-NH bending vib.), 1488, 1450 (aromatic ring skleton); MS *m/z* = 307 (M^+); Anal. Calcd. for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67; O, 20.82. Found: C, 58.60; H, 6.82; N, 13.63; O, 20.78%.

***N'-3-nitrobenzoyl-2-propyl pentanehydrazide (SPAM-06)*:**

Yield: 82%, IR (KBr, cm^{-1}): 3446 (-NH stretching vib.), 3046, 2944 ($-CH_3$), 2842 ($-CH_2$), 1666 (-C=O), 1608 (-NH bending vib.), 1494, 1438 (aromatic ring skleton); MS *m/z* = 307 (M^+); Anal. Calcd. for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67; O, 20.82. Found: C, 58.61; H, 6.85; N, 13.61; O, 20.81%.

***N'-2-nitrobenzoyl-2-propyl pentanehydrazide (SPAM-07)*:**

Yield: 80%, IR (KBr, cm^{-1}): 3448 (-NH stretching vib.), 3028, 2950 ($-CH_3$), 2846 ($-CH_2$), 1670 (-C=O), 1602 (-NH bending vib.), 1502, 1444 (aromatic ring skleton); MS *m/z* = 307 (M^+); Anal. Calcd. for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67; O, 20.82. Found: C, 58.60; H, 6.84; N, 13.63; O, 20.80%.

***N'-2-methylbenzoyl-2-propylpentanehydrazide (SPAM-08)*:**

Yield: 82%, IR (KBr, cm^{-1}): 3456 (-NH stretching vib.), 3042, 2956 ($-CH_3$), 2838 ($-CH_2$), 1674 (-C=O), 1604 (-NH bending vib.), 1490, 1456 (aromatic ring skleton); MS *m/z* = 276 (M^+); Anal. Calcd. for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14; O, 11.58. Found: C, 69.51; H, 8.71; N, 10.12; O, 11.52%.

***N'-3-chlorobenzoyl-2-propyl pentanehydrazide (SPAM-09)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3448 (-NH stretching vib.), 3038, 2954 ($-CH_3$), 2822 ($-CH_2$), 1670 (-C=O), 1600 (-NH bending vib.), 1474, 1456 (aromatic ring skleton); MS

m/z = 296 (M^+), 298 (M^{+2}); Anal. Calcd. for $C_{15}H_{21}ClN_2O_2$: C, 60.70; H, 7.13; N,9.44; O, 10.78. Found: C, 60.68; H, 7.10; Cl, 11.92; N, 9.42; O, 10.71%.

***N'-2-chlorobenzoyl-2-propyl pentanehydrazide (SPAM-10)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3458 (-NH stretching vib.), 3020, 2946 (-CH_3), 2832 (-CH_2), 1658 (-C=O), 1608 (-NH bending vib.), 1488, 1454 (aromatic ring skleton); MS *m/z* = 296 (M^+), 298 (M^{+2}); Anal. Calcd. for $C_{15}H_{21}ClN_2O_2$: C, 60.70; H, 7.13; N, 9.44; O, 10.78. Found: C, 60.66; H, 7.10; N, 9.43; O, 10.74%.

3.C.8 RESULTS AND DISCUSSION

Several 2-propylpentanohydrazide were prepared by reacting hydrazine hydrate with Methyl-2-propylpentanoate. Methyl-2-propylpentanoate was prepared by the esterification of 2-propylpentanoic acid which is also known as *Valproic acid*.

In Section-A the targeted compounds such as N'-(2-(substituted amino)-acetyl)-2-propylpentanehydrazide were synthesized from N'-(2-chloroacetyl)-2-propylpentanehydrazide by using base catalyst. These types of the compounds were synthesized at room temperature.

In Section-B the targeted compounds such as N'-(1-(substituted phenyl) ethylidene) 2-propylpentanehydrazide were synthesized from 2-propylpentanohydrazide by using glacial acetic acid as a catalyst. These types of the compounds were synthesized using Microwave assisted method which was found to be much faster than the conventional and the % yield was found to be higher than conventional methods.

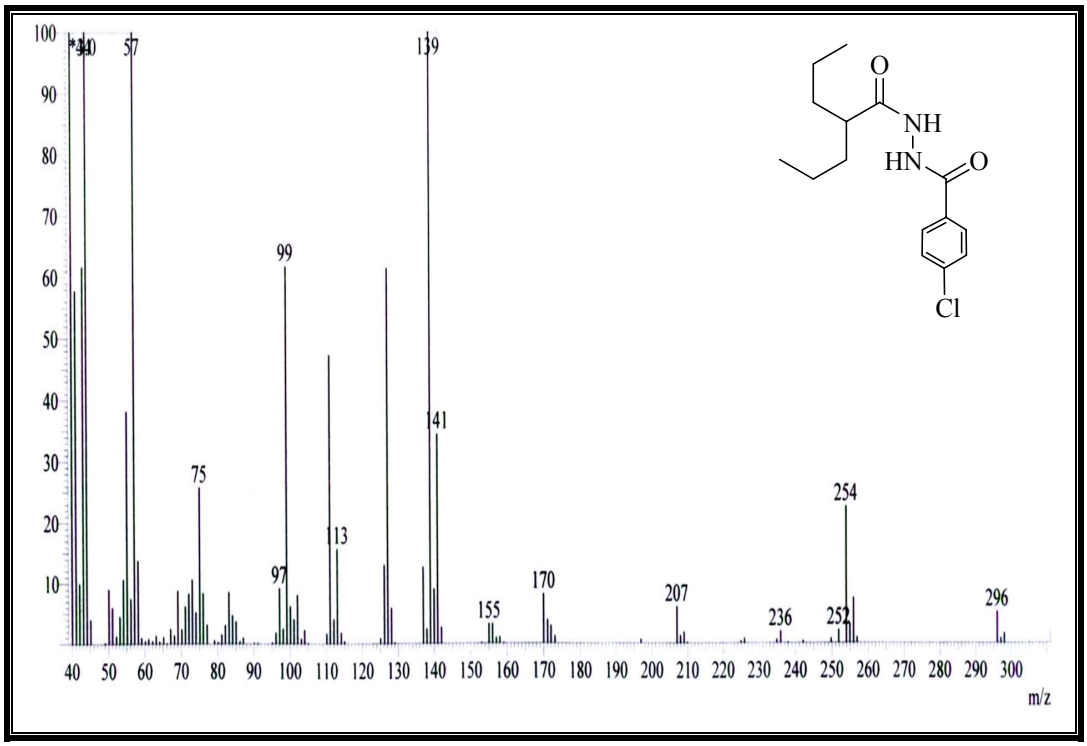
In Section-C the targeted compounds such as N'-substituted benzoyl-2-propyl pentanehydrazide were synthesized from 2-propylpentanohydrazide by using base catalyst. These types of the compounds were synthesized at room temperature.

3.C.9 CONCLUSION

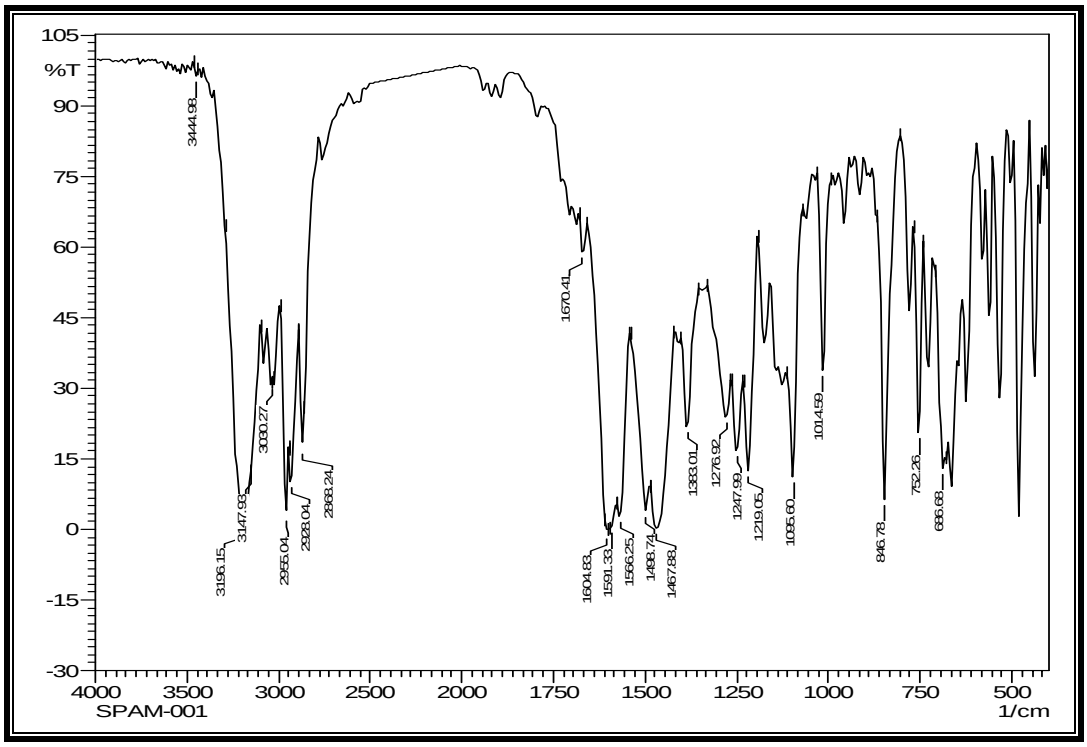
In conclusion, the compounds enlisted in this chapter are novel bioactive compounds for various biological activities.

3.C.10 REPRESENTATIVE SPECTRA

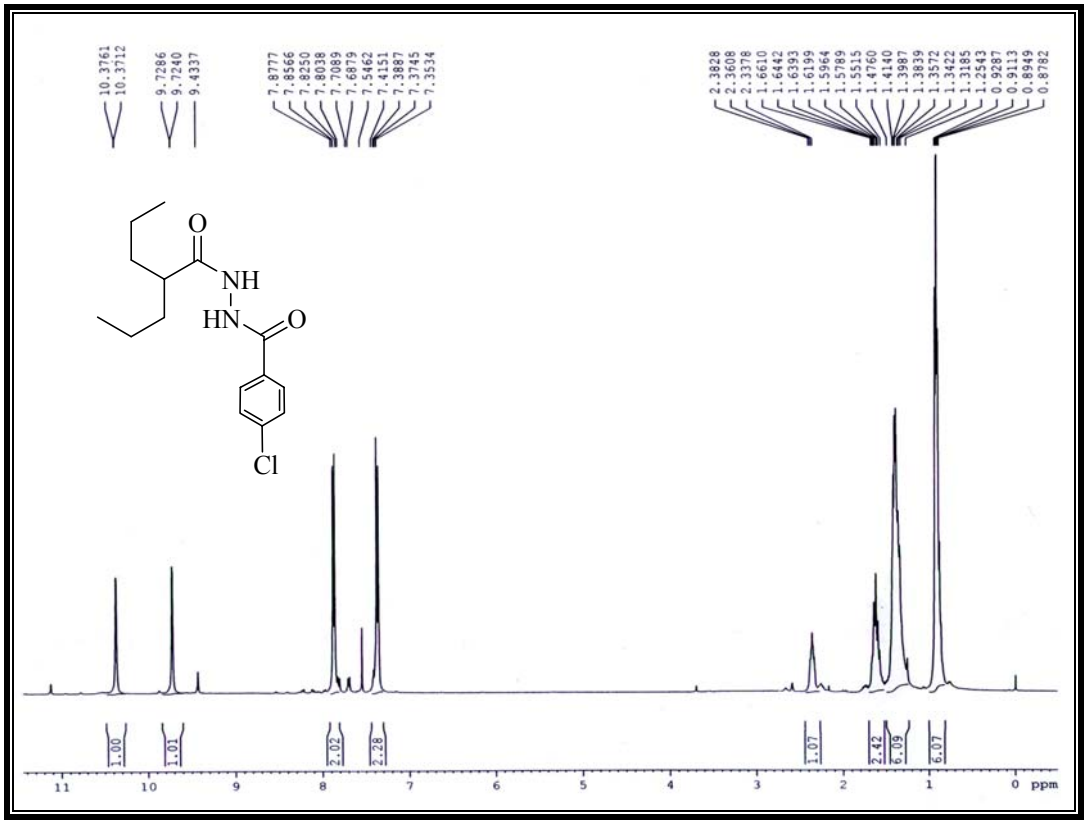
3.C.10.1 Mass Spectrum of SPAM-01



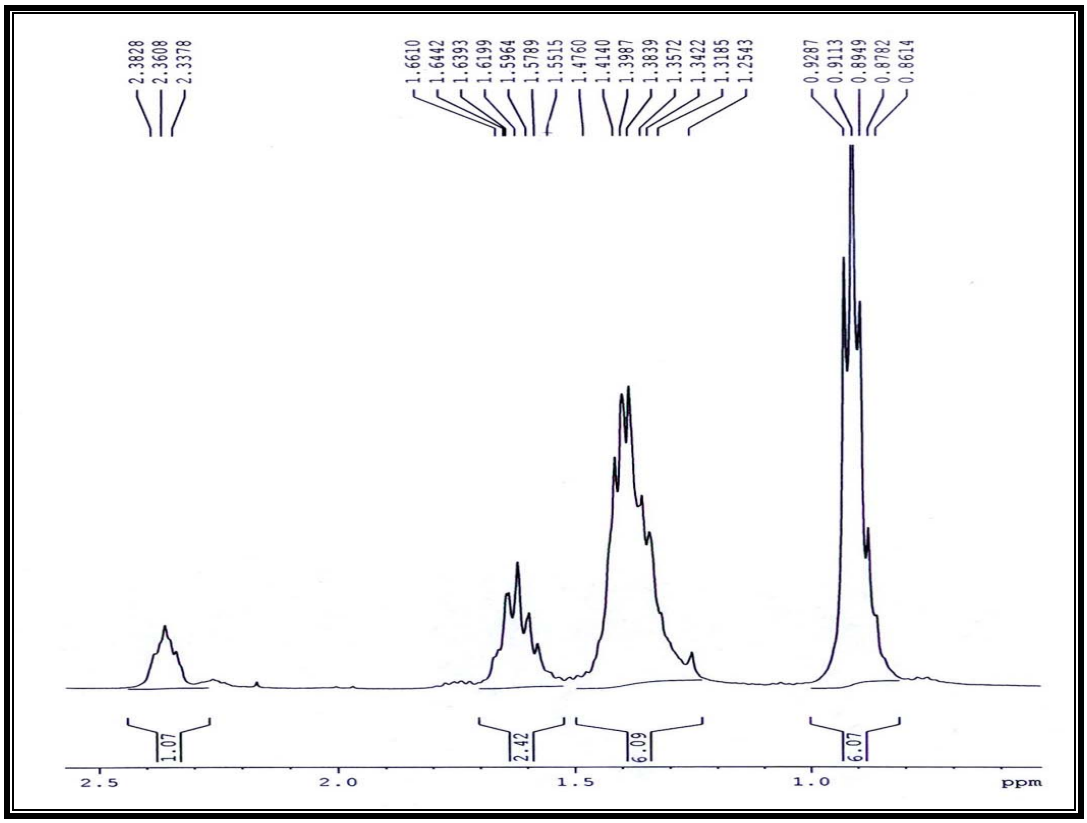
3.C.10.2 IR Spectrum of SPAM-01



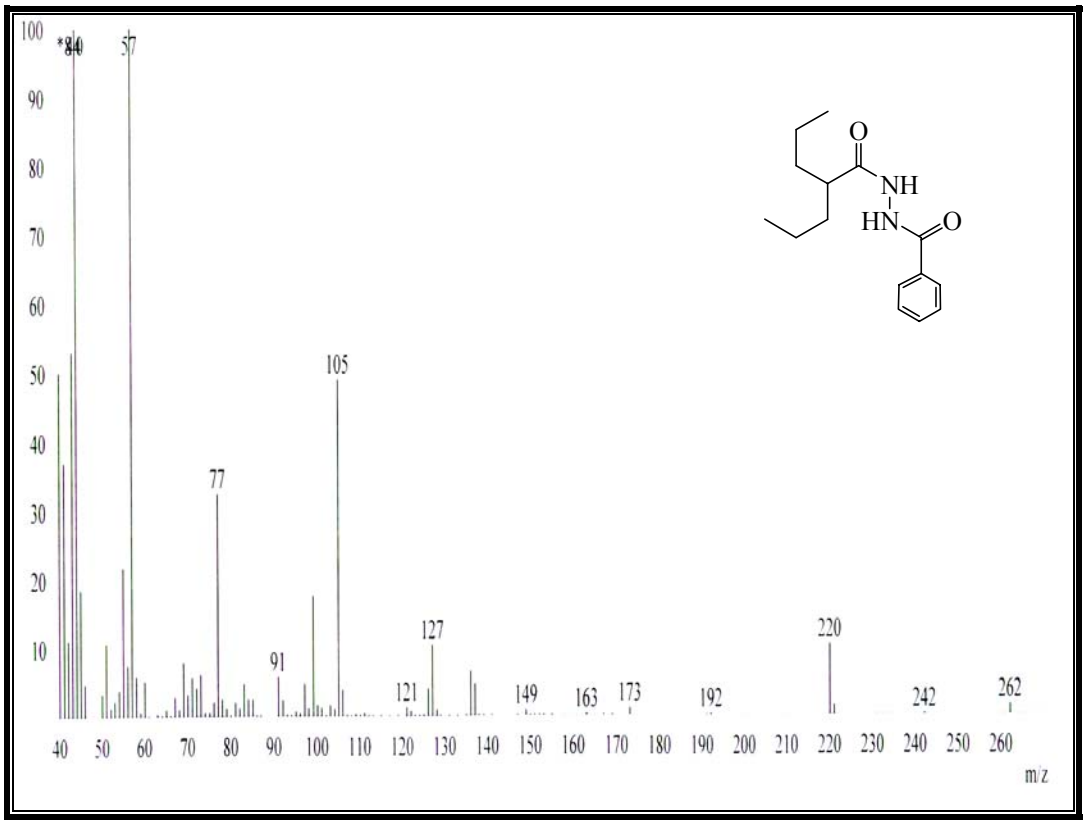
3.C.10.3 ^{1}H NMR Spectrums of SPAM-01



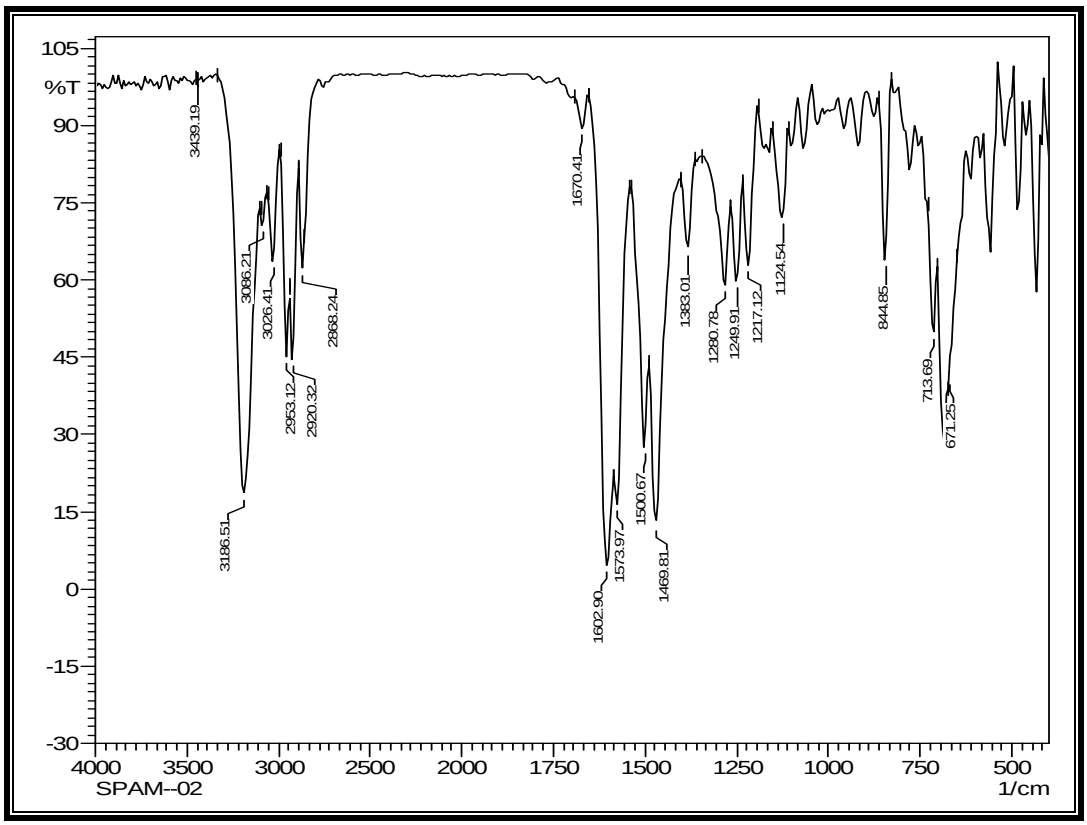
EXPANDED ^{1}H-NMR SPECTRUM OF SPAM-01



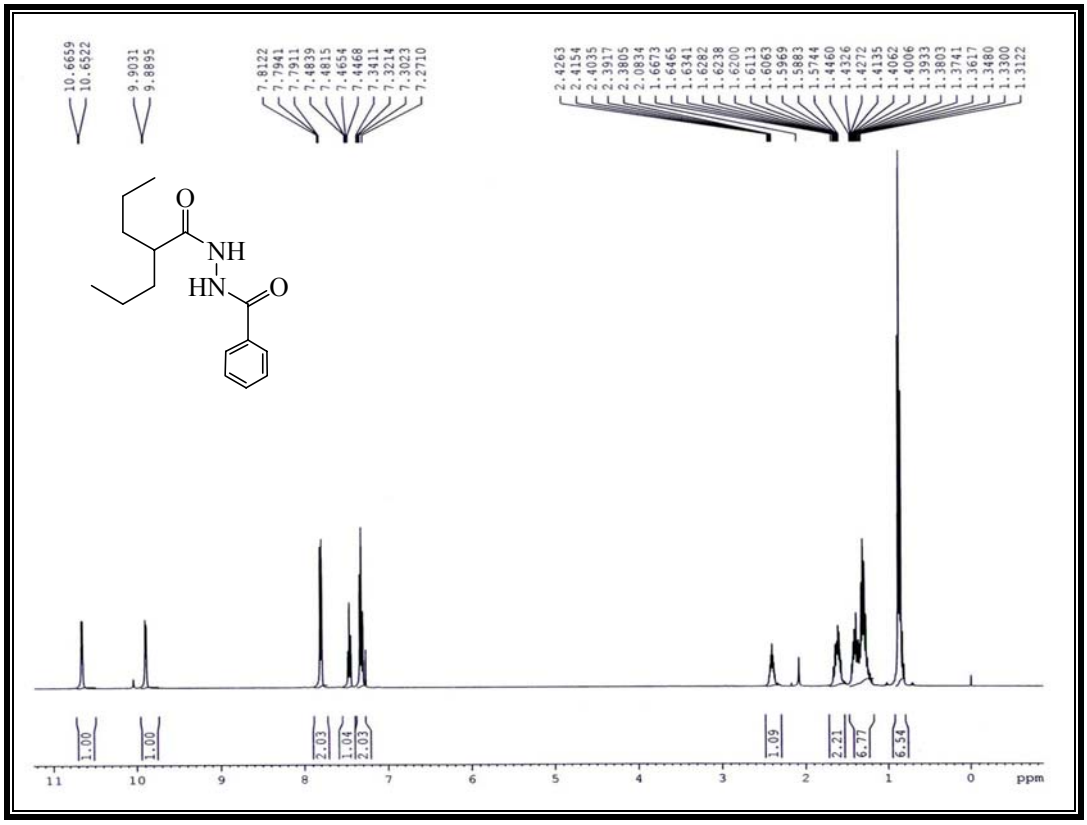
3.C.10.4 Mass Spectrum of SPAM-02



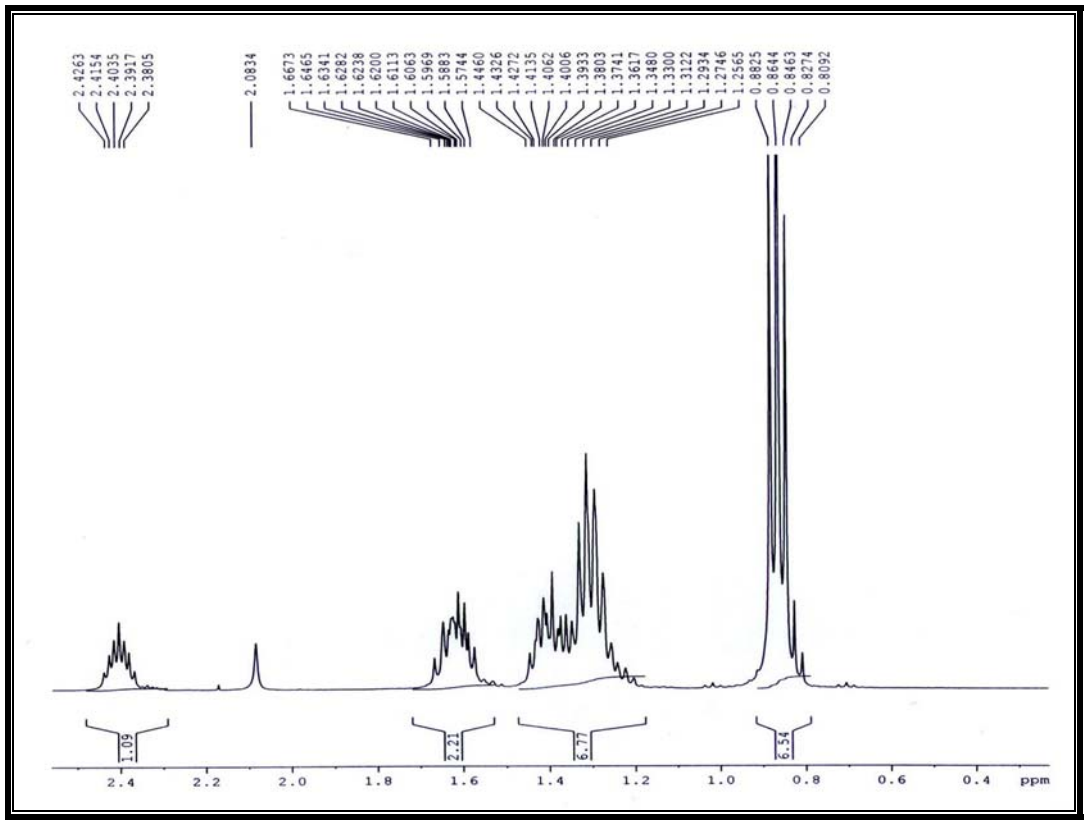
3.C.10.5 IR Spectrum of SPAM-02



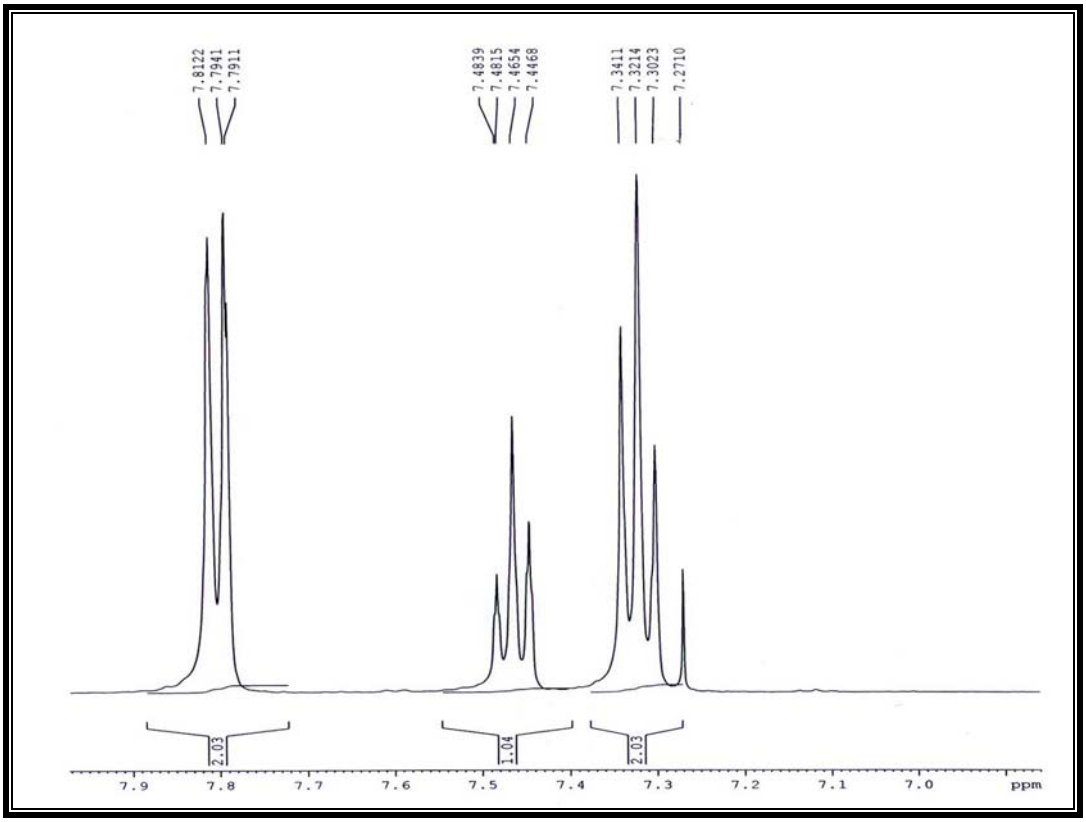
3.C.10.6 ^{1}H NMR Spectrum of SPAM-02



EXPANDED ^{1}H-NMR SPECTRUM OF SPAM-02



EXPANDED ^{1}H-NMR SPECTRUM OF SPAM-02



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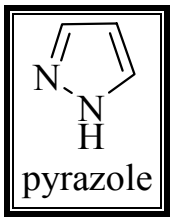
CHAPTER - 4

SECTION - A

A RAPID MICROWAVE ASSISTED SYNTHESIS OF 1-METHYL-3-((1, 3 (SUBSTITUTED)-DIPHENYL-1H-PYRAZOL-4-YL)METHYLENE) QUINOLINE -2,4-(1H,3H)-DIONE

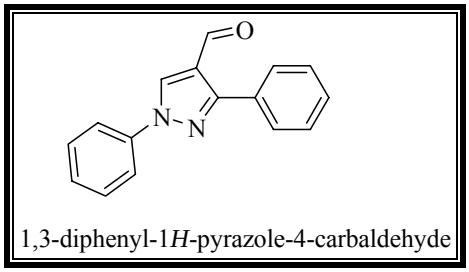
4.A.1 PYRAZOLES : A VERSATILE SYNTHON

Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a five-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature.



pyrazole

The synthesis of pyrazoles remains of great interest owing to the wide applications in pharmaceutical and agrochemical industry due to their herbicidal, fungicidal, insecticidal, analgesic, antipyretic and anti-inflammatory properties.[1, 2] Some methods have been developed in recent years, though the most important method is the reaction between hydrazines and β-dicarbonyl compounds.[3] This reaction involves the double condensation of 1, 3-diketones or α, β-unsaturated ketones with hydrazine or its derivatives.[4, 5] However, the appealing generality of this method is somewhat vitiated by the severe reaction conditions or the multistep sequences usually required to access the starting materials.[6] Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies for this class of compounds.[7]



1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde

The application of Vilsmeier–Haack (VH) reagent ($POCl_3$ / DMF) for formylation of a variety of both aromatic and heteroaromatic substrates is well

documented.[8] Besides this, the reagent has also been extensively used for effecting various chemical transformations from other classes of compounds. Many of these reactions have led to novel and convenient routes for the synthesis of various heterocyclic compounds.[9] A notable example that finds significant application in heterocyclic chemistry is the synthesis of 4-formylpyrazoles from the double formylation of hydrazones with Vilsmeier-Haack (VH) reagent.[10,11] These observations, coupled with the recent developments on the simple synthesis of pyrazole derivatives,[1,2] especially 4-functionalized 1, 3-diphenylpyrazoles as antibacterial,[12] anti-inflammatory,[13,14] antiparasitic,[15] and antidiabetic[16] drugs, prompted chemistry research to undertake the synthesis of pyrazole-4-carboxldehyde derivatives using Vilsmeier-Haack (VH).[17-19] The study is particularly aimed at developing a one-pot synthesis of pyrazole-4-carboxaldehyde oximes starting from acetophenone phenylhydrazones.

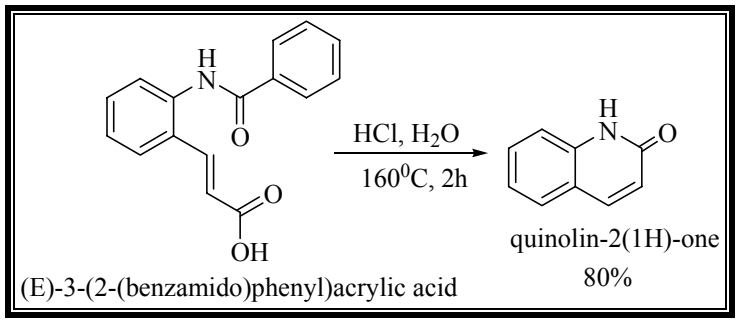
The subsequent details are regarding several approaches related to quinolone nucleus, its modification and synthetic methods reported.

4.A.2 METHODS FOR PREPARATION : 2-QUINOLONES

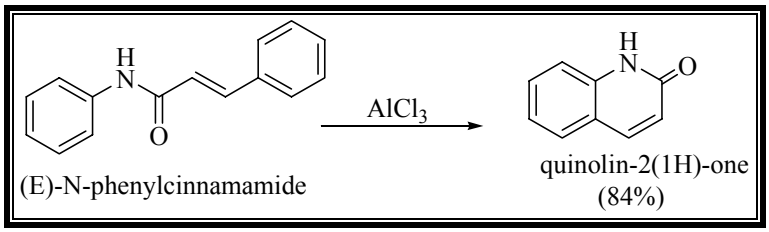
2-quinolones were prepared by various routes. However, we are discussing a few:-

Following methods are used as most common and recent preparation strategies.

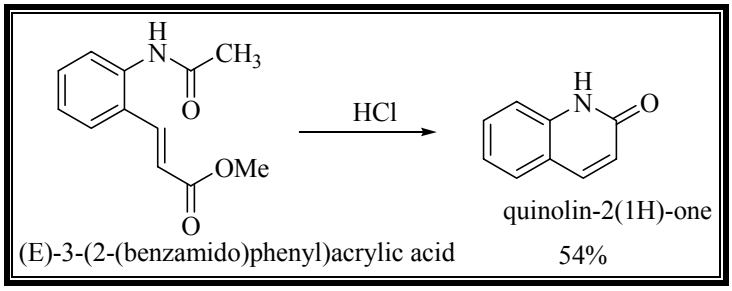
Cyclization of o-benzamide cinnamic acid was discussed using HCl involving steps of condensation and deacylation.[20]



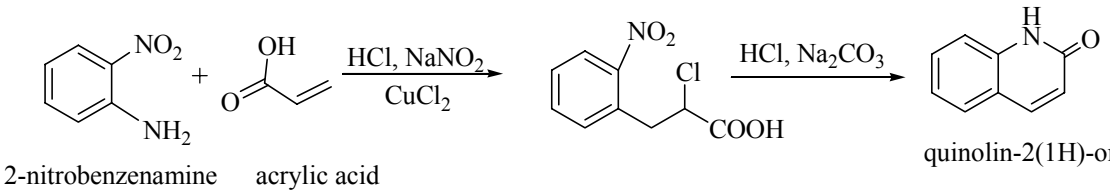
Cyclization of N-phenylcinnamamide in presence of $AlCl_3$[21]



Hiroshi et al[22] discuss ortho vinylation of aromatic anilines in presence of HCl via mechanism of cyclopalldation to prepare various nitrogen heterocycles including 2-quinolones

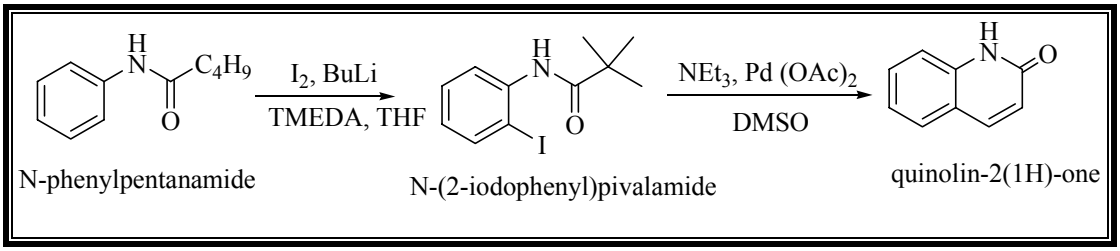


McCord et al[23] discuss synthesis of 2-quinolones and substituted quinolones and other heterocycles by using 2-nitro aniline and substituted 1-propionic acid in presence of HCl and Na_2CO_3



2-nitrobenzenamine acrylic acid quinolin-2(1H)-one

2-quinolones can also be prepared by iodination of 2,2-dimethyl-N-phenyl propanamide in presence of iodine and butyl lithium and N,N,N',N'-tetramethyl ethylene diamine. The product obtained was further cyclised using 2-Propenoic acid, methyl ester in presence of triethylamine and palladium acetate[24]

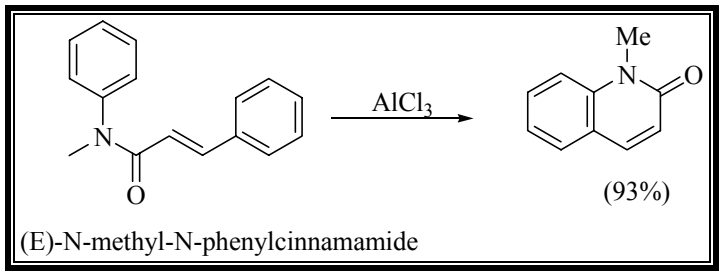


N-phenylpentanamide N-(2-iodophenyl)pivalamide quinolin-2(1H)-one

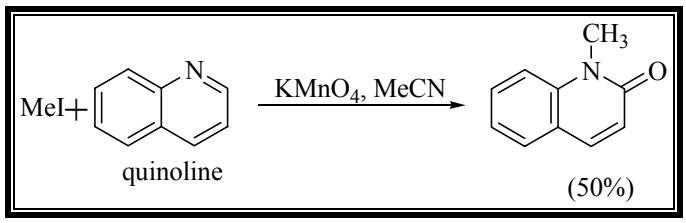
4.A.3 METHODS FOR PREPARATION : N-METHYL-2-QUINOLONES

Several methods are reported for the preparation of 4-methyl quinolone. Many of these are synthesized bellow.

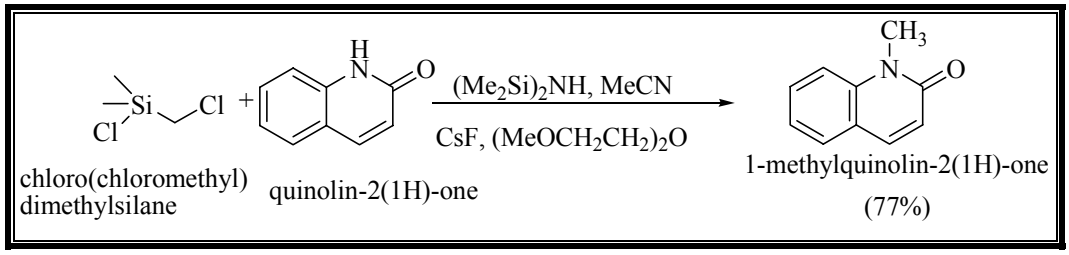
1) It was prepared by cyclisation of N-methyl-N-phenylcinnamamide in presence of $AlCl_3$[25]



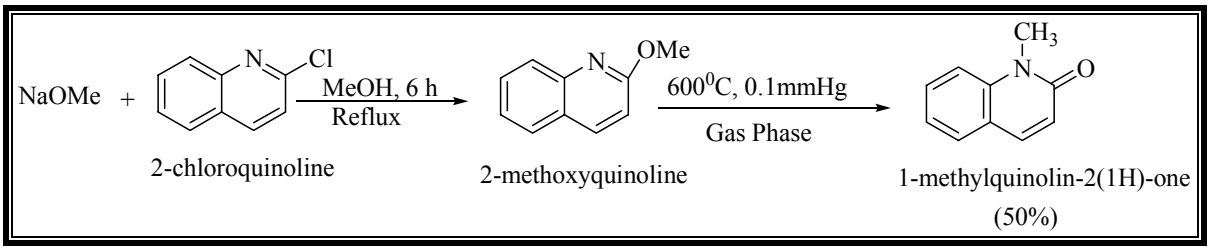
2) Oxidation of quinoline in presence of $KMnO_4$ and Methyl Iodide results in 50% of N-methyl-2-quinolone.[26]



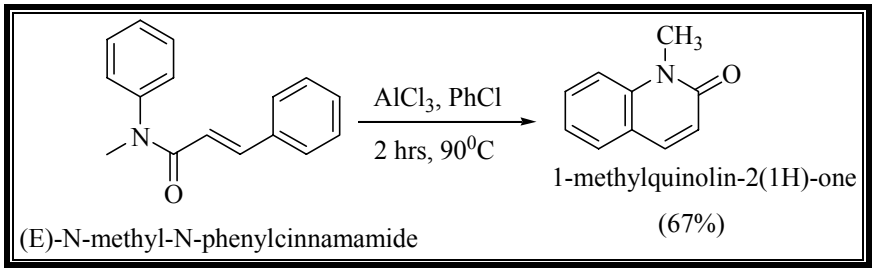
3) Chemoselective methylation of amides and heterocycles using chloromethyldimethylsilyl chloride in presence of other nucleophiles selectively yielded 77% of N-methyl-2-quinolone.[27]



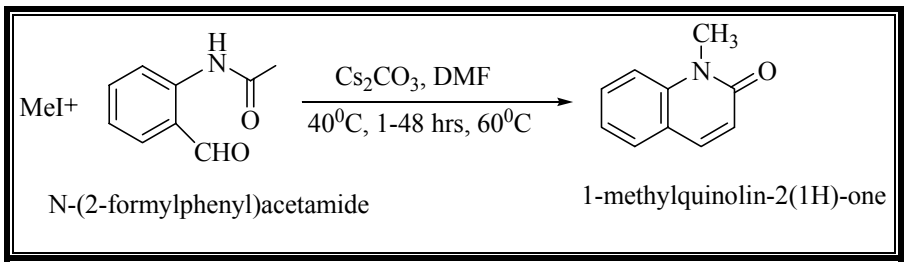
4) A rearrangement reaction of 2-methoxypyridine under Flash Vacuum Pyrolysis (FVP) conditions involving high temperature, low pressure and gas phase conditions to yield 50% of N-methyl-2-quinolone[28]



5) Cyclisation reaction of N-aryl cinnamamides in presence of $AlCl_3$ yielded desired products in 67% yield. Use of Bismuth Chloride gives selective cyclization[29]

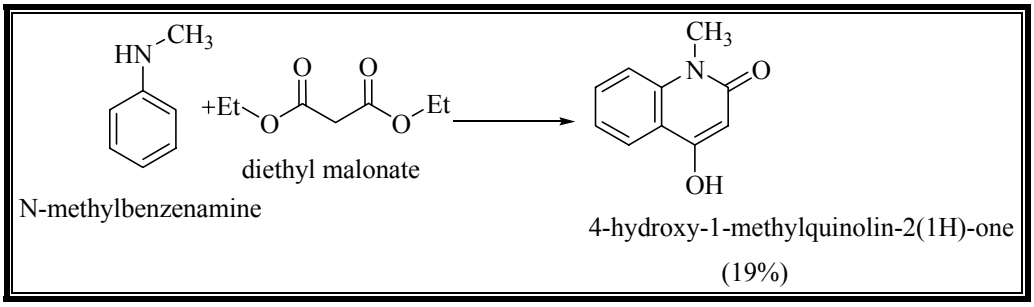


6) Similarly, cyclization reaction yielding N-methyl-2quinolones by 2-Nitrobenzaldehydes. It involved the first step of reduction of 2-Nitrobenzaldehydes to 2-aminobenzaldehyde, which was reacted immediately with acylchlorides to give 2-carboxyamidobenzaldehydes. Subsequent reaction with a base yielded 3-substituted quinolinones. Further, treatment with Iodine in Methyl Iodide in basic medium gave 3-substituted 1-methyl 2-quinolone.[30]

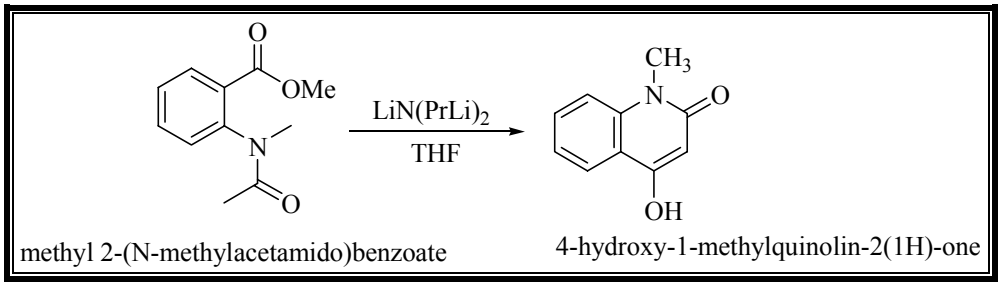


4.A.4 METHODS FOR PREPARATION : 4-HYDROXY-N-METHYL-2-QUINOLONES

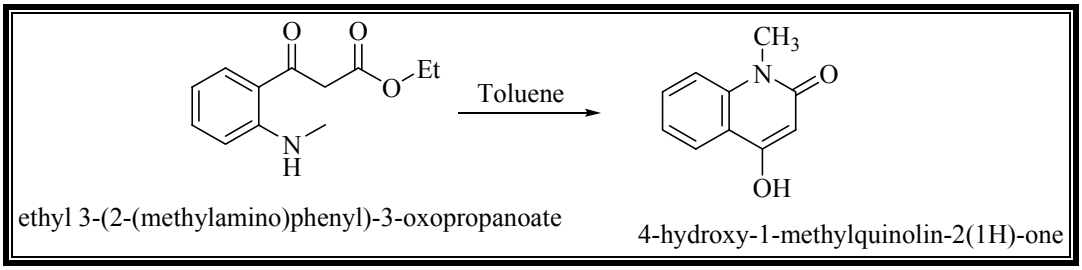
1) Condensation of methylaniline and malonic acid diethyl ester yielded 19% of 4-Hydroxy-N-methyl-2-quinolone[31]



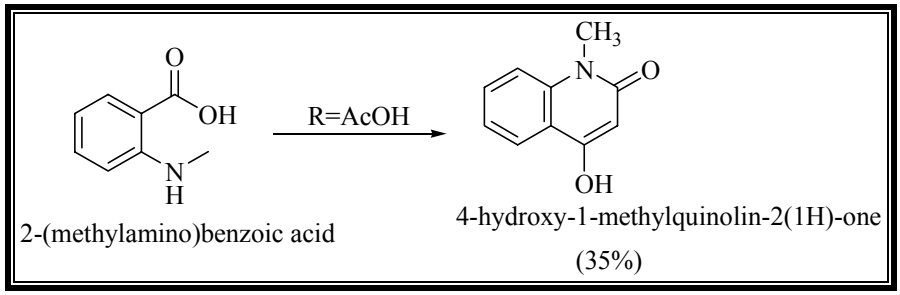
2) Cyclization reaction of N-acetyl-N-methyl anthranilic acid methyl ester in presence of LDA and THF gave the desired compound[32]



3) Another cyclization reaction of compound ethyl 3(2-(methylamino) phenyl)-3-oxopropanoete in toluene yields 4-hydroxy-N-methyl-2-quinolone.[33]



4) Some authors used mushroom tyrosinase as catalyst to synthesize coumestans, benzofurans and other heterocyclic compounds. Similarly, they used N-methylanthranilic acid and acetic acid in presence of mushroom tyrosinase[34]

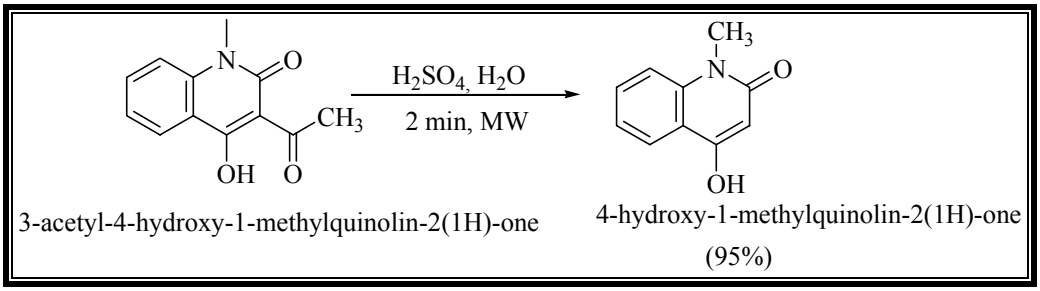


2-(methylamino)benzoic acid

4-hydroxy-1-methylquinolin-2(1H)-one

(35%)

5) Deacetylation under acidic conditions under MicroWave irradiation for 2 mins resulted in 95% if 4-hydroxy-N-methyl-2-quinolone.[35]

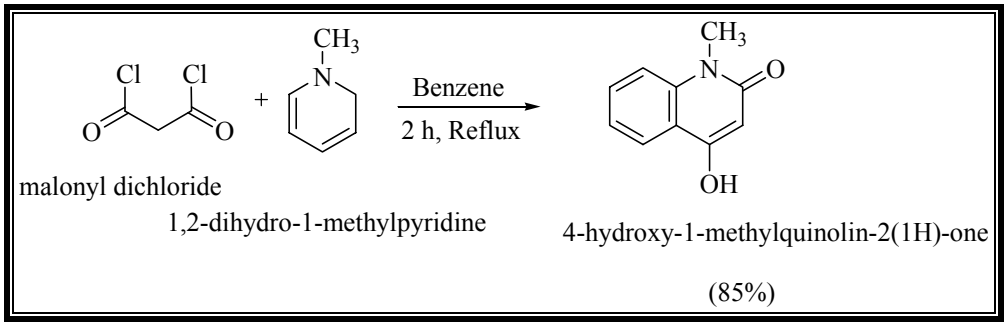


3-acetyl-4-hydroxy-1-methylquinolin-2(1H)-one

4-hydroxy-1-methylquinolin-2(1H)-one

(95%)

6) A convenient one-pot synthesis of 2,4-quinolone by condensation of malonyl chloride with N-methylaniline in presence of benzene under 2 hours reflux resulted in 85 % of 4-hydroxy-N-methyl-2-quinolone[36]



malonyl dichloride

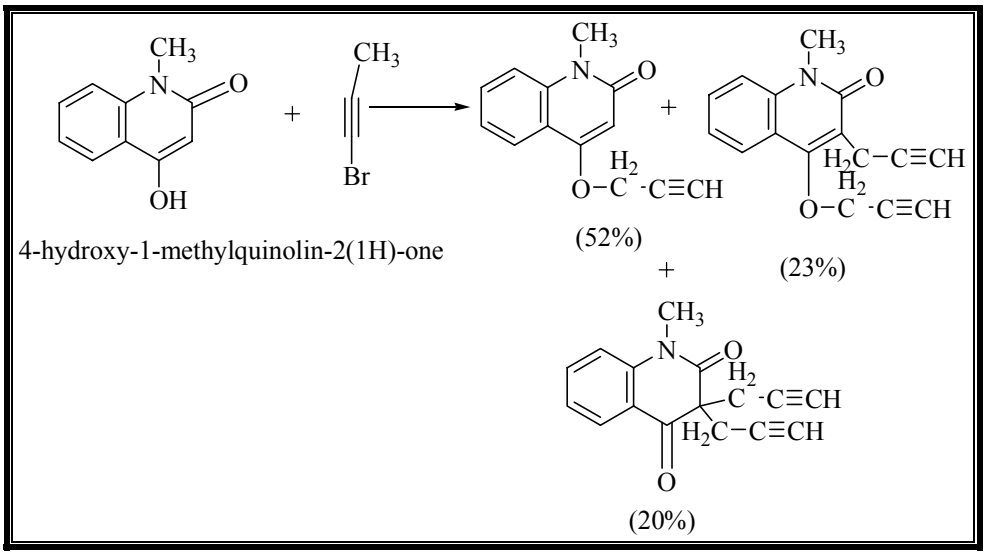
1,2-dihydro-1-methylpyridine

4-hydroxy-1-methylquinolin-2(1H)-one

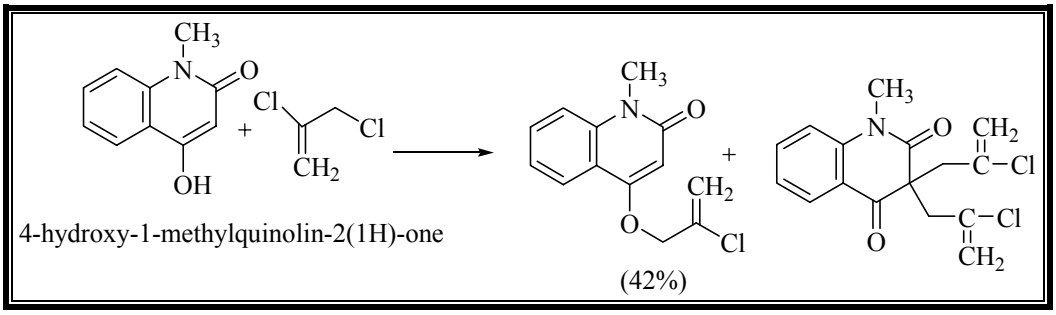
(85%)

4.A.5 REACTIONS OF 4-HYDROXY-N-METHYL-2-QUINOLONES

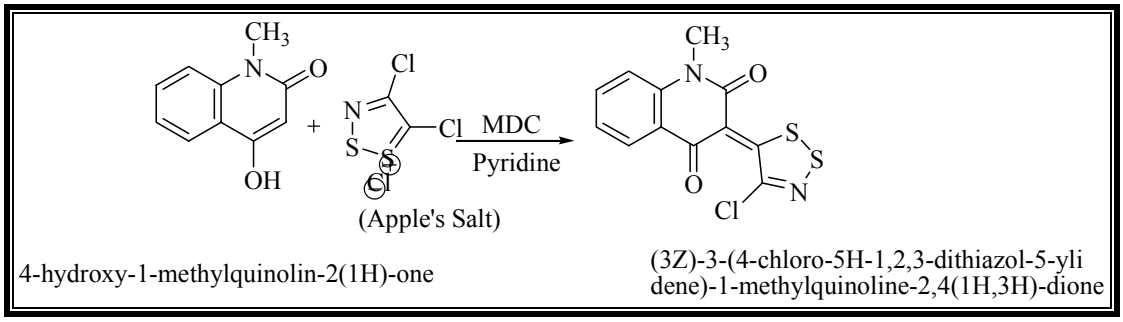
1) Reaction of 4-hydroxy-2-quinolone with 3-bromopropyne in presence of K_2CO_3 and dimethylketone resulted in mixture of three products as shown below[37]



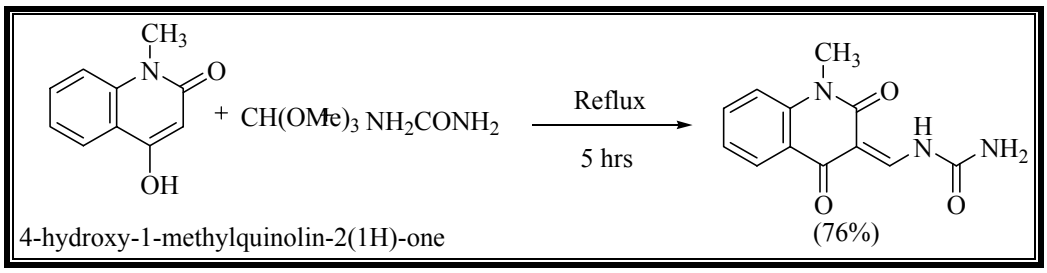
2) Reaction of 4-hydroxy-2-quinolone with 2,3-dichloropropene in presence of potassium carbonate resulted in a mixture of 4-((2-chloro-2-propenyl)oxy)-1-methyl -2,4-quinolinedione and 3,3-bis-((-2-chloro-2-propenyl)oxy)-1-methyl-2,4-quinolinediones.[38]



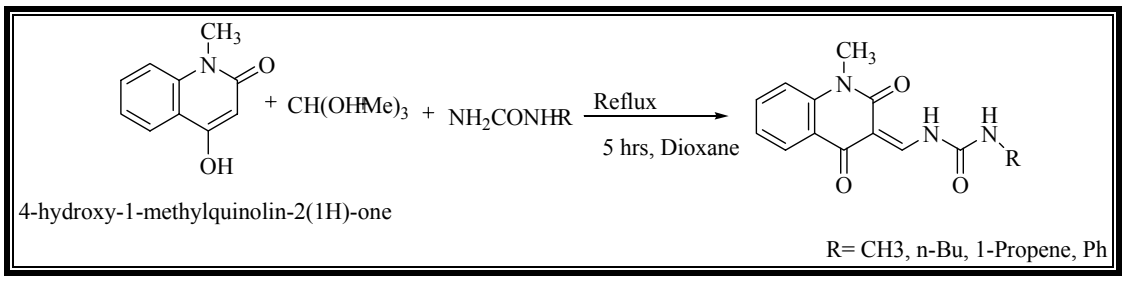
3) 4-hydroxy-2-quinolone reacts with Apple's salt in presence of pyridine in Methylene dichloride to gave (3Z)-3-(4-chloro-5H-1, 2, 3-dithiazole-5-ylidene)1-methylquinoline-2,4(1H,3H)-dione.[39]



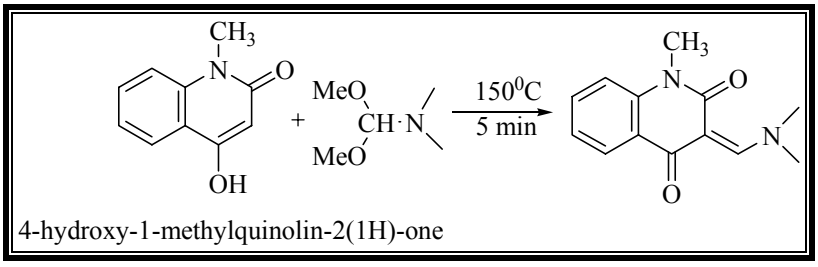
4) The preparation of 3-methylurea derivatives of 4-hydroxy-2-quinolone in presence of urea and triethylorthoformate in dioxan is reported.[40]



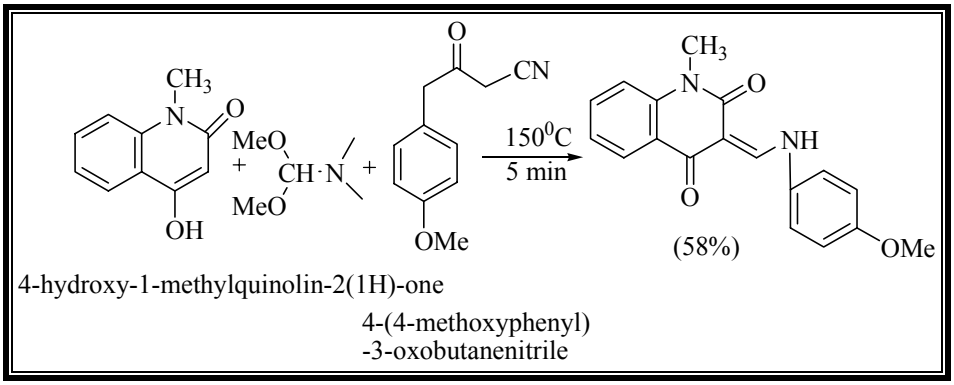
5) Reaction of 4-hydroxy-N-methyl-2-quinolone with substituted ureas in presence of triethyl orthoformate and using dioxane as solvent were also afforded 67-96% yield (4-5 reactions to be compiled in one)[41]



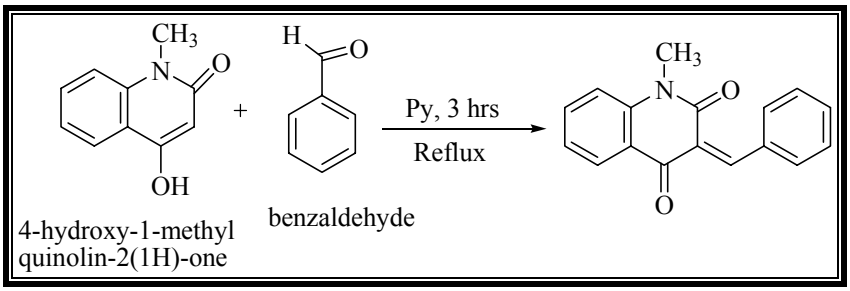
6) One-pot, microwave assisted synthesis of 3-substututed-N-Methyl-2, 4-Quinolinedione in solution phase using 1,2-dimethoxytrimethylamine were investigated.[42]



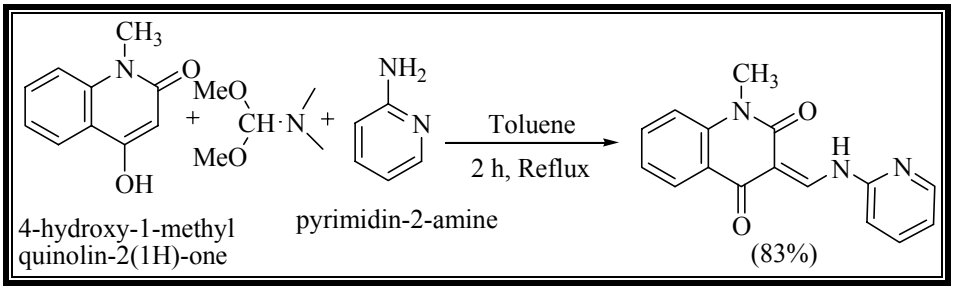
Similarly, using the above scheme, dimethoxy triethylamine with 4-hydroxy-1-methylquinoline-2(1H)-one gave 58% of 3-substituted product as shown below



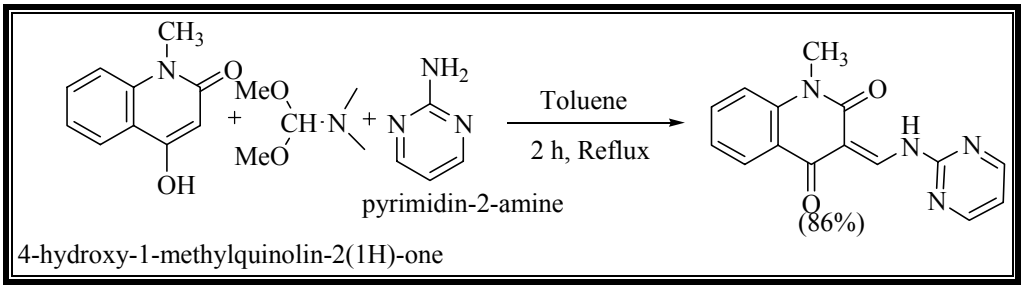
7) Knoevanagel condensation of 4-hydroxy-N-methyl-2-quinolone in presence of aldehyde and pyridine was (refluxed for 3 hours) yielded 78% of N-methyl-3(-1-phenylmethylene)-2, 4-quinolinedione.[43]



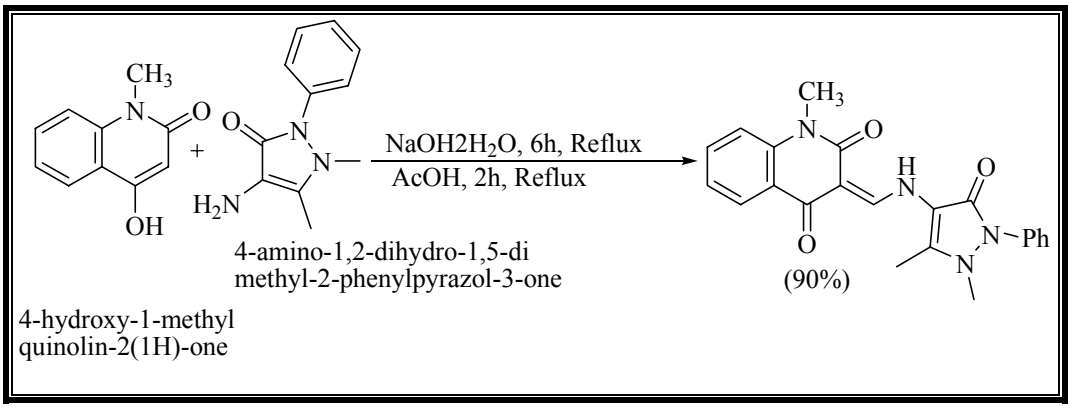
8) 4-hydroxy-N-methyl-2-quinolone in presence of o-aminopyridine and 1,1-dimethoxytrimethylamine in toluene under reflux conditions yielded 83% of N-methyl-3-[-2-pyridynylamino)methylene)-2,4-quinolinedione[44]



Similarly, using 3-aminopyridine and 2-aminopyrimidine gave corresponding products in 83-86% yield.

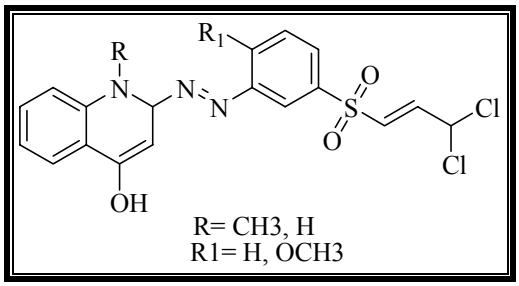


9) Reimer-Tiemann reaction reported between 4-hydroxy-N-methyl-2-quinolone and 4-amino antypyrine two were refluxed under alkaline conditions followed by overnight reflux in AcOH yielded 90% of 3-substituted product[45]

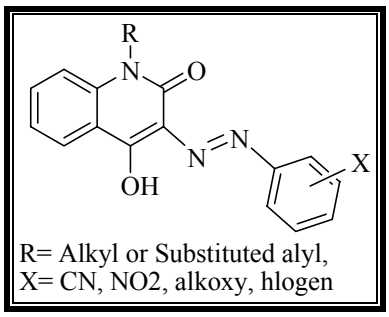


4.A.6 3-DIAZO ANALOGUES OF 4-HYDROXY-N-METHYL-2-QUINOLONES

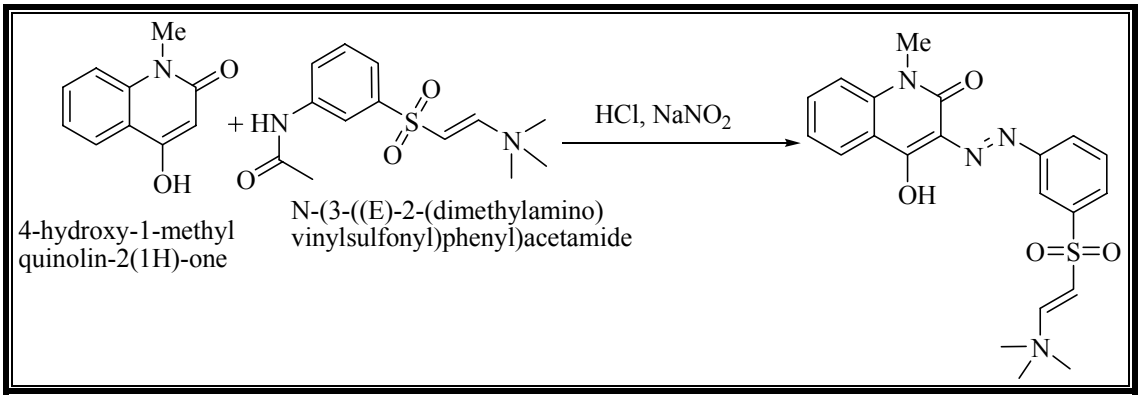
1) The authors reported two sublimation fast polyester dyes prepared from diazotization reaction of (1) with 4-hydroxy-N-methyl-2-quinolone at pH >8 for polyester and polyamide fibers.[46] Similarly, diazotization of 3-amino-$C_6H_3SO_2CH{=}CHCl_2$ with 4-hydroxy-N-methyl-2-quinolone at pH 9-10 resulted in monoazo dyes.



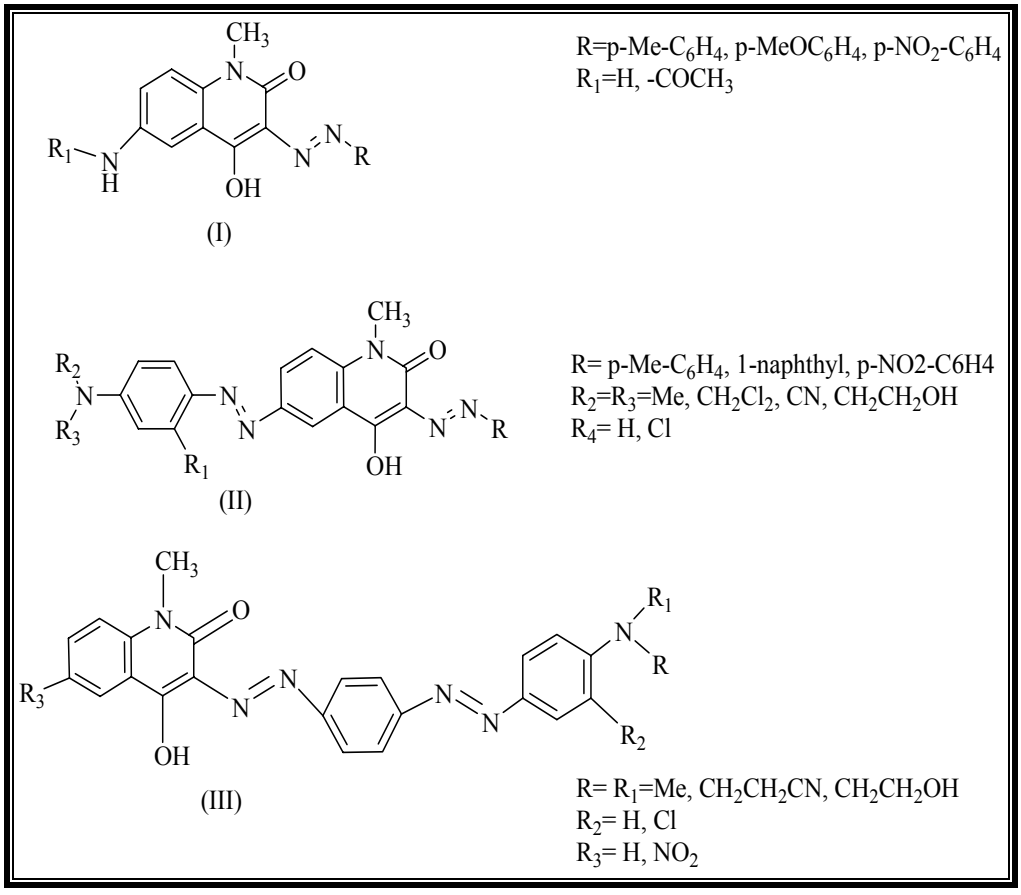
2) Sato et al have reported that halogenation of 3-phenylazo-4-hydroxy-2-quinolone yielded a better quality of dyes for polyesters fiber.[47]



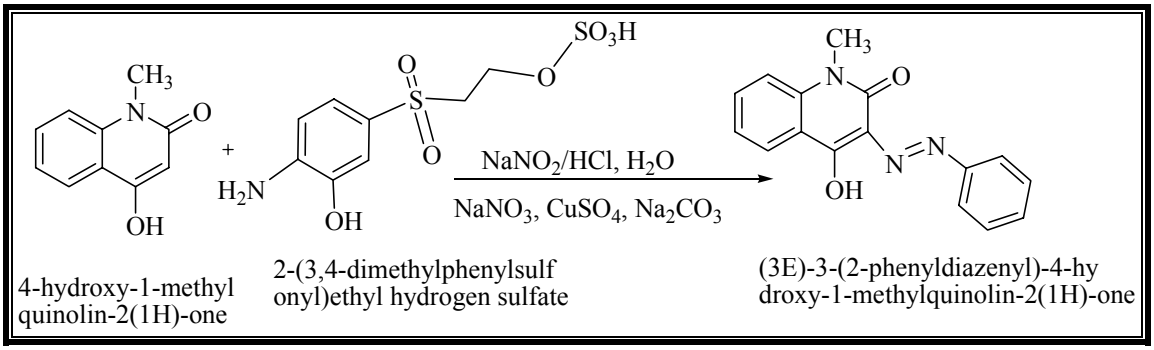
3) Some authors used standard diazotization techniques using HCl and $NaNO_2$ as below[48]



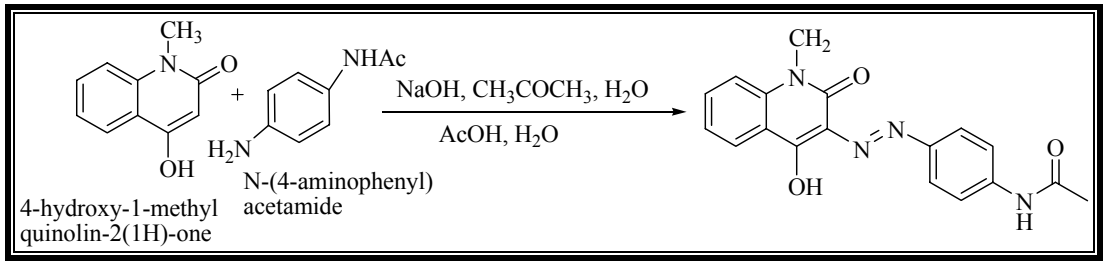
4) Synthesis and dying performance of mono and bis-azo 4-hydrxy-N-methyl-2-quinolones of structures I, II, III are well documented[49]



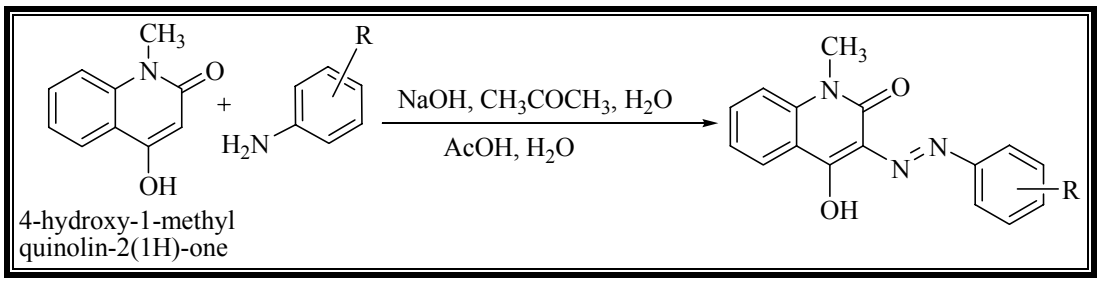
5) The inventors of patent claim preparation and uses of sulphonated quinolone dyes and it's use as fiber reactive dyes as per scheme shown below[50]



6) Preparation of various bis-azo disperse dyes for polyester by reaction of n-(4-aminophenyl)-acetamide with N-methyl-4-hydroxy-2-quinolone in dimethylketone in basic medium is reported[51]



7) These authors specifically prepared 12 3-(arylazo)-4-hydroxy-1-methyl-2-(1H)-Quinolone dyes by coupling reaction between 4-hydroxy-1-methyl-2(1H)-Quinolone with 12 different diazo compounds as depicted below[52]

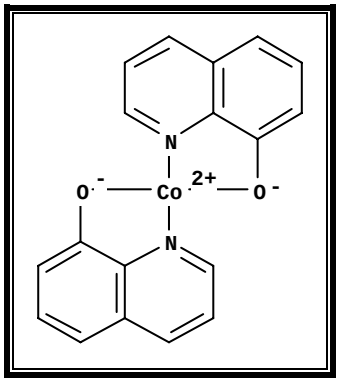


4.A.7 BIOLOGICAL IMPORTANCE

4.A.7a QUINOLONE AND ITS DERIVATIVES:

Quinolones act as Local anesthetic,[53] Quinolone substituted by pipirazine can act as cardiotonic agents[54] also useful in treatment like Peptic Ulcer treatment[55] Anticoagulants[56] Thrombosis and Phosphodiesterase inhibitors[57] Antidiabetic[58] Somatostatin decreasing inhibitor[59] Oxytocin antagonists[60] Antipsorasis[61] Antiinfective[62] Inhibitors of necrosis factor as well as interleukin 6 production[63] Neutrophil induced inflammation inhibitor[64] Rejection inhibitor for transplants[65] Hepatocyte growth factor enhancers[66] Virus replication inhibitor[67] Cardiac Fibroblast inhibitors[68] Anti multiple sclorosis[69] For mood disorders in addition to serotponin uptake inhibitors[70] For accelerating salivation,[71] It is also discussed to have B-adrenoreceptor agonist properties.[72]

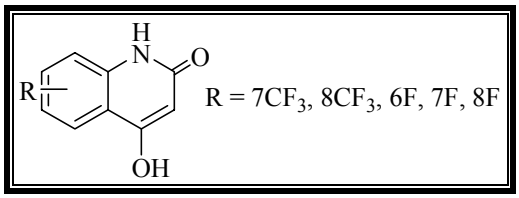
US 3493464 disclose the use of metallic quinolinolate complex formed in-situ to have fungus static activity. They prepared Ferrous, Manganese, Copper, Nickel and Zinc Complexes[73]



Mutagenicity of quinolines and various quinolones were studied in Salmonella Typhurium.[74] Quinolones are also known to bind to RNA, DNA and pol nucleotides in presence of NADPH and rat liver microsomes and it metabolises into various substituted quinolones incl 2-quinolone.[75]

Similarly, 4-hydroxy-2-quinolone were also reported to have many biological activities. Shah A et al discussed antimicrobial/antitubercular screening of substituted 2,4-dihydroxyquinolones.[76] Naturally occurring 4-hydroxy-2-quinolones were isolated from Haplophyllum A. Juss. and were checked for cytotoxic activity of 2,4-quinolone on human cancer cell line[77]

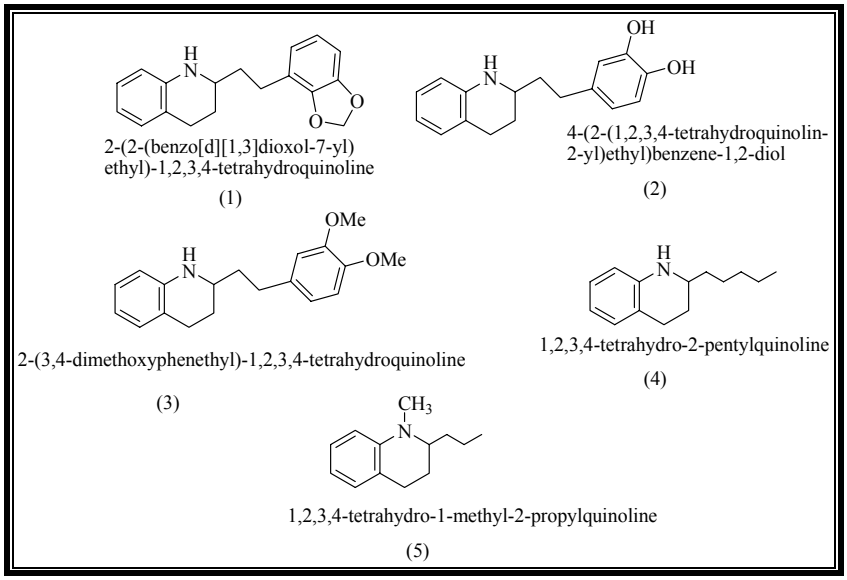
It was found that 4–hydroxy-2-quinolone having trimethyl flouro or flouro substituent are important for pharmacological and synthetic targets and also act as basic synthon for number of antibacterial fluoroquinolones and are promising potent and selective glycine site NMDA receptors. A simple one step MW assisted synthesis was described and it's phytotoxicity as well as cytotoxicity was evaluated against Leukemia and adrenocarcinome derived cell line.[78]



4.A.7b N-METHYL 2-QUINOLONES:

1) N-methyl-2-quinolone and was reported as fungistats and also posseses fungicidal properties[79], 2) In 1981, N-methyl-2-quinolone was reported as the bitter constituent of heamolymph in aposematic beetle[80]. 3) In some of the antifungal studies it was found that N-methyl-2-quinolone were non-toxic to the mycelial growth of *Pyrucularia Oryzae* which causes rice blast disease in vitro and they did not inhibit conodial formations too. However, the studies indicated that it did block melanization of appresoria and prevented the penetration of infected hyphae through the epidermal wall. The reason was that these compounds blocked pathway of melanin synthesis between scylactone and vermelone[81]. 4) N-methyl-2-quinolone was reported to covert into 2,4-dione analogue in presence of milk xanthine oxidase and hence acts as competitive inhibitor of the same enzyme[82]. *5)* It is found out that N-methyl-2-quinolone inhibits 1,8-dihydroxynaphthalene-melanin biosynthesis and in turn melanin synthesis in the conodia of Penicillium and Aspergillus species.[83] 6) In 1999, it was extracted from *Galipea Officianallis* (This species is the only known species

which contains tetrahydroquinoline alkaloids) The ethnolic extract was found to be active against *Mycobacterium Tuberculosis*[84] 7) The alkaloids from *Galipea Officianallis* were analysed using GC-MS and five new tetrahydroquinolines were identified[85]



4.A.7c 4-HYDROXY N-METHYL 2-QUINOLONES:

1) Biotransformation of alkaloids through intermediate stage of 4-hydroxy-quinolones by aid of cell suspension cultures of *Ruta Graveolens* was reported[86]

2) It was reported as hair dye coupler and similar applications[87-89]

3) It was isolated from fermentation broth of Dactylosporangium species and it acted as Chymase inhibitor.[88]

5) Benzalacetone synthase enzyme derived from *Rheum Palmatum* efficiently catalysed the condensation of anthraninoyl-COA with Malonyl-COA to produce 4-hydroxy-quinolones – an alkaloid scaffold normally produced by type III polyketide[90]

4.A.8 AIM OF CURRENT WORK

4-hydroxy 2-quinolones and aromatic aldehydes are known to produce 2, 4-chromane diones (arylidine at C_3 position) and coumarin dimers under reflux, with or without base. Thus, few new chromane diones were prepared using pyrazole aldehydes.

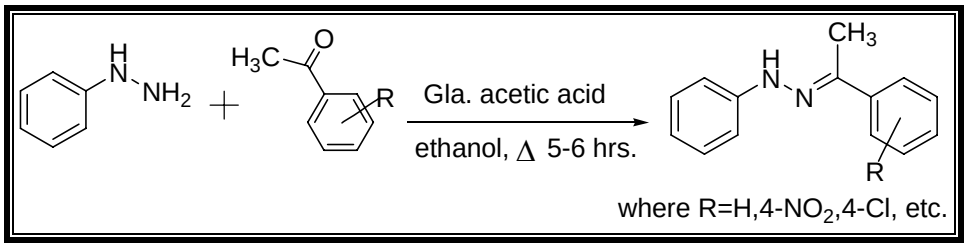
Though the chemistry of the synthesized compounds is known, the compounds are reported herein for the first time. Biological importance of such important compounds is the rational behind the current work done in chapter-7.

The current chapter is related to preparation of small molecules based on Quinolones and Pyrazoles together.

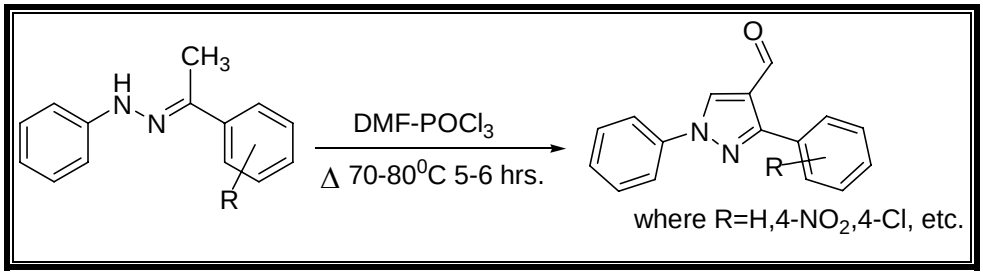
4.A.9 REACTION SCHEMES

4.A.9.1 PREPARATION OF PYRAZOLE ALDEHYDES

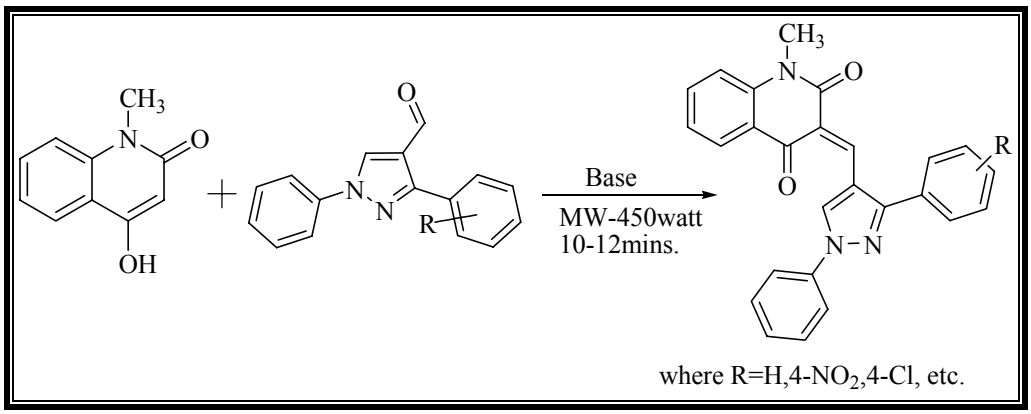
STEP – 1



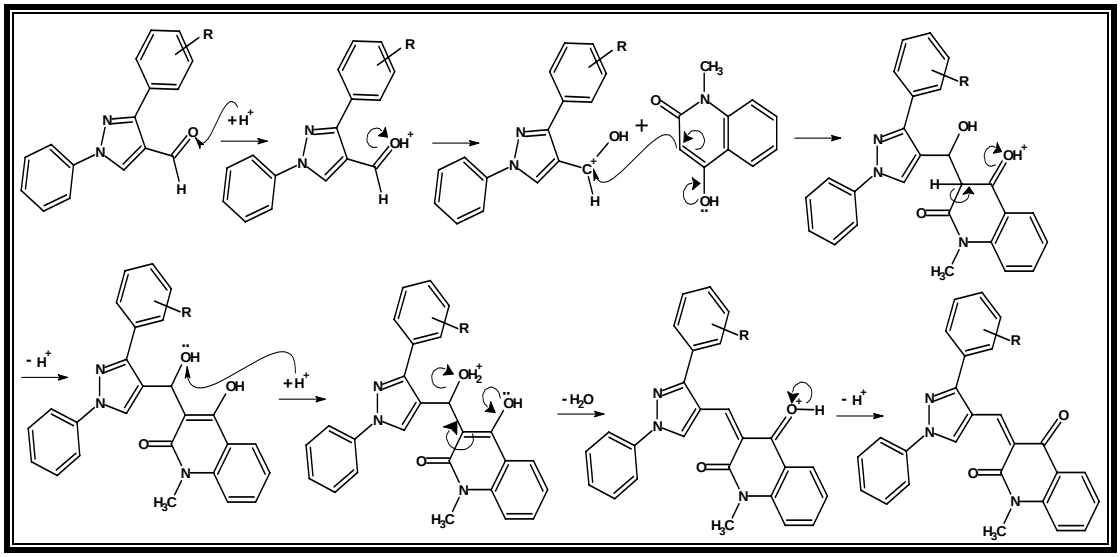
STEP – 2



4.A.9.2 PREPARATION OF (*E*)-1-METHYL-3-((1, 3 (SUBSTITUTED)-DIPHENYL-1H-PYRAZOL-4-YL)METHYLENE) QUINOLINE -2,4-(1H,3H)-DIONES



4.A.10 PLAUSIBLE REACTION MECHANISM



4.A.11 EXPERIMENTAL

4.A.11.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in **Samsung MW83Y Microwave Oven** which was locally modified for carrying out chemical reactions. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in $CDCl_3$ solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

4.A.11.2 PREPARATION OF PYRAZOLE ALDEHYDE : GENERAL METHOD

STEP – [1] PREPARATION OF ACETOPHENONE PHENYL HYDRAZONES

Substituted acetophenone (0.1 mole) was dissolved in 50 ml of ethanol into 250 ml round bottom flask. and Phenyl hydrazine (0.1 mole) was added to above flask along with 3-4 drops of glacial acetic acid. The reaction mixture was refluxed for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the crystalline product was separated by filtration. The product was washed with ethanol and dried to give substituted acetone phenyl hydrazone in good yield which was pure enough to use as such for the next step.

Code No.	MF	MW (g/m)	MP (°C)	% Yield
APH-01	$C_{14}H_{13}N_3O_2$	255	134-136	85
APH-02	$C_{14}H_{13}ClN_2$	244	-----	80
APH-03	$C_{14}H_{14}N_2$	210	126-128	78
APH-04	$C_{14}H_{13}N_3O_2$	255	106-108	79
APH-05	$C_{14}H_{13}ClN_2$	244	-----	72
APH-06	$C_{15}H_{16}N_2$	224	-----	72

STEP – [2] PEPARATION OF PYRAZOLE ALDEHYDES

25 ml of dry dimethylformamide was transferred into 250 ml flat bottom flask. 3 ml of phosphorous oxychloride was added drop wise to above flask under stirring at 0-5°C. After completion of the addition, the mixture was stirred at this temperature for 10-15 min. Acetophenone phenyl hydrazone (0.03 mole) freshly prepared was added to above mixture and the content was heated on water bath for 5-6 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using toluene : ethyl acetate (7 : 3) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the content of the flask was poured on crushed ice to isolate the product. The separated product was filtered off and it was washed with cold water to remove acidity. It was dried at 65°C and recrystallized from the mixture of DMF-Methanol to give crystalline pyrazole aldehyde in good yield.

Similarly other compounds are also prepared.

TABLE-4.A.1

Code No.	MF	MW (g/m)	MP (°C)	% Yield
PA-01	$C_{16}H_{11}N_3O_3$	293	162-164	75
PA-02	$C_{16}H_{11}ClN_2O$	282	142-144	71
PA-03	$C_{16}H_{12}N_2O$	248	144-146	68
PA-04	$C_{16}H_{11}N_3O_3$	293	176-178	72
PA-05	$C_{16}H_{11}ClN_2O$	282	140-142	71
PA-06	C17H14N2O	262	152-154	70

4.A.11.3 PREPARATION OF 1-METHYL-3-((1, 3 (SUBSTITUTED)-DIPHENYL-1H-PYRAZOL-4-YL)METHYLENE) QUINOLINE -2,4-(1H,3H)-DIONES (SP-40-SP-45) : GENERAL METHODS

[A] CONVENTIONAL METHOD

N-methyl4-hydroxyquinolone (0.01 mole) was dissolved in 20 ml of dimethylformamide into 100 ml round bottom flask. Substituted pyrazole-4-carboxaldehyde (0.01 mole) was added to the above flask along with few drops of piperidine. The reaction mixture was heated on water bath for 4-5 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the product was separated by filtration. The product was washed with methanol and dried to give desired product in good yield which was recrystallized by DMF. Physical data of the synthesized end products are summarized in the Table No. 4.A.3. Similarly other compounds are also prepared.

[B] MICROWAVE METHOD

N-methyl4-hydroxyquinolone (0.01 mole) was dissolved into 20 ml of dimethylformamide into 100 ml microwave flask. Substituted pyrazole-4-carboxaldehyde (0.01 mole) was added to the above flask along with few drops of piperidine. The reaction mixture was irradiated under microwave irradiation using Qpro-M microwave synthesizer for the desired time at 450 W. The progress and the completion of the reaction were checked at interval of every one min. by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was scratched into 30 ml of methanol. The separated product was filtered off and washed with methanol and it was dried to give desired product in good yield which was recrystallized by DMF. Physical data of the synthesized end products are summarized in the Table No. 4.A.3. Similarly other compounds are also prepared.

COMPARATIVE RESULTS OF METHOD (A) AND METHOD (B) ARE SUMMARIZED AS UNDER.

TABLE-4.A.2

Code No.	Reaction Condition					% Yield	
	Method (A)		Method (B)			Method (A)	Method (B)
	Temp. (°C)	Time (hrs.)	Watt (W)	Temp. (°C)	Time (min.)		
SP – 40	150-160	4.5	450	110	3.0	68	85
SP – 41	150-160	5.0	450	110	3.3	64	80
SP – 42	150-160	4.8	450	110	3.1	70	83
SP – 43	150-160	5.5	450	110	3.2	72	81
SP – 44	150-160	5.3	450	110	3.0	66	79
SP – 45	150-160	5.1	450	110	3.2	62	76

4.A.12 PHYSICAL DATA

PHYSICAL DATA OF 1-METHYL-3-((1, 3 (SUBSTITUTED)-DIPHENYL-1H-PYRAZOL-4-YL)METHYLENE) QUINOLINE -2,4-(1H,3H)-DIONES (SP-40 TO SP-45)

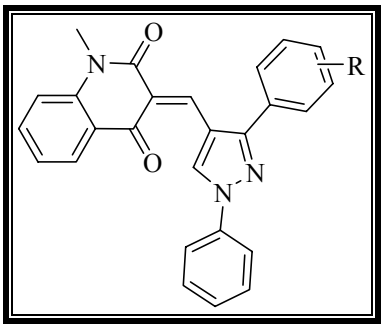


TABLE-4.A.3

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	M.P. ^{0}C	R_f Value
01	SP-40	4-NO_2	$C_{26}H_{18}N_4O_4$	450	162-164	0.48
02	SP-41	4-Cl	$C_{26}H_{18}ClN_3O_2$	439	170-172	0.44
03	SP-42	H	$C_{26}H_{19}N_3O_2$	405	178-180	0.52
04	SP-43	3-$N0_2$	$C_{26}H_{18}N_4O_4$	450	168-170	0.40
05	SP-44	3-Cl	$C_{26}H_{18}ClN_3O_2$	439	156-158	0.46
06	SP-45	4-CH_3	$C_{27}H_{21}N_3O_2$	419	174-176	0.44

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (6 : 4)

4.A.13 SPECTRAL DISCUSSION

4.A.13.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. SP-40 and SP-41 of Mass spectra are given on page no. 154 and 156.

4.A.13.2 IR SPECTRA

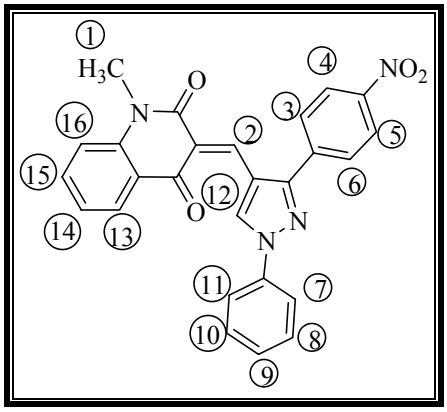
IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

In case of SP-40 to SP-45 of –CH stretching vib. of aromatic were found near 3040 cm^{-1} . –CH stretching of assym were found near 2960 $cm^{-1.}$ –CH stretching of sym. were found near 2870 cm^{-1}. Two Carbonyl stretching frequencies were obtained near 1700 cm^{-1}. –CH deformation assym were found near 1460 cm^{-1}. –CH deformation sym. were found near 1370 cm^{-1}. SP-40 and SP-41 of IR spectra are given on page no. 154 and 156.

4.A.13.3 ^{1}H NMR SPECTRA

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H NMR spectral interpretation can be discussed as under.

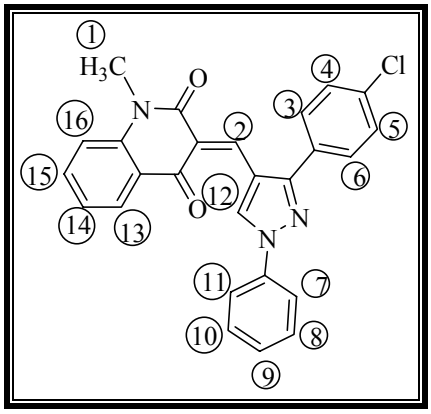
^{1}H NMR OF 1-METHYL-3-((3-(4-NITROPHENYL)-1-PHENYL-1H-PYRAZOL-4-YL)METHYLENE)QUINOLINE-2,4(1H,3H)-DIONE (SP-40)



1. The Proton no. 1 of N-Methyl group gave a characteristic singlet at 2.13 δ ppm.
2. Arylidine proton of Proton no. 2 became deshielded and gave a characteristic singlet at 6.43 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
3. The Proton no. 3 and 6 of phenyl ring gave a doublet at 7.66 δ ppm – 7.68 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
4. The Proton no. 4 and 5 of phenyl ring gave a doublet at 7.79 δ ppm – 7.81 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
5. The Proton no. 13 and 16 of two proton gave a multiplet at 7.26 δ ppm – 7.33 δ ppm.
6. The Proton no. 8, 9 and 10 of three proton gave a multiplet at 7.36 δ ppm – 7.47 δ ppm.
7. The Proton no. 7 ,11 and 12 of three proton gave a multiplet at 8.06 δ ppm – 8.12 δ ppm.
8. The Proton no. 14 and 15 of two proton gave a multiplet at 7.57 δ ppm – 7.61 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SP-40 has been confirmed for cis geometrical. Spectrum is given on page no. 155.

^{1}H NMR OF 3-((3-(4-CHLOROPHENYL)-1-PHENYL-1H-PYRAZOL-4-YL)METHYLENE)-1-METHYLQUINOLINE-2,4(1H,3H)-DIONE (SP-41)



1. The Proton no. 1 of N-Methyl group gave a characteristic singlet at 2.13 δ ppm.
2. Arylidine proton of Proton no. 2 became deshielded and gave a characteristic singlet at 6.37 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
3. The Proton no. 3 and 6 of phenyl ring gave a doublet at 6.82 δ ppm – 6.84 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
4. The Proton no. 4 and 5 of phenyl ring gave a doublet at 7.17 δ ppm – 7.19 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
5. The Proton no. 13 and 16 of two proton gave a multiplet at 7.22 δ ppm – 7.26 δ ppm.
6. The Proton no. 8, 9 and 10 of three proton gave a multiplet at 7.40 δ ppm – 7.44 δ ppm.
7. The Proton no. 7 and 11 of two proton gave a multiplet at 7.61 δ ppm – 7.65 δ ppm.
8. The Proton no. 14 and 15 of two proton gave a doublet at 7.75 δ ppm – 7.77 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.
9. The Proton no. 12 of pyrazole ring gave a singlet at 8.00 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SP-41 has been confirmed for cis geometrical. Spectrum is given on

page no. 157.

4.A.13.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

4.A.14 ANALYTICAL DATA

***1-methyl–3-((3-(4-nitrophenyl)-1-phenyl-1H–pyrazol-4-yl)methylene)quinoline-2,4 (1H,3H)-dione (SP-40)*:**

IR (KBr, cm^{-1}): 3032 (Ar-H, str.), 2946 (-CH str. Assym.), 2876 (-CH str. Sym.), 1692 (>CO str.), 1624 (C=C alip.), 1540 (C=C, Ar.), 1451 (-CH def. Assym.), 1368 (-CH def. Sym.), 1122, 1095 (N-N str.), 754 (1,4-di sub); ^{1}H NMR (400 MHz, DMSO): δ (ppm) 2.13 (3H, S), 6.43 (1H, S), 7.33 (2H, m,), 7.47 (3H, m), 7.61 (2H, m), 7.68 (2H, d, J=8.0 Hz), 7.81 (2H, d, J=8.0 Hz), 8.12 (3H, m); MS *m/z* = 450 (M^+); Anal. Calcd. for $C_{26}H_{18}N_4O_4$: C, 69.33; H, 4.03; N, 12.44; O, 14.21. Found: C, 69.30; H, 4.01; N, 12.39; O, 14.20%.

***3-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-methylquinoline-2,4(1H,3H)-dione (SP-41)*:**

IR (KBr, cm^{-1}): 3034 (Ar-H, str.), 2941 (-CH str. Assym.), 2866 (-CH str. Sym.), 1697 (>CO str.), 1626 (C=C alip.), 1541 (C=C, Ar.), 1452 (-CH def. Assym.), 1371 (-CH def. Sym.), 1126, 1093 (N-N str.), 756 (1,4-di sub); ^{1}H NMR (400 MHz, DMSO): δ (ppm) 2.13 (3H, S), 6.37 (1H, S), 6.84 (2H, d, J=8.0 Hz), 7.19 (2H, d, J=8.0 Hz), 7.26 (2H, m), 7.44 (3H, m), 7.65 (2H, m), 7.77 (2H, d, J=8.0 Hz), 8.00 (1H, S); MS *m/z* = 439 (M^+), 441(M^{+2}) ; Anal. Calcd. for $C_{26}H_{18}ClN_3O_2$: C, 70.99; H, 4.12; N, 9.55; O, 7.27. Found: C, 70.93; H, 4.10; N, 9.55; O, 7.22%.

***1-methyl-3-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)quinoline-2,4(1H,3H)-dione (SP-42)*:**

IR (KBr, cm^{-1}): 3039 (Ar-H, str.), 2956 (-CH str. Assym.), 2874 (-CH str. Sym.), 1681 (>CO str.), 1627 (C=C alip.), 1545 (C=C, Ar.), 1454 (-CH def. Assym.), 1371 (-CH def. Sym.), 1147, 1095 (N-N str.); MS *m/z* = 405 (M^+); Anal. Calcd. for $C_{26}H_{19}N_3O_2$: C, 77.02; H, 4.72; N, 10.36; O, 7.89. Found: C, 77.00; H, 4.71; N, 10.33; O, 7.82%.

***1-methyl-3-((3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)quinoline-2,4(1H,3H)-dione (SP-43)*:**

IR (KBr, cm^{-1}): 3038 (Ar-H, str.), 2946 (-CH str. Assym.), 2864 (-CH str. Sym.), 1690 (>CO str.), 1626 (C=C alip.), 1548 (C=C, Ar.), 1450 (-CH def. Assym.), 1371 (-CH def. Sym.), 1124, 1093 (N-N str.), 752 (1,4-di sub); MS *m/z* = 450 (M^+); Anal. Calcd. for $C_{26}H_{18}N_4O_4$: C, 69.33; H, 4.03; N, 12.44; O, 14.21. Found: C, 69.30; H, 4.00; N, 12.43; O, 14.19%.

***3-((3-(3-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)-1-methylquinoline-2,4(1H,3H)-dione (SP-44)*:**

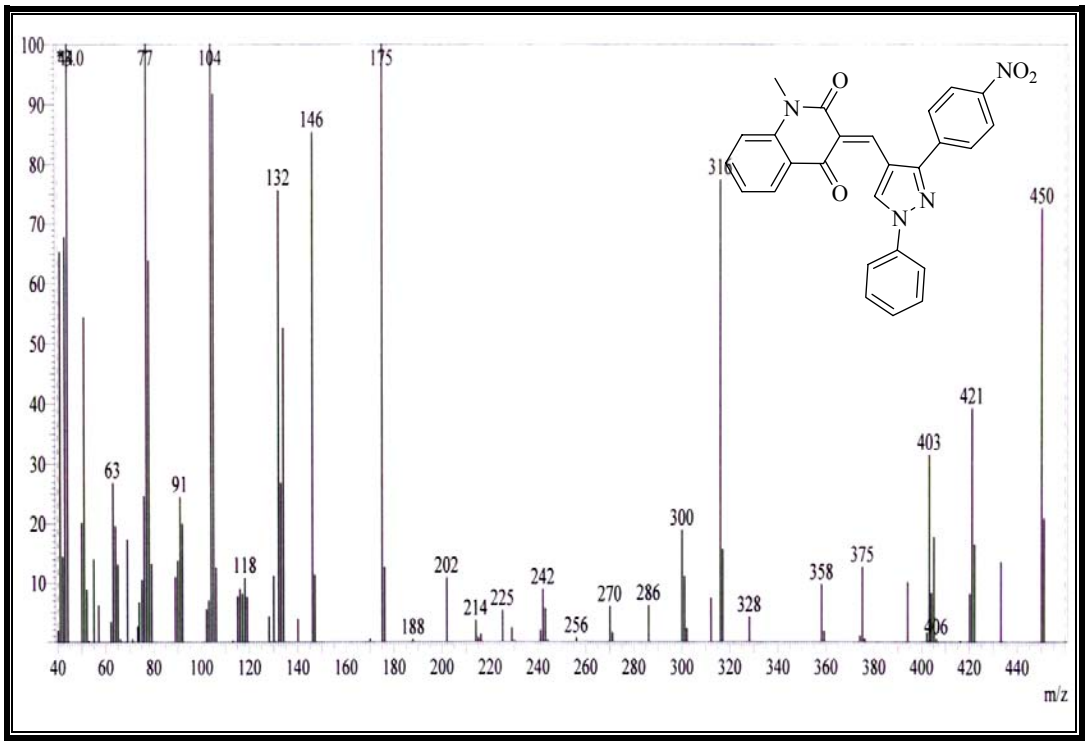
IR (KBr, cm^{-1}): 3044 (Ar-H, str.), 2942 (-CH str. Assym.), 2863 (-CH str. Sym.), 1688 (>CO str.), 1626 (C=C alip.), 1538 (C=C, Ar.), 1451 (-CH def. Assym.), 1371 (-CH def. Sym.), 1122, 1093 (N-N str.), 754 (1,4-di sub); MS *m/z* = 439 (M^+), 441(M^{+2}); Anal. Calcd. for $C_{26}H_{18}ClN_3O_2$: C, 70.99; H, 4.12; N, 9.55; O, 7.27. Found: C, 70.94; H, 4.11; N, 9.52; O, 7.23%.

***1-methyl-3-((1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methylene)quinoline-2,4(1H,3H)-dione (SP-45)*:**

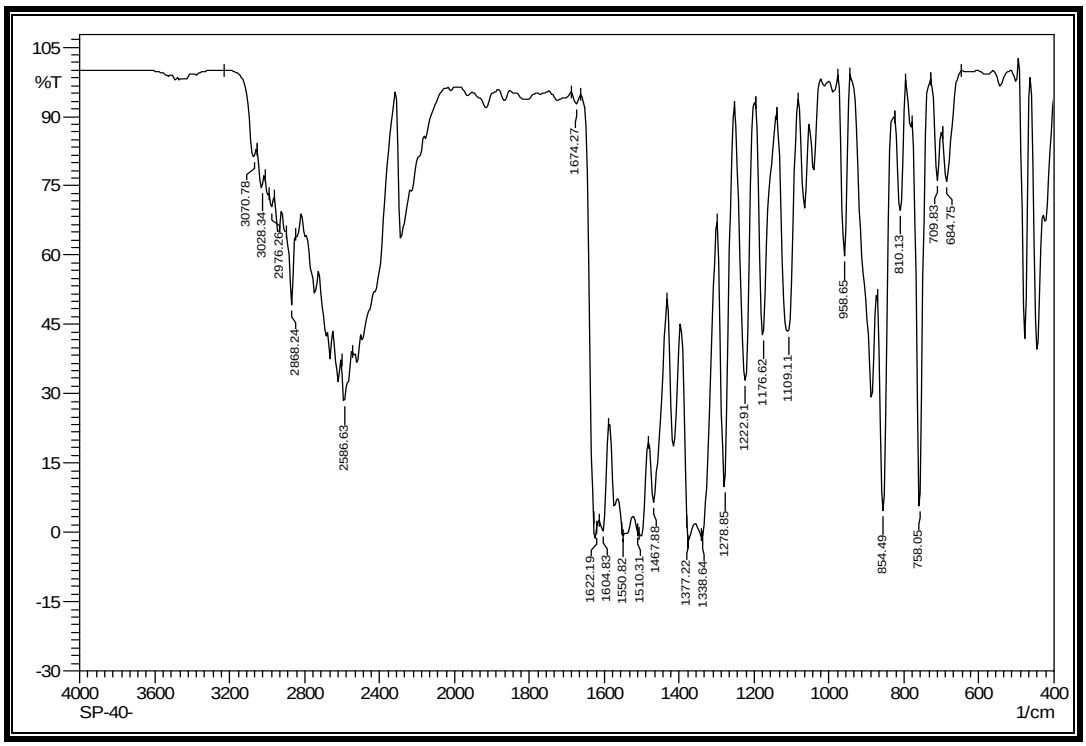
IR (KBr, cm^{-1}): 3030 (Ar-H, str.), 2944 (-CH str. Assym.), 2868 (-CH str. Sym.), 1694 (>CO str.), 1624 (C=C alip.), 1541 (C=C, Ar.), 1456 (-CH def. Assym.), 1370 (-CH def. Sym.), 1124, 1095 (N-N str.), 752 (1,4-di sub); MS *m/z* = 419 (M^+); Anal. Calcd. for $C_{27}H_{21}N_3O_2$: C, 77.31; H, 5.05; N, 10.02; O, 7.63. Found: C, 77.30; H, 5.01; N, 10.00; O, 7.61%.

4.A.15 REPRESTANTIVE SPECTRA

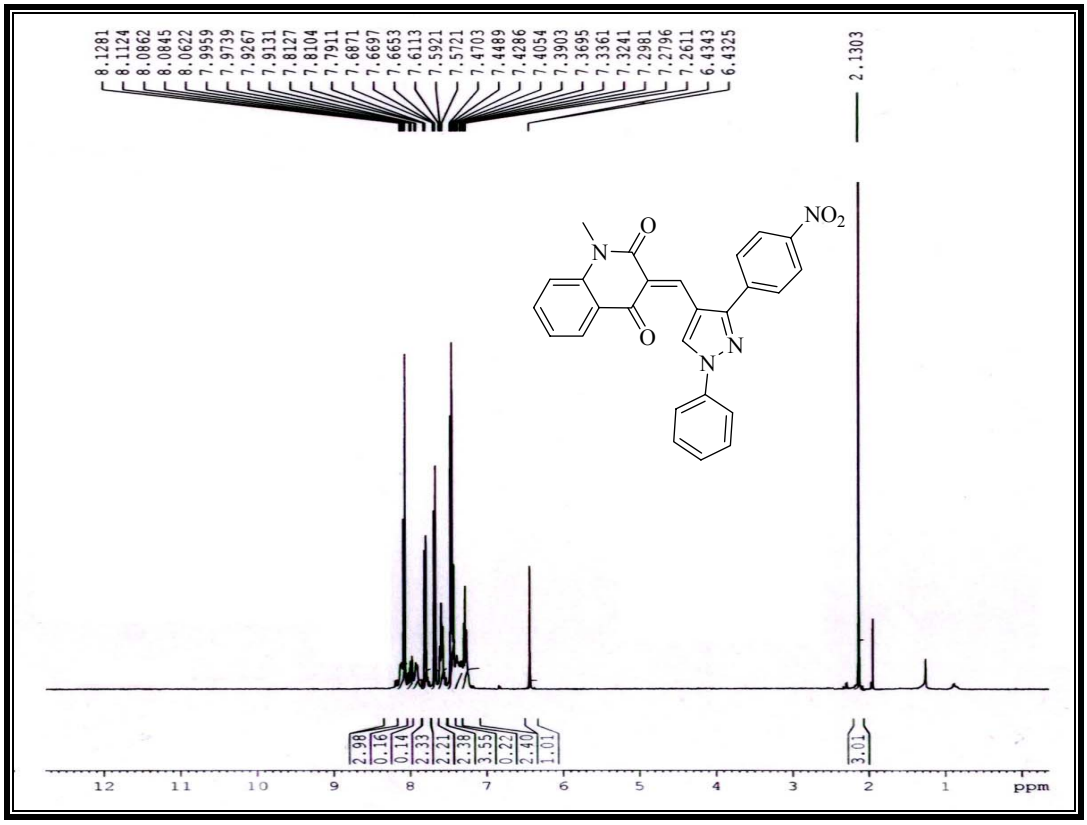
4.A.15.1 Mass Spectrum of SP-40



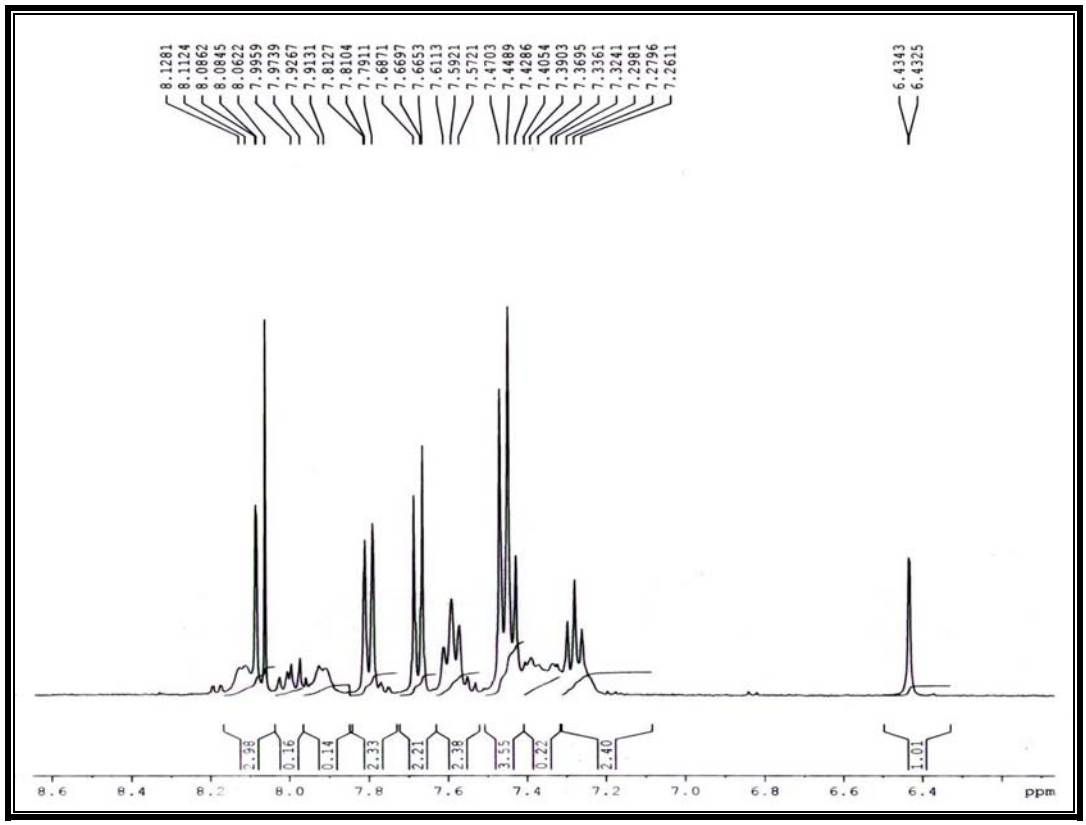
4.A.15.2 IR Spectrum of SP-40



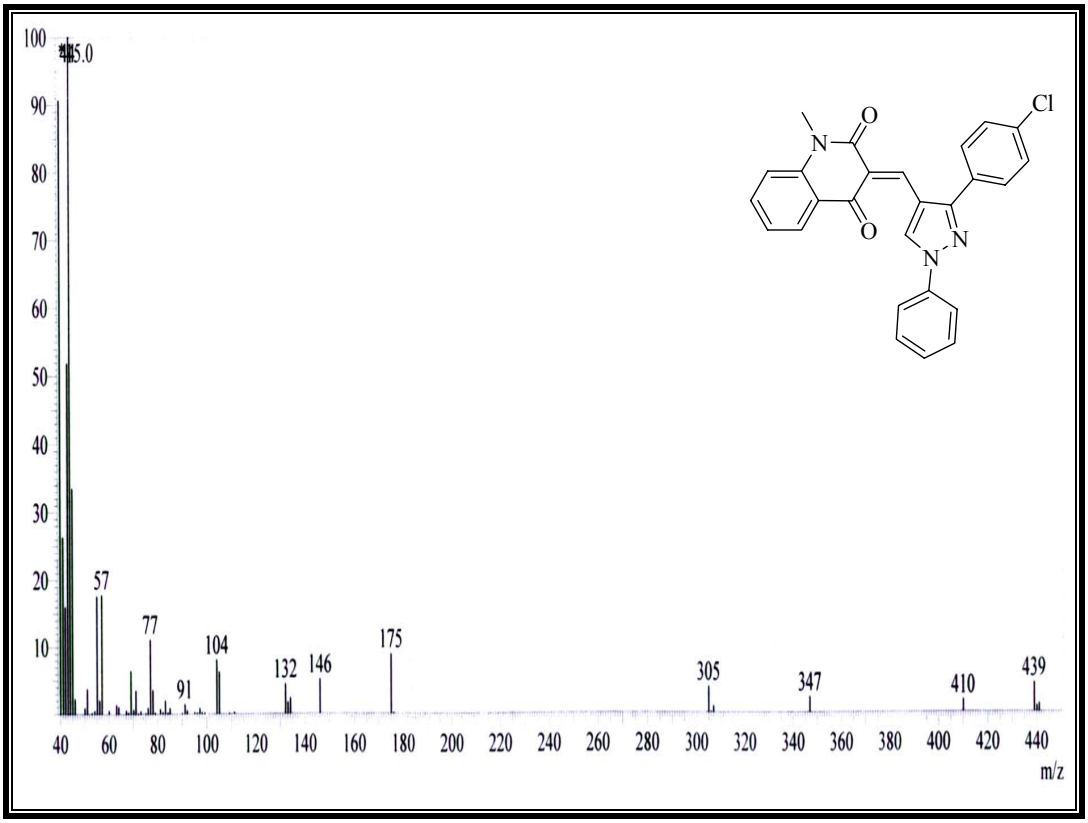
4.A.15.3 ^{1}H NMR Spectrum of SP-40



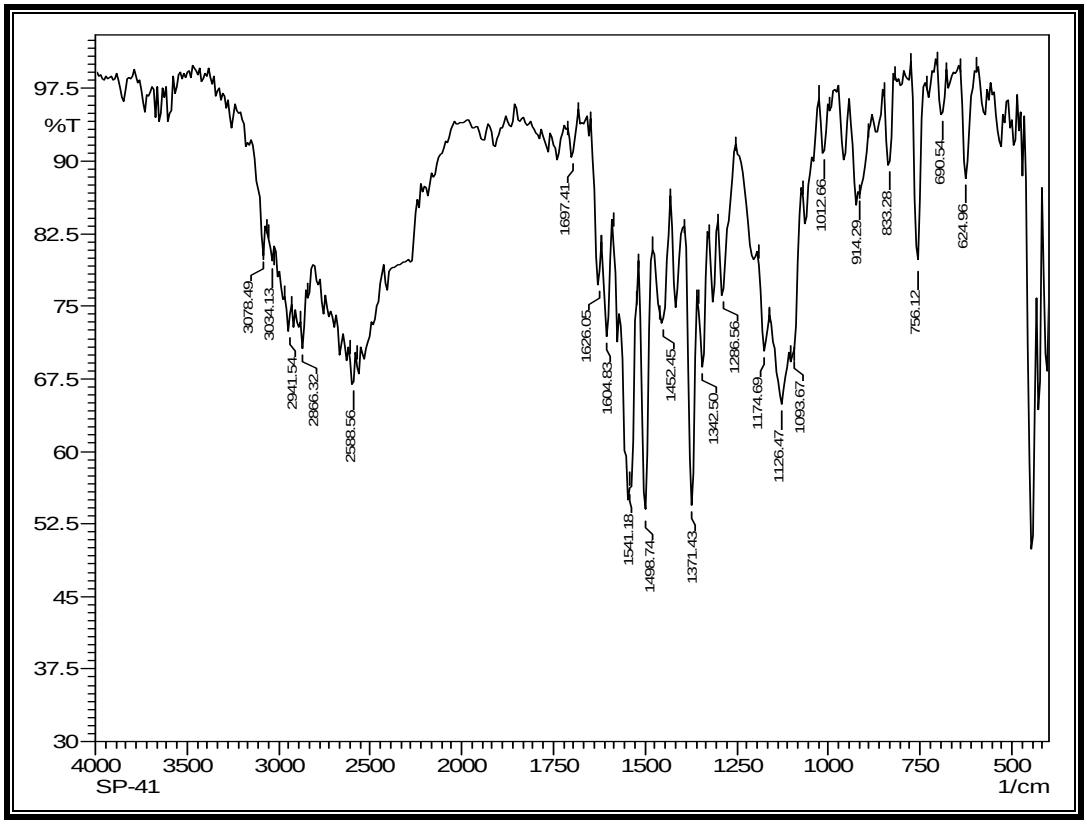
EXPANDED ^{1}H-NMR SPECTRUM OF SP-40



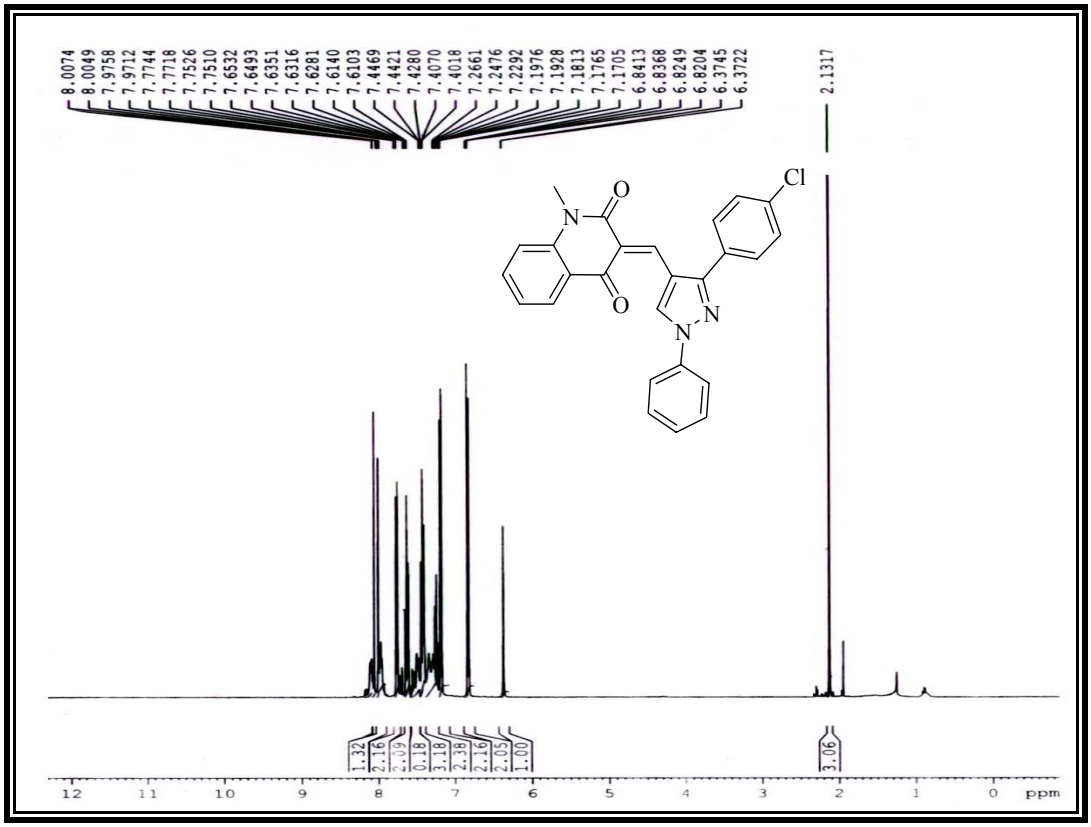
4.A.15.4 Mass Spectrum of SP-41



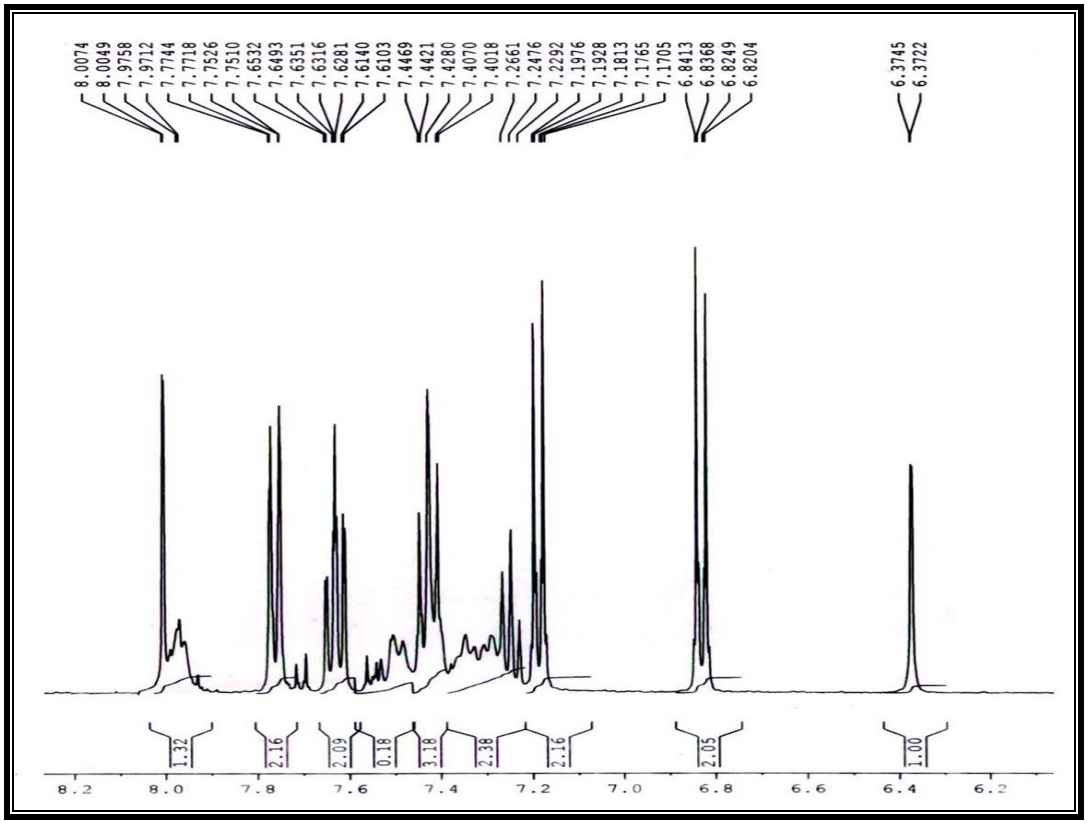
4.A.15.5 IR Spectrum of SP-41



4.A.15.6 ^{1}H NMR Spectrum of SP-41



EXPANDED ^{1}H-NMR SPECTRUM OF SP-41



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CHAPTER - 4

SECTION - B

A RAPID MICROWAVE ASSISTED SYNTHESIS OF 3-SUBSTITUTED BENZYLIDENE-8-METHYL-3H-CHROMENE-2, 4-DIONE

4.B.1 COUMARINS

Coumarin is a chemical compound (2*H*-chromen-2-one, 1-benzopyran-2-one, benzopyrone; $C_9H_6O_2$); a toxin found in many plants, notably in high concentration in the tonka bean, vanilla grass, woodruff, mullein and bison grass. It has a sweet scent, readily recognised as the scent of newly-mown hayand has been used in perfumes since 1882. It has clinical medical value as the precursor for several anticoagulants, notably warfarin and is used as a gain medium in some dye lasers.

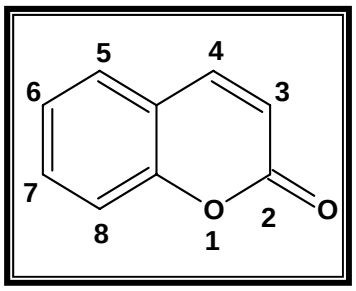


Fig. 4.1

The isolation of coumarin was first reported by Vogel[1] in 1820. He isolated coumarin from tonka beans, bearing the characteristic aroma of cutted grass. The name of coumarin originates[2] from a Caribbean word "*coumarou*" for the tonka tree, which was known botanically at one time as *Coumarouna odorta Aubl*, coumarin is now the accepted trival name. Coumarin was first synthesized in 1868 on treatment of sodium salt of o-hyroxy benzaldehyde with acetic anhydride.[3a,3b] Compounds containing coumarin subunit possess a wide range of activities and show an interesting reactivity.[4-7] This is consequence of the rich electronic structure of coumarin which offers abundant possibilities for diversified activity and reactivity of the system.

4.B.2 SYNTHESIS OF 4-HYDROXY COUMARINS

Perkin[3] synthesized coumarin and then several methods are reported for the synthesis of 4-hydroxy coumarins and their 4-hydroxy substituted derivatives namely:

1 Anschutz method[8]
2 Pauli Lockemann synthesis[9]
3 Sonn's synthesis[10]
4 Mentzer's synthesis[11]
5 Robertson synthesis[12]
6 Ziegler and Junek method[13]
7 Garden's method[14]
8 Shah, Bose and Shah's method[15]
9 Kaneyuki method[16]
10 Resplandy's method[17]
11 Jain, Rohatagi and Sheshadri's method[18]
12 Shah, Bhatt and Thakor's method[19]

Shah and co-workers[15, 19] have prepared 4-hydroxy coumarin derivatives in good yield by condentation of different phenols with malonic acid in the presence of zinc chloride and phosphorous oxychloride. The method is useful as single step preparation of 4-hydroxy coumarin derivatives substituted in benzenoid part.

Recently many researchers[20-51] have reported synthetic strategies for 4-hydroxy coumarin.

4.B.3 CHROMANE DIONES

Tautomeric form of 4-hydroxycoumarin was first established by J. Kolsa.[52] He proposed that if 4-hydroxy coumarin is a 2, 4-dioxochroman than its $–COCH_2CO$ group should be reactive and easily condense with ester but show no reaction with diethylcarbonate, ethylcyanoacetate, methylacetoacetate, ethylacetoacetate and benzylacetoacetate in presence of sodium and sodium ethoxide. Thus it was concluded that 4-hydroxycoumarin enolises at the lactone carbonyl and exists mainly in coumarin tautomerism in which the enol form of coumarin is favored. (Fig. 4.2)

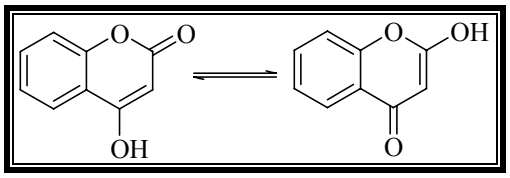


Fig. 4.2

Various methods are utilized to prepare 2, 4-chromandiones and 2, 3, 4-chromantriones shows ketonic nature of 4-hydroxycoumarin and therefore they were thought of interest to investigate their chemical reactivity and other properties. So far, 2, 4-chromandiones have been isolated as crystalline solids. Solid derivatives of 2, 3, 4-chromantriones have been reported usually as derivatives of the 3-keto group.

Extensive work has been reported on 2, 4-chromandiones and 2, 3, 4-chromanetriones. They are regarded as analogues of 4-chromanones and 3, 4-chromanediones respectively.[53] (Fig. 4.3)

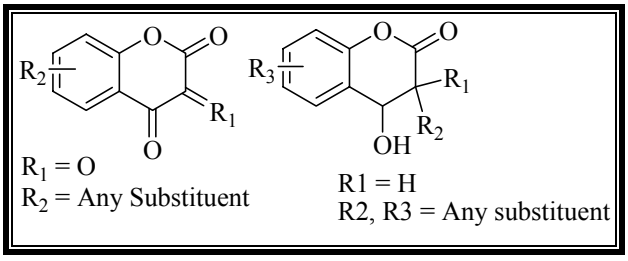


Fig. 4.3

A brief survey of literature on some 2, 4-chromandione and 2, 3, 4-chromantrione derivatives is summarized.

4-hydroxycoumarins frequently react with aromatic aldehydes to give 3-benzylidene-2, 4-chromandiones.[54-57] (Fig. 4.4)

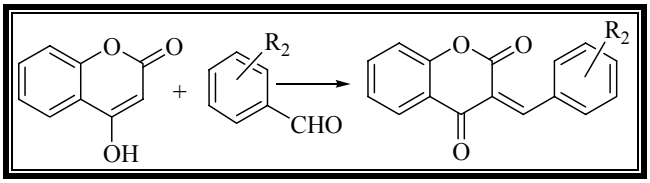


Fig. 4.4

however, reactions using salicylaldehyde or its analogues multicyclic compounds were obtained either solely or in addition to salicylidene derivatives of type as shown in (Fig. 4.5).[58]

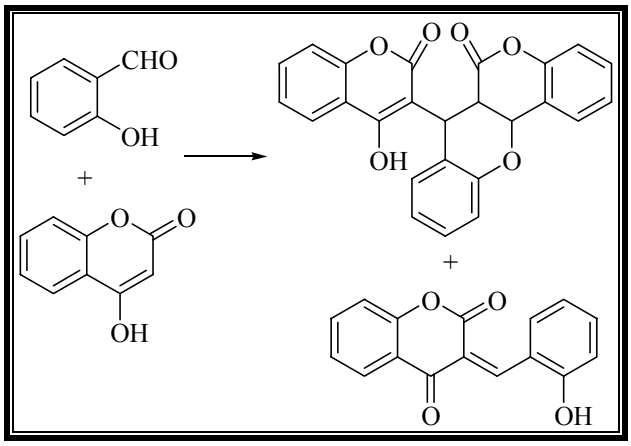


Fig. 4.5

The proportions of the products were dependent on reaction conditions used e.g. when salicylaldehyde and 4-hydroxycoumarin refluxed in ethanol; a dimeric type of structure in addition to benzylidene derivative was obtained.[59]

When two moles of salicylaldehyde were reacted with 4-hydroxycoumarins, it gave appropriate benzylidene derivative only (Fig. 4.6). However, one mole of salicylaldehyde with two moles of 4-hydroxycoumarin gave the dicoumarinyl structure.[60] (Fig. 4.7)

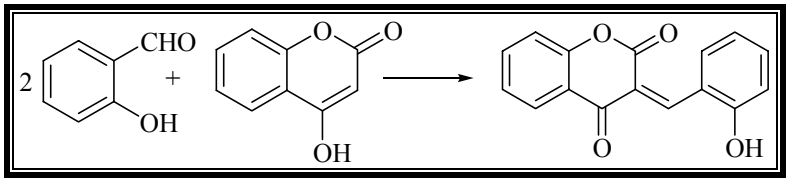


Fig. 4.6

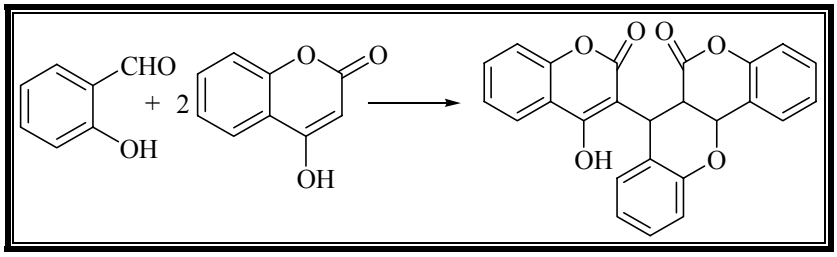


Fig. 4.7

Similarly, reaction of 4-hydroxycoumarin with acetylated aldehydohexoses in ethanol for 24 hours gave substance shown in (Fig. 4.8).[61]

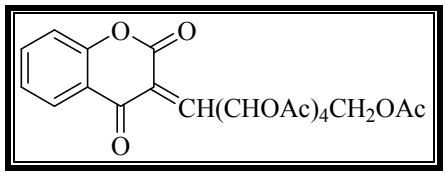


Fig. 4.8

Reaction between 4-hydroxycoumarin and hydroxylamine hydrochloride gave corresponding 2, 4-chromadione-4-oximes.[62] (Fig. 4.9)

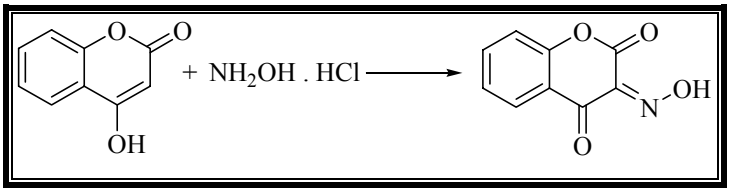


Fig. 4.9

Reaction of chlorine with 4-hydroxycoumarins in suitable solvent or sulfuryl chloride led to the formation 3, 3-dichloro-2, 4-chromandiones.[63-67] Halogenations of 3-substituted 4-hydroxycoumarin afforded 3-chloro-2, 4-chromandiones. When 3, 3' methylenebis (4-hydroxycoumarin) was treated with sufuryl chloride, 3, 3' methylenebis (3-chloro-2, 4-chromandione) was isolated. (Fig. 4.10)

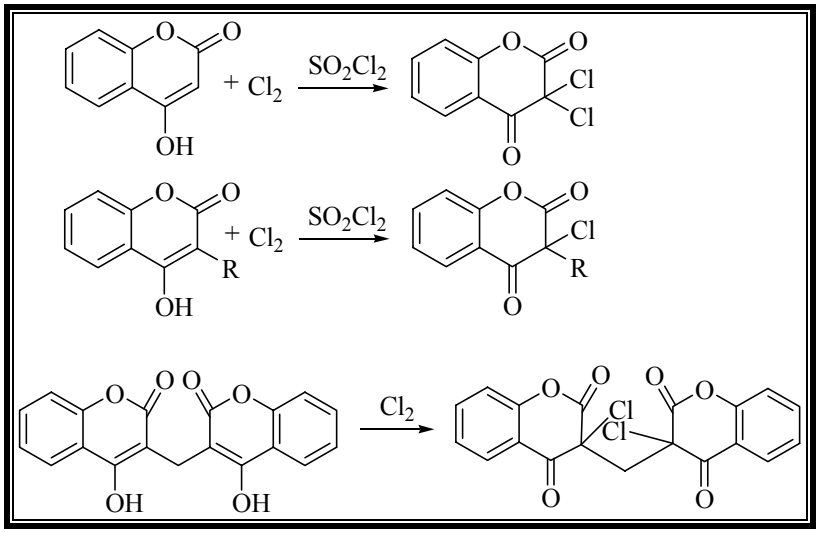


Fig. 4.10

When 3-amino-4-hydroxycoumarin was reacted with nitrous acid, it gave 3-diazo-2, 4-chromandiones. The same product was also obtained in 72% yield when sodium nitrite in dilute hydrochloric acid was added to 3-amino-4-hydroxycoumarin.[68] (Fig. 4.11)

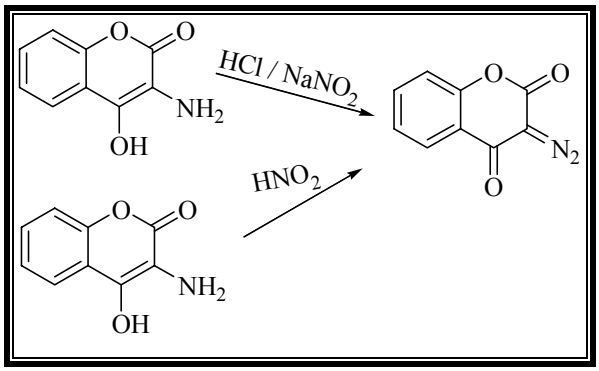


Fig. 4.11

However, reaction of 4-hydroxycoumarin with aqueous sodium nitrite afforded 2, 3, 4-chromantrione-3-oxime which forms a silver salt.[69] (Fig. 4.12)

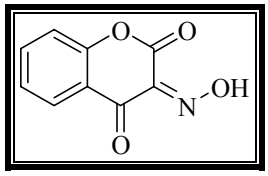
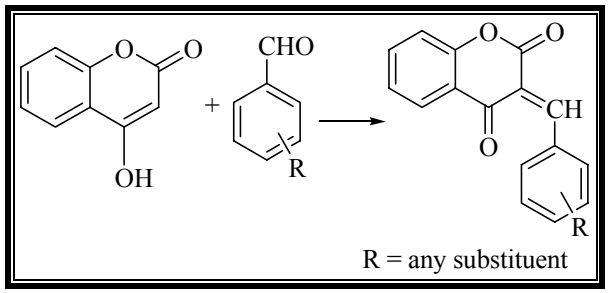


Fig. 4.12

4.B.4 MANNICH BASES FROM COUMARINS AND QUINOLONES

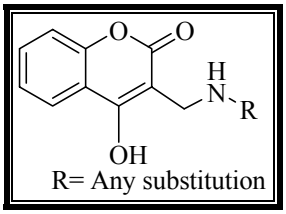
Mannich reaction is a reaction between a molecule with an active hydrogen atom, an aldehyde or an amine yielding "Mannich bases"[70]

4-hydroxy coumarin frequently reacts with aldehydes to give 3-benzylidene-2,4-chromandiones. The conditions used were heating with acetic anhydride in presence of small amount of piperidine[71], Ethanol at room temperature[72] or at reflux[73]



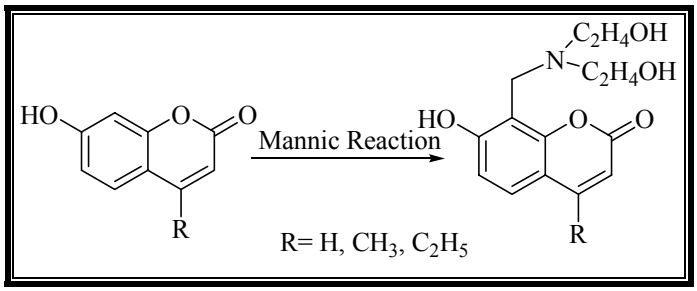
Mannich[74] has reported many primary amines like methylamine, ethylamine. Ethanolamine, β-phenylethylamine etc which are used for the formation of bases under Mannish reaction conditions.

Robertson and Link[75] prepared 3-substituted aminomethyl-4-hydroxycoumarins by reaction between amine and formalin in absolute ethanol. To this solution, 4-hydroxycoumarin in ethanol was added and the product was obtained after cooling the reaction mixture.

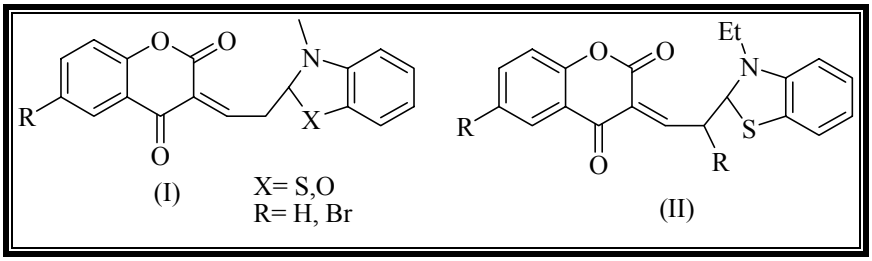


They also prepared 3,3'-methylenebis-4-hydroxycoumarin by reaction between secondary amine with 4-hydroxycoumarin against the expected product as Mannich bases.

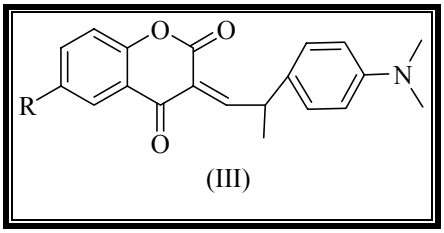
Elderfield and Mehta[76] prepared Mannich bases from 4-substituted-7-hydroxycoumarin derivatives – which were converted to nitrogen mustard as potential anticancer agents.



A number merocyanine dyes of type (I) have been synthesized frpm 4-hydroxy coumarin and 2-acetanilidovinylbenzothiazole methiodide and related compounds by refluxing for few minutes in acetic anhydride and triethylamine. Analogous coumpounds (II) have been prepared by treatment of 4-hydroxy coumarin with 2-(2-chloro propenyl)-3-ethylbenzothiazolonium chloride.[77]

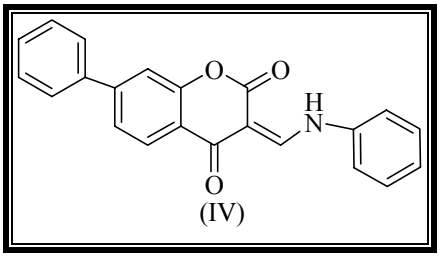


Webster and McGolgin[78] have prepared dimethylaminophenylpropylidene-2,4-chromandione and it was used in the production of visible laser radiation.

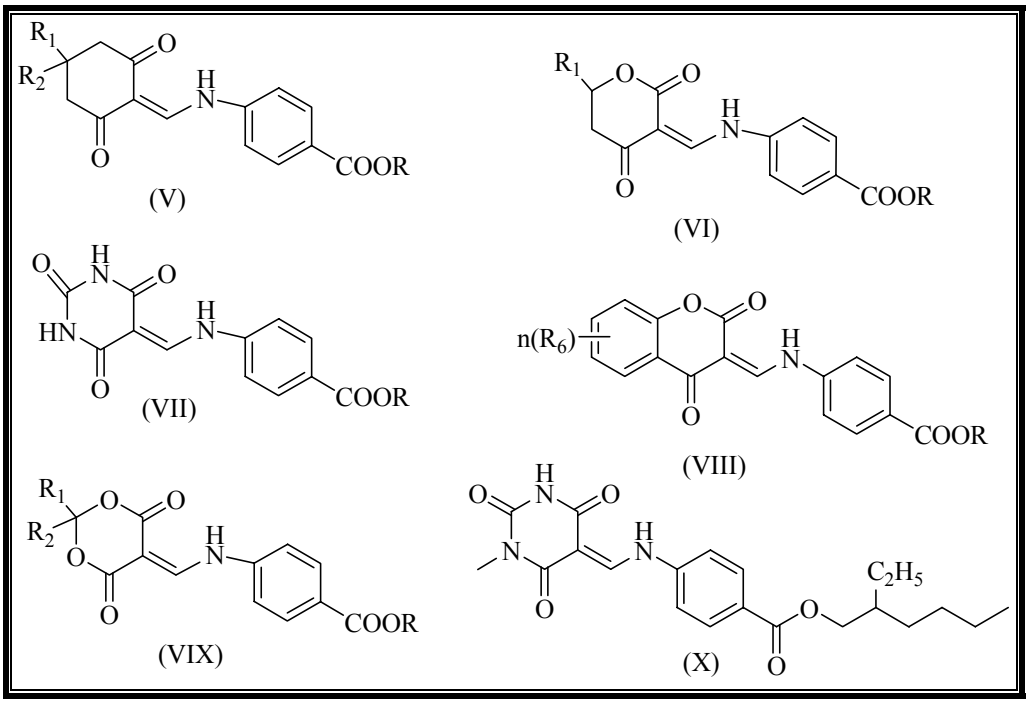


4-hydroxy-6-phenyl-2-pyrone when condensed with triethyl orthoformate and aniline in ethanol, gave the compound of the type (IV), which was cleaved in

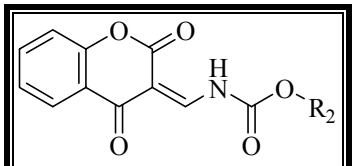
refluxing aqueous acetic acid-hydrochloride acid into 2-phenyl-4-pyrone in 62% yield.[79]


(IV)

Yasude and Hukuoka[80] have reported many compounds of the type (V-X) which were claimed as UV absorbing agents, weather-resistant organic polymer compound and a cosmetic ingredient also (VI-X). It was also observed that these derivatives have an excellent UV absorbtion ability and a high light stability.

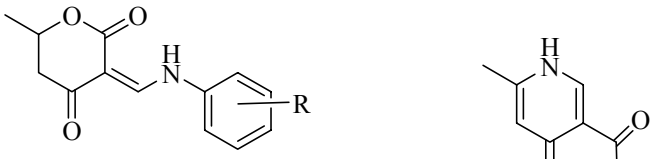

(V) (VI) (VII) (VIII) (VIX) (X)

Stankovicova and co-workers have studied the skeletons of the compounds of the type (XXII). The semiemperical PM3 method has also been used for the calculation of the heats of formation and optimal structure of 4-chromones (XI).



(XI)

Zhengming and co-workers[81] have reported that when pyranones (XII) were refluxed with aqueous hydrochloride acid for 1 hour., afforded pyridine derivatives (XIII) with 46-72 percent yield.

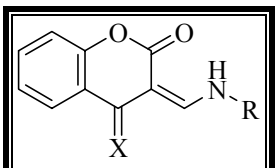


(XII)

(XIII)

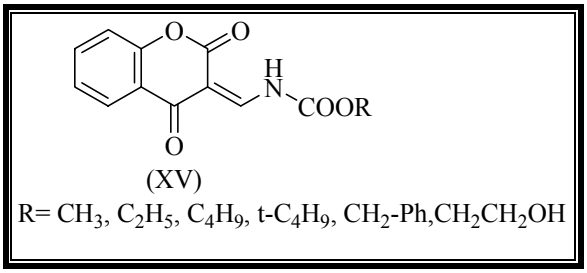
R= 4-Br, 4-Cl, 4-NO_2, 4-CH_3, 2-Cl, 2-NO_2, 2,4-Dichloro

The equilibrium between Z and E isomers of the type (XIV, where X=S, Se) derived from 3-formyl-4-thio (seleno)coumarin resulting from the hindered rotation around the exocyclic carbon-carbon bond in the stable ketoamnie tautomeric forms have been studied by means of IR, 1D & 2D, NMR spectroscopy and also by ab initio and semi empirical calculation.[82]



(XIV)

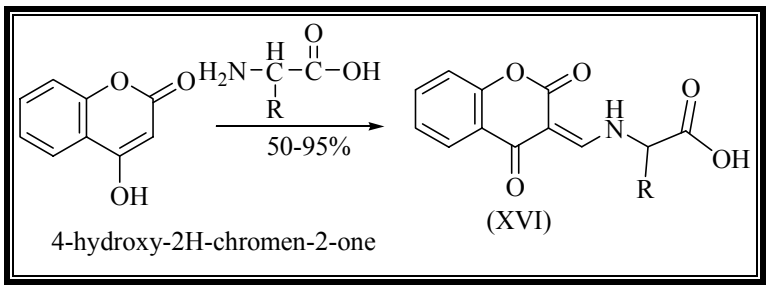
Hamdi and co workers[83] synthesized many new compounds N-(methylene-4-oxo-coumarinyl) carbamates (XV) by the condensation of carbamates with 4-hydroxy coumarin in the presence of triethylorthoformate with good yields.



(XV)

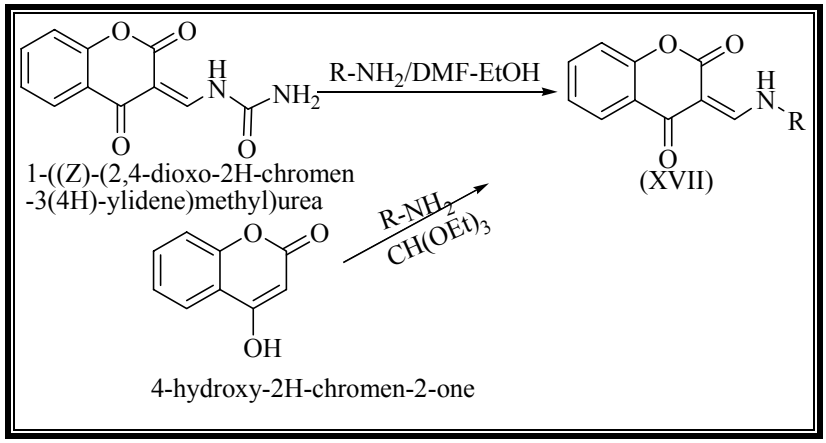
R= CH_3, C_2H_5, C_4H_9, t-C_4H_9, CH_2-Ph,CH_2CH_2OH

Further, when 4-hydroxy coumarin reacted with a-amino acids in the presence of excess triethylorthoformate gave the compounds of type (XVI) with good yield. The amino acids like glycine, alanine, leucine, phenyl alanine, tyrosine, tryptophan, L-DOPA, serine, cysteine, glutamic acid and glutamine were used.[84]



4-hydroxy-2H-chromen-2-one (XVI)

It was also revealed that N-(methylene-4-oxo-coumarinyl) amines of the type (XVII) have been prepared by aliphatic and aromatic amines with 3-ureidomethylenecoumarins. The compounds of the type (XVII) were prepared by the reactions of amines with 4-hydroxy coumarin and triethyl orthoformate with 40-90 % yield.[85]

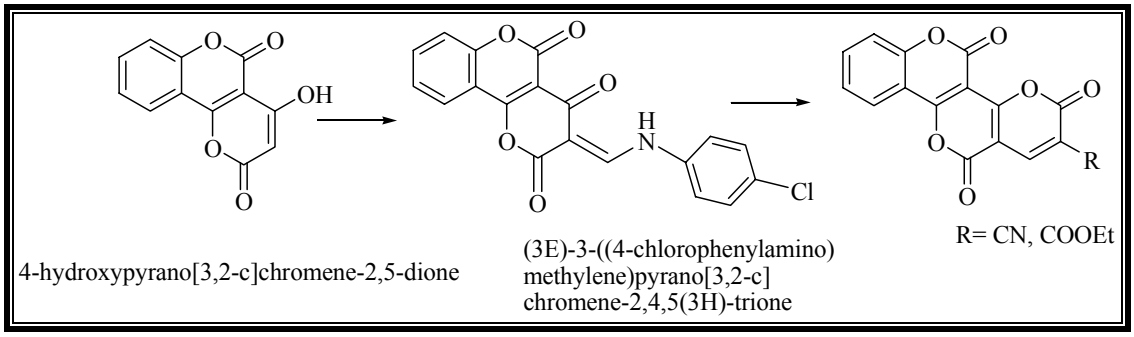


4-hydroxy-2H-chromen-2-one (XVII)

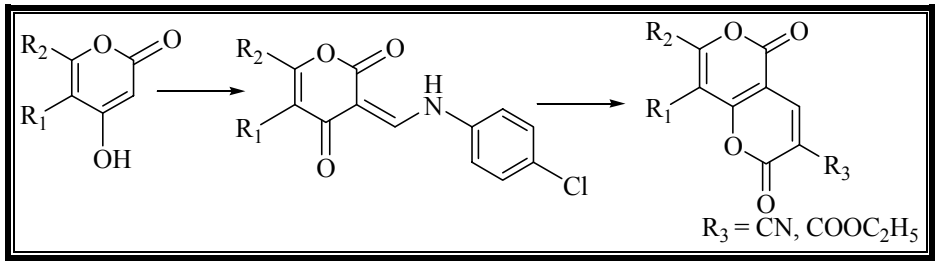
It was also observed that the addition of the amine at site C3 of the unsaturated a-B-ketone system with elimination of urea indicated that the –NHR group is a poor leaving group. This reaction can also be carried out with substituted 3-ureidomethylene coumarin with elimination of substituted urea.

Numerous applications of methylene oxopyrone amines as an intermediate have been found from different literature and are summerised as below –

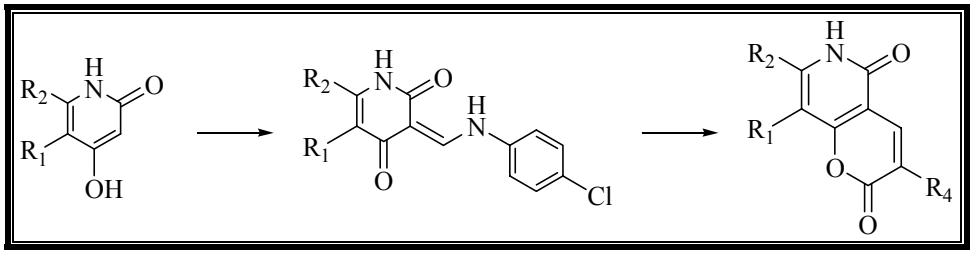
a) Zeigler, E and coworkers [86]



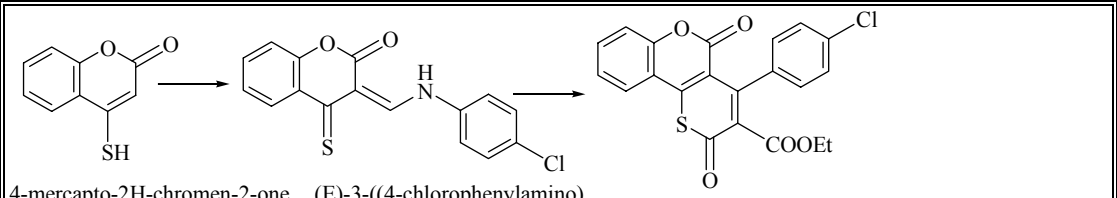
b) Butt, M.A. and coworkers [87, 88]



c) Ziegler, E and coworkers [89,90,91,92]

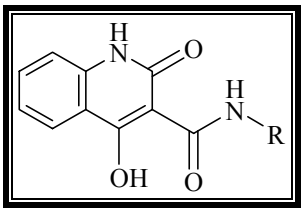


d) Peinhardt, et al[93]

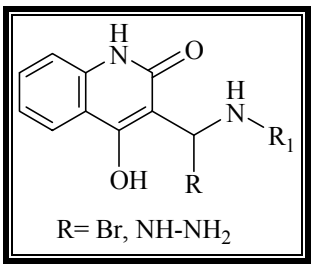


4-mercapto-2H-chromen-2-one (E)-3-((4-chlorophenylamino) methylene)-4-thioxochroman-2-one ethyl 4-(4-chlorophenyl)-2,5-dihydro-2,5-dioxothiopyrano[3,2-c]chromene-3-carboxylate

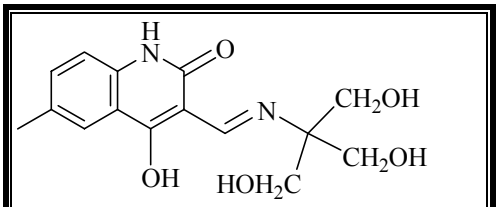
discloses 4-hydroxy-2-oxo-3(1H)-quinoline carboxoamides and their analogues as herbicides.[94]



The authors synthesized a series of heterocycles including target compounds and found them to be active against gram positive, gram negative as well as yeast.[95]

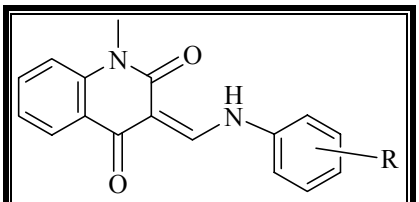


The authors screened a chemolibrary for drug like substances having the ability to activate reactive oxygen species production in murine phagocytes and identified the new molecules with potent neutrophil activating properties.[96]



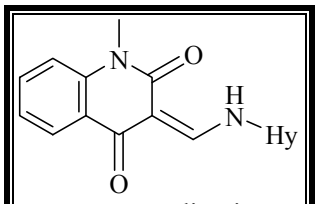
3-((E)-(1,3-dihydroxy-2-(hydroxy methyl)propan-2-ylimino)methyl)-4-hydroxy-6-methylquinolin-2(1H)-one

The authors used CoMSIA and CoMFA to study three dimensional QSAR of 21 compounds of 2, 4-quinolindione derivatives against wheat-leaf-rust.[97]



R= Et, CH_3, OH

The authors prepared a series of 3-(heteroarylaminomethylene) quinolonediones in 72-79% yields and studied their molluscidal and larvicidal activities.[98]



Hy= Heterocyclic Ring

4.B.5 BIOLOGICAL ACTIVITIES

Numerous biological activities have been associated with simple coumarins and its analogues. Among them, antimicrobial, antiviral, anticancer, enzyme inhibition, anti-inflammatory, antioxidant, anticoagulant and effect on central nervous system are most prominent. Coumarin nucleus possesses diversified biological activities that can be briefly summarized as under:

1 Antimicrobial and Molluscicidal[99-120]
2 Antiviral[121-125]
3 Anticancer[126-136]
4 As Enzyme Inhibition[137-142]
5 Antioxidant[143-146]
6 Anti-inflammatory[147-151]
7 Anticoagulant and Cardiovascular[152-155]
8 Effect on Central Nervous System[156-158]

4-hydroxycoumarin is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 4-hydroxycoumarin has led to the extensive use of this compound in organic synthesis. 4-hydroxy coumarin shows diversified chemical reactivity. Preparation of 3-acetyl-4-hydroxycoumarinyl chalcones, condensation reaction of 4-hydroxycoumarin with aldehydes and use of 4-hydroxycoumarin as a β-keto ester in dihydropyrimidine synthesis have been discussed herein.

4.B.6 AIM OF CURRENT WORK

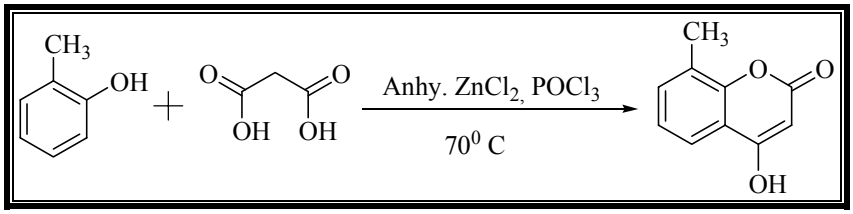
4-hydroxycoumarins and aromatic aldehydes are known to produce 2, 4-chromane diones (arylidine at C_3 position) and coumarin dimers under reflux, with or without base. Thus, few new chromane diones were prepared using aldehydes.

Besides this, microwave chemistry and especially microwave assisted organic synthesis has drawn a remarkable attention of all chemistry researchers towards its advantages. There are many reactions reported involving microwave assisted organic synthesis. Microwave chemistry is one of the best tools to carry out different reactions on desired molecules with lesser reaction time, lesser energy, easy work up and higher yields with better purity. In this chapter microwave assisted organic synthesis has been adopted to minimize the reaction time as compared to conventional heating and to optimize the results.

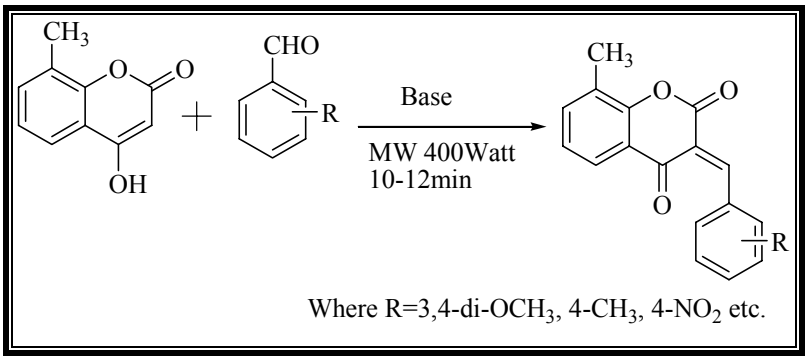
Though the chemistry of the synthesized compounds is known, the compounds are reported herein for the first time. Biological importance of such important compounds is the rational behind the current work done in this chapter.

4.B.7 REACTION SCHEMES

4.B.7.1 PREPARATION OF 8-METHYL-4-HYDROXYCOUMARIN

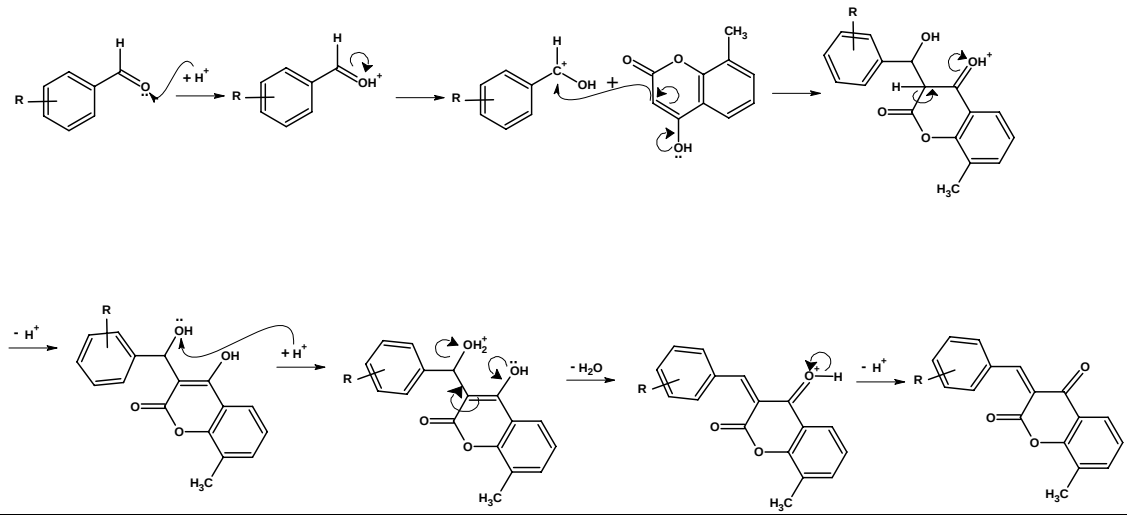


4.B.7.2 PREPARATION OF 3-SUBSTITUTED BENZYLIDENE-8-METHYL-3H-CHROMENE-2, 4-DIONES



4.B.8 PLAUSIBLE REACTION MECHANISM

SPC Series



4.B.9 EXPERIMENTAL

4.B.9.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. All the reactions were carried out in **Samsung MW83Y Microwave Oven** which was locally modified for carrying out chemical reactions. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in $CDCl_3$ solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

4.B.9.2 PREPARATION OF 8-METHYL-4-HYDROXYCOUMARIN

It was prepared according to the method reported by Shah *et. al.* [a]

O-Cresol (0.1 mole) and malonic acid (10.4 gm; 0.1 moles) were added to a mixture of phosphorous oxychloride (40 ml) and anhydrous zinc chloride (30 gms) which was preheated to get rid of any moisture. The reaction mixture was heated on a water bath at 70 ^{o}C for 8-10 hours. It was cooled and decomposed with ice and water to afford buff-yellow coloured solid.

The solid was then filtered and washed thoroughly with water. It was then triturated with 10% sodium carbonate solution and filtered. The filterate was slowly acidified with dilute HCl till the effervescence ceased. The product was filtered and dried and recrystallised with methanol.

[a] A. K. Shah, N. S. Bhatt and V. M. Thakor; *Curr. Sci.*, **1984**, *53(24)*, 1289.

4.B.9.3 PREPARATION OF 3-SUBSTITUTED BENZYLIDENE-8-METHYL-3H-CHROMENE-2, 4-DIONES (SPC-01 TO SPC-20) : GENERAL METHODS

[A] CONVENTIONAL METHOD

8-Methyl 4-hydroxycoumarin (0.01 mole) was dissolved in 30 ml of methanol into 100 ml round bottom flask. Substituted Benzaldehydes (0.01 mole) was added to the above flask along with few drops of piperidine. The reaction mixture was heated on water bath for 4-5 hours. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (6: 4) as a mobile phase. After the reaction to be completed, the reaction mixture was cooled to room temperature and the product was separated by filtration. The product was washed with methanol and dried to give desired product in good yield which was recrystallized by DMF. Physical data of the synthesized end products are summarized in the table.

Similarly other compounds are also prepared.

[B] MICROWAVE METHOD

8-Methyl 4-hydroxycoumarin (0.01 mole) was dissolved into 20 ml of methanol into 100 ml microwave flask. Substituted Benzaldehydes (0.01 mole) was added to the above flask along with few drops of piperidine. The reaction mixture was irradiated under microwave irradiation using Qpro-M microwave synthesizer for the desired time at 400 Watt. The progress and the completion of the reaction were checked at interval of every one min. by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (6 : 4) as a mobile phase. After the reaction to be completed, the reaction mixture was scratched into 30 ml of methanol. The separated product was filtered off and washed with methanol and it was dried to give desired product in good yield which was recrystallized by DMF. Physical data of the synthesized end products are summarized in the table.

Similarly other compounds are also prepared.

COMPARATIVE RESULTS OF METHOD (A) AND METHOD (B) ARE SUMMARIZED AS UNDER.

TABLE-4.B.1

Code No.	Reaction Condition					% Yield	
	Method (A)		Method (B)			Method (A)	Method (B)
	Temp. (°C)	Time (hrs.)	Watt (W)	Temp. (°C)	Time (min.)		
SPC-01	90-95	4.5	400	110	3.0	68	85
SPC-02	90-95	5.0	400	110	3.3	64	80
SPC-03	90-95	4.8	400	110	3.1	70	83
SPC-04	90-95	5.5	400	110	3.2	72	81
SPC-05	90-95	5.3	400	110	3.0	66	82
SPC-06	90-95	5.0	400	110	3.4	70	86
SPC-07	90-95	4.5	400	110	3.0	74	90
SPC-08	90-95	5.2	400	110	3.2	68	88
SPC-09	90-95	5.0	400	110	3.0	72	86
SPC-10	90-95	4.8	400	110	3.1	76	84
SPC-11	90-95	4.6	400	110	3.3	70	82
SPC-12	90-95	4.8	400	110	3.7	64	88
SPC-13	90-95	5.2	400	110	3.1	68	80
SPC-14	90-95	5.5	400	110	4.2	76	84
SPC-15	90-95	4.8	400	110	3.8	72	86
SPC-16	90-95	4.6	400	110	3.6	70	82
SPC-17	90-95	4.2	400	110	3.1	74	94
SPC-18	90-95	5.2	400	110	3.0	78	86
SPC-19	90-95	5.0	400	110	3.5	68	80
SPC-20	90-95	4.7	400	110	3.3	76	88

4.B.10 PHYSICAL DATA

PHYSICAL DATA OF 3-SUBSTITUTED BENZYLIDENE-8-METHYL-3H-CHROMENE-2, 4-DIONES (SPC-01 TO SPC-20)

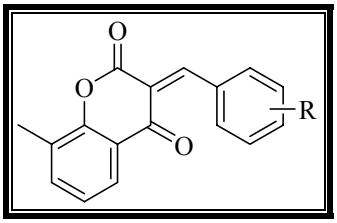


TABLE-4.B.2

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	M.P. ^{0}C	R_f Value
01	SPC-01	3,4-di-OCH_3	$C_{19}H_{16}O_5$	324	220-222	0.46
02	SPC-02	4-CH_3	$C_{18}H_{14}O_3$	278	200-202	0.52
03	SPC-03	4-Cl	$C_{17}H_{11}ClO_3$	298	212-214	0.44
04	SPC-04	2-OH,4-OCH_3	$C_{18}H_{14}O_5$	310	180-182	0.48
05	SPC-05	4-NO_2	$C_{17}H_{11}NO_5$	309	172-174	0.50
06	SPC-06	2-CH_3	$C_{18}H_{14}O_3$	278	188-190	0.40
07	SPC-07	2-NO_2	$C_{17}H_{11}NO_5$	309	164-166	0.44
08	SPC-08	3-Cl	$C_{17}H_{11}ClO_3$	298	162-164	0.48
09	SPC-09	4-OH	$C_{17}H_{12}O_4$	280	170-172	0.42
10	SPC-10	2-OCH_3	$C_{18}H_{14}O_4$	294	178-180	0.46
11	SPC-11	3,4-di-OH	$C_{17}H_{12}O_5$	296	184-186	0.42
12	SPC-12	2-OH,5-OCH_3	$C_{18}H_{14}O_5$	310	192-194	0.54
13	SPC-13	3-CH_3	$C_{18}H_{14}O_3$	278	190-192	0.50
14	SPC-14	3-NO_2	$C_{17}H_{11}NO_5$	309	160-162	0.48
15	SPC-15	2-Cl	$C_{17}H_{11}ClO_3$	298	166-168	0.40
16	SPC-16	2-OH	$C_{17}H_{12}O_4$	280	172-174	0.46
17	SPC-17	H	$C_{17}H_{12}O_3$	264	192-194	0.44
18	SPC-18	3-OH	$C_{17}H_{12}O_4$	280	188-190	0.48
19	SPC-19	4-Br	$C_{17}H_{11}BrO_3$	343	178-180	0.40
20	SPC-20	4-F	$C_{17}H_{11}FO_3$	282	176-178	0.42

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (6 : 4)

4.B.11 SPECTRAL DISCUSSION

4.B.11.1 MASS SPECTRAL STUDY

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. Characteristic M^{+2} ion peaks with one-third intensity of molecular ion peak were observed in case of compounds having chlorine atom. SPC-02 and SPC-03 of Mass spectra are given on page no. 193 and 195.

4.B.11.2 IR SPECTRAL STUDY

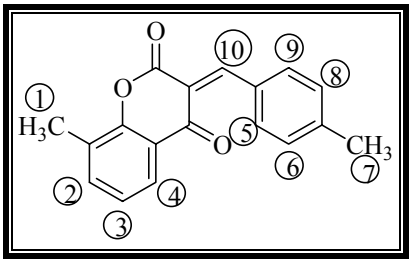
IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

In case of SPC-01 to SPC-20 series of compounds, stretching and bending frequency for aromatic and stretching frequencies for methyl, methylene groups were found near 3050 cm^{-1}, 1400-1640 cm^{-1}, 2950 cm^{-1} and 2850 cm^{-1} respectively in all the compounds. Two Carbonyl stretching frequencies were obtained near 1700 cm^{-1} and 1670 cm^{-1} in all the compounds. Frequencies for ether linkage were obtained near 1050 cm^{-1} and in all the compounds. SPC-02 and SPC-03 of Mass spectra are given on page no. 193 and 195.

4.B.11.3 ^{1}H NMR SPECTRAL STUDY

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from ^{1}H NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H NMR spectral interpretation can be discussed as under.

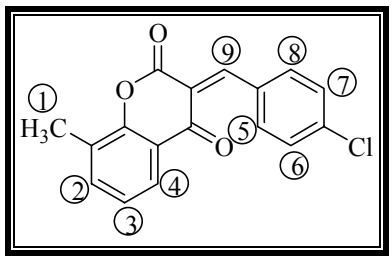
^{1}H NMR OF 3-(4-METHYLBENZYLIDENE)-8-METHYL-3H-CHROMENE-2, 4-DIONE (SPC-02)



1. Proton no. 1 and 7 of total 6H gave a doublet at 2.47 δ ppm-2.50 δ ppm.
2. Arylidine proton of Proton no. 10 became deshielded and gave a characteristic singlet at 6.07 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
3. Aromatic ring proton no. 5, 6, 8 and 9 of four proton gave a multiplet at 7.09 δ ppm-7.14 δ ppm.
4. Proton no. 2 and 3 of two proton gave a multiplet at 7.25 δ ppm-7.30 δ ppm.
5. Proton no. 4 gave a dublet at 7.45 δ ppm - 7.47 δ ppm. and *J* value of this proton is 8.0 Hz. It suggests ortho coupling.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPC-02 has been confirmed for cis geometrical. Spectrum is given on page no. 194.

^{1}H NMR OF 3-(4-CHLOROBENZYLIDENE)-8-METHYL-3H-CHROMENE-2,4-DIONE (SPC-03)



1. Proton no. 1 of three proton gave a characteristic singlet at 2.46 δ ppm.
2. Arylidine proton of Proton no. 9 became deshielded and gave a characteristic singlet at 6.32 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
3. Proton no. 6 and 7 gave a doublet at 7.18 δ ppm-7.20 δ ppm. and *J* value of this proton is 8.0 Hz. it suggests ortho coupling.It suggests an ortho coupling.
4. Proton no. 5 and 8 gave a doublet at 7.46 δ ppm-7.48 δ ppm. and *J* value of this proton is 8.0Hz. It suggests ortho coupling.It suggests an ortho coupling.
5. Proton no. 2 and 3 of two proton gave a multiplet at 7.24 δ ppm-7.28 δ ppm.
6. Proton no.4 gave a doublet at 7.83 δ ppm-7.85 δ ppm. and *J* value of this proton is 8.0Hz. it suggests ortho coupling.It suggests an ortho coupling.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPC-03 has been confirmed for cis geometrical. Spectrum is given on page no. 196.

4.B.11.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

4.B.12 ANALYTICAL DATA

***3-(3,4-dimethoxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-01)*:**
IR (KBr, cm^{-1}): 3020 (-CH_3 str.), 2866 (-CH_2 str.), 1670 (>CO str.), 1604, 1572, 1510 (Ar-H, ben), 1091 (C-O-C); MS *m/z* = 324 (M^+); Anal. Calcd. for $C_{19}H_{16}O_5$: C, 70.36; H, 4.97; O, 24.67. Found: C, 70.30; H, 4.94; O, 24.62.

***3-(4-methylbenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-02)*:**
IR (KBr, cm^{-1}): 3026 (-CH_3 str.), 2866 (-CH_2 str.), 1670 (>CO str.), 1602, 1573, 1508 (Ar-H, ben), 1091 (C-O-C); ^{1}H NMR (400 MHz, DMSO): δ (ppm) 2.50 (6H, d), 6.07 (1H, S), 7.14 (4H, m,), 7.30 (2H, m), 7.47 (1H, m, *J* = 0.56 Hz); MS *m/z* = 278 (M^+); Anal. Calcd. for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07; O, 17.25. Found: C, 77.60; H, 5.02; O, 17.21.

***3-(4-chlorobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-03)*:**
IR (KBr, cm^{-1}): 3032 (-CH_3 str.), 2918 (-CH_2 str.), 1664 (>CO str.), 1602, 1570, 1489 (Ar-H, ben), 1095 (C-O-C), 779 (C-Cl); ^{1}H NMR (400 MHz, DMSO): δ (ppm) 2.46 (3H, s), 6.32 (1H, s), 7.20 (2H, d, *J* = 8.56 Hz), 7.28 (2H, m), 7.48 (2H, d, *J* = 7.24 Hz), 7.85 (1H, d, J=7.96 Hz); MS *m/z* = 298 (M^+), 300 (M^{+2}); Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71; O, 16.07. Found: C, 68.31; H, 3.66; O, 16.01

***3-(2-hydroxy-4-methoxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-04)*:**
IR (KBr, cm^{-1}): 3014 (-CH_3 str.), 2870 (-CH_2 str.), 1674 (>CO str.), 1602, 1570, 1508 (Ar-H, ben), 1091 (C-O-C); MS *m/z* = 310 (M^+); Anal. Calcd. for $C_{18}H_{14}O_5$: C, 69.6 7; H, 4.55; O, 25.78. Found: C, 69.61; H, 4.50; O, 25.72.

***3-(4-nitrobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-05)*:**

IR (KBr, cm^{-1}): 3016 (-CH_3 str.), 2874 (-CH_2 str.), 1670 (>CO str.), 1604, 1566, 1506 (Ar-H, ben), 1094 (C-O-C); MS *m/z* = 309 (M^+); Anal. Calcd. for $C_{17}H_{11}NO_5$: C, 66.02; H, 3.58; N, 4.53; O, 25.87. Found: C, 66.02; H, 3.50; N, 4.51; O, 25.82.

***3-(2-methylbenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-06)*:**

IR (KBr, cm^{-1}): 3020 (-CH_3 str.), 2872 (-CH_2 str.), 1672 (>CO str.), 1603, 1571, 1508 (Ar-H, ben), 1088 (C-O-C); MS *m/z* = 278 (M^+); Anal. Calcd. for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07; O, 17.25. Found: C, 77.62; H, 5.01; O, 17.21.

***3-(2-nitrobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-07)*:**

IR (KBr, cm^{-1}): 3010 (-CH_3 str.), 2866 (-CH_2 str.), 1670 (>CO str.), 1601, 1567, 1512 (Ar-H, ben), 1090 (C-O-C); MS *m/z* = 309 (M^+); Anal. Calcd. for $C_{17}H_{11}NO_5$: C, 66.02; H, 3.58; N, 4.53; O, 25.87. Found: C, 66.00; H, 3.53; N, 4.50; O, 25.84.

***3-(3-chlorobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-08)*:**

IR (KBr, cm^{-1}): 3024 (-CH_3 str.), 2877 (-CH_2 str.), 1670 (>CO str.), 1611, 1572, 1511 (Ar-H, ben), 1092 (C-O-C); MS *m/z* = 298 (M^+), 300 (M^{+2}); Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71; O, 16.07. Found: C, 68.31; H, 3.70; O, 16.04.

***3-(4-hydroxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-9)*:**

IR (KBr, cm^{-1}): 3024 (-CH_3 str.), 2860 (-CH_2 str.), 1664 (>CO str.), 1606, 1560, 1508 (Ar-H, ben), 1090 (C-O-C); MS *m/z* = 280 (M^+); Anal. Calcd. for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32; O, 22.83. Found: C, 72.82; H, 4.30; O, 22.80.

***3-(2-methoxybenzylidene)-8-methyl-3H-chromene-2,4-dione(SPC-10)*:**

IR (KBr, cm^{-1}): 3018 (-CH_3 str.), 2876 (-CH_2 str.), 1671 (>CO str.), 1612, 1572, 1508 (Ar-H, ben), 1088 (C-O-C); MS *m/z* = 294 (M^+); Anal. Calcd. for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79; O, 21.75. Found: C, 73.42; H, 4.71; O, 21.71.

***3-(3,4-dihydroxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-11)*:**

IR (KBr, cm^{-1}): 3022(-CH_3 str.), 2878 (-CH_2 str.), 1670 (>CO str.), 1601, 1573, 1511 (Ar-H, ben), 1093 (C-O-C); MS *m/z* = 296 (M^+); Anal. Calcd. for $C_{17}H_{12}O_5$: C, 68.92; H, 4.08; O, 27.00. Found: C, 68.88; H, 4.02; O, 27.02.

***3-(2-hydroxy-5-methoxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-12)*:**

IR (KBr, cm^{-1}): 3015 (-CH_3 str.), 2873 (-CH_2 str.), 1671 (>CO str.), 1602, 1572, 1508 (Ar-H, ben), 1095 (C-O-C); MS *m/z* = 310 (M^+); Anal. Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; O, 25.78. Found: C, 69.63; H, 4.51; O, 25.74.

***3-(3-methylbenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-13)*:**

IR (KBr, cm^{-1}): 3012 (-CH_3 str.), 2870 (-CH_2 str.), 1672 (>CO str.), 1602, 1571, 1507 (Ar-H, ben), 1093 (C-O-C); MS *m/z* = 278 (M^+); Anal. Calcd. for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07; O, 17.25. Found: C, 77.62; H, 5.02; O, 17.20.

***3-(3-nitrobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-14)*:**

IR (KBr, cm^{-1}): 3023 (-CH_3 str.), 2871 (-CH_2 str.), 1675 (>CO str.), 1602, 1571, 1511 (Ar-H, ben), 1091 (C-O-C); MS *m/z* = 309 (M^+); Anal. Calcd. for $C_{17}H_{11}NO_5$: C, 66.02; H, 3.58; N, 4.53; O, 25.87. Found: C, 66.00; H, 3.54; N, 4.51; O, 25.83.

***3-(2-chlorobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-15)*:**

IR (KBr, cm^{-1}): 3011 (-CH_3 str.), 2873 (-CH_2 str.), 1676 (>CO str.), 1601, 1570, 1518 (Ar-H, ben), 1095 (C-O-C); MS *m/z* = 298 (M^+), 300 (M^{+2}); Anal. Calcd. for $C_{17}H_{11}ClO_3$: C, 68.35; H, 3.71; O, 16.07. Found: C, 68.30; H, 3.62; O, 16.04.

***3-(2-hydroxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-16)*:**

IR (KBr, cm^{-1}): 3020 (-CH3), 2877 (-CH_2), 1671 (>CO), 1610, 1574, 1506 (Ar-H, ben), 1090 (C-O-C); MS *m/z* = 280 (M^+); Anal. Calcd. for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32; O, 22.83. Found: C, 72.81; H, 4.30; O, 22.81.

***3-benzylidene-8-methyl-3H-chromene-2,4-dione (SPC-17)*:**

IR (KBr, cm^{-1}): 3010 (-CH_3 str.), 2874 (-CH_2 str.), 1674 (>CO str.), 1606, 1570, 1509 (Ar-H, ben), 1090 (C-O-C); MS *m/z* = 264 (M^+); Anal. Calcd. for $C_{17}H_{12}O_3$: C, 77.26; H, 4.58; O, 18.16. Found: C, 77.24; H, 4.52; O, 18.14.

***3-(3-hydroxybenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-18)*:**

IR (KBr, cm^{-1}): 3017 (-CH_3 str.), 2874 (-CH_2 str.), 1670 (>CO str.), 1611, 1570, 1505 (Ar-H, ben), 1088 (C-O-C); MS *m/z* = 280 (M^+); Anal. Calcd. for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32; O, 22.83. Found: C, 72.81; H, 4.30; O, 22.81.

***3-(4-bromobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-19)*:**

IR (KBr, cm^{-1}): 3012 (-CH_3 str.), 2875 (-CH_2 str.), 1674 (>CO str.), 1603, 1576, 1508 (Ar-H, ben), 1091 (C-O-C); MS *m/z* = 343 (M^+), 345 (M^{+2}); Anal. Calcd. for $C_{17}H_{11}BrO_3$: C, 59.50; H, 3.23; O, 13.99. Found: C, 59.46; H, 3.20; O, 13.96.

***3-(4-fluorobenzylidene)-8-methyl-3H-chromene-2,4-dione (SPC-20)*:**

IR (KBr, cm^{-1}): 3016 (-CH_3 str.), 2875 (-CH_2 str.), 1666 (>CO str.), 1603, 1568, 1509 (Ar-H, ben), 1088 (C-O-C); MS *m/z* = 282 (M^+), 284 (M^{+2}); Anal. Calcd. for $C_{17}H_{11}FO_3$: C, 72.34; H, 3.93; O, 17.00. Found: C, 72.32; H, 3.90; O, 17.04.

4.B.13 RESULTS AND DISCUSSION

This chapter is mostly related to modification in the previous work done by our group as well as others but the scaffolds reported here are new. Earlier 4-hydroxycoumarin and N-methyl 4-hydroxy quinolone derivatives were known to be prepared by conventional method. Moreover, microwave assisted method was also employed to compare both the methods in order to acquire best results. Microwave assisted method found much faster than the conventional one and % yield found to be higher than conventional but the purity was similar in both the methods.

It is very well known that C_3 position of 4-hydroxycoumarin and N-methyl 4-hydroxy quinolone is highly reactive. In section-A N-methyl 4-hydroxy quinolone and different pyrazole aldehydes were refluxed in methanol under alkaline condition. In section-B, 4-hydroxycoumarin and different aldehydes were refluxed in methanol under alkaline condition, in order to prepare coumarin and quinolone dimers, but the N-methyl 4-hydroxyquinolone tautomarize into 4(3*H*)-dione and due to the in situ generation of active methylene group it gave arylidine at C_3-position.

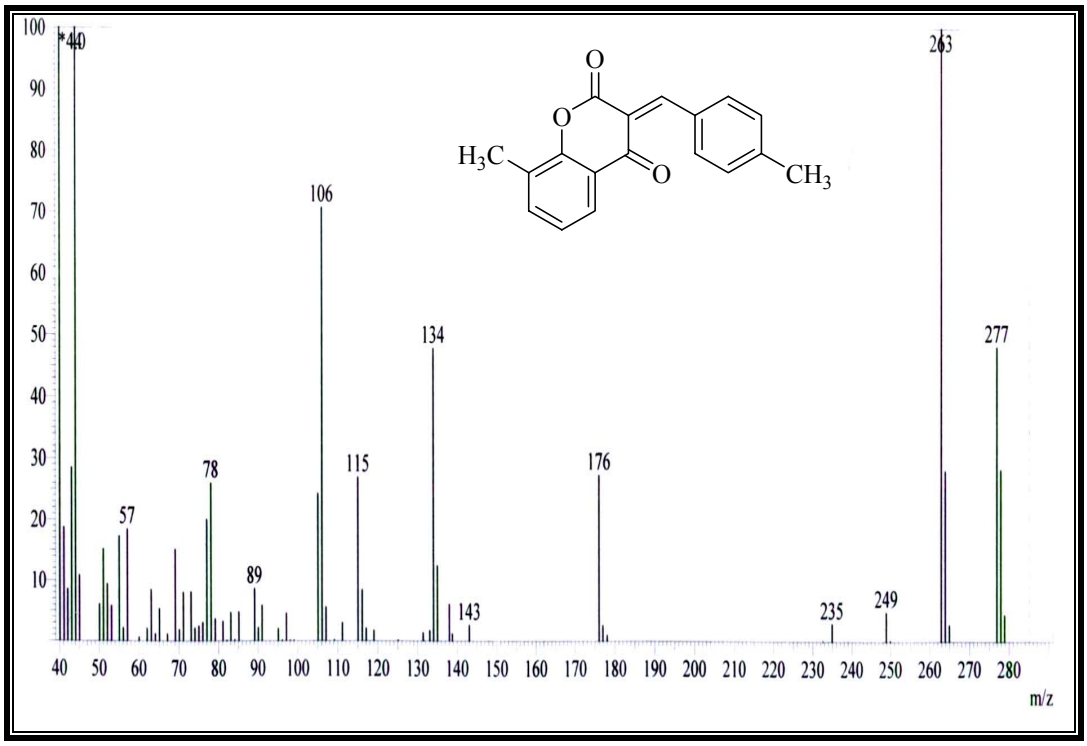
4.B.14 CONCLUSION

4-hydroxycoumarin and N-methyl-4-hydroxy quinolone is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 4-hydroxycoumarin and N-methyl-4-hydroxy quinolone has led to the extensive use of this compound in organic synthesis. It was of interest to study the biological activities of newly synthesized coumarin and quinolone derivatives.

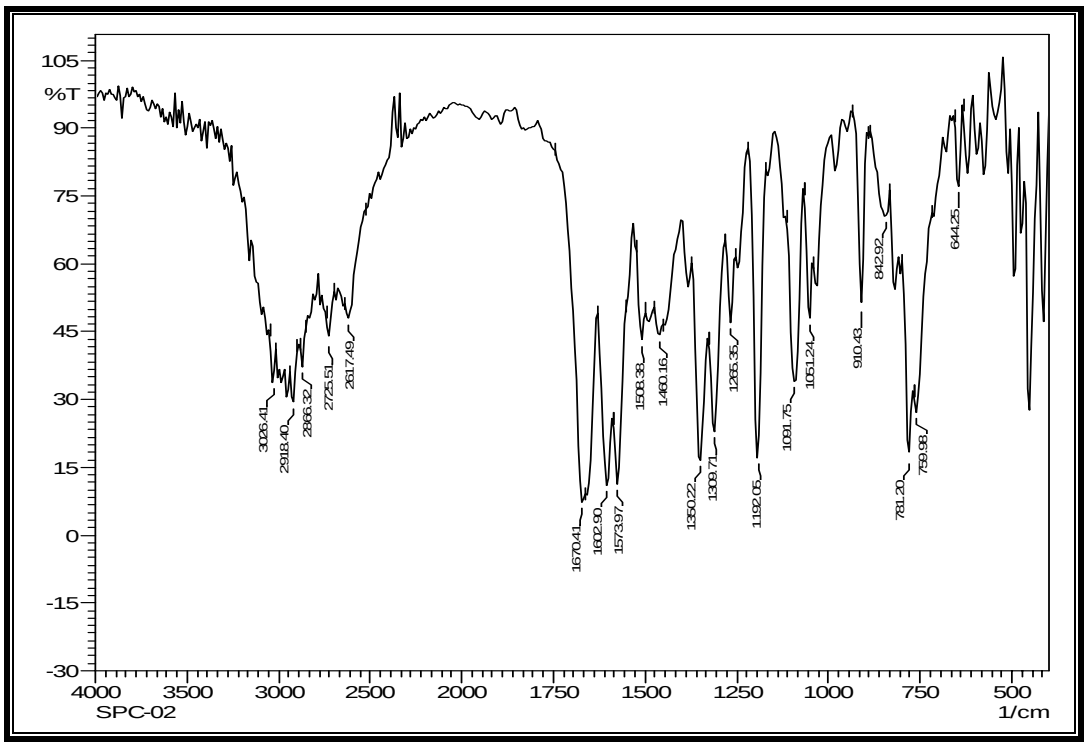
These types of the compounds were synthesized using Microwave assisted method which was found to be much faster than the conventional and the % yield was found to be higher than conventional methods.

4.B.15 REPRESENTATIVE SPECTRA

4.B.15.1 Mass Spectrum of SPC-02

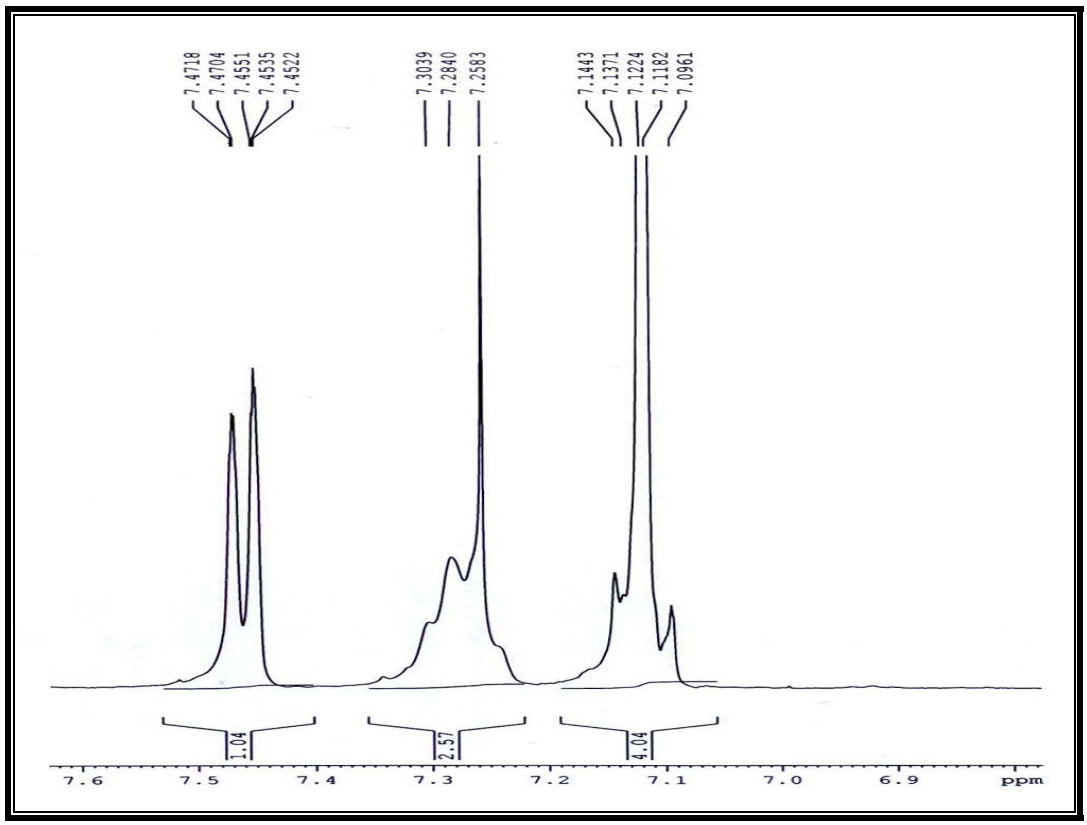


4.B.15.2 IR Spectrum of SPC-02

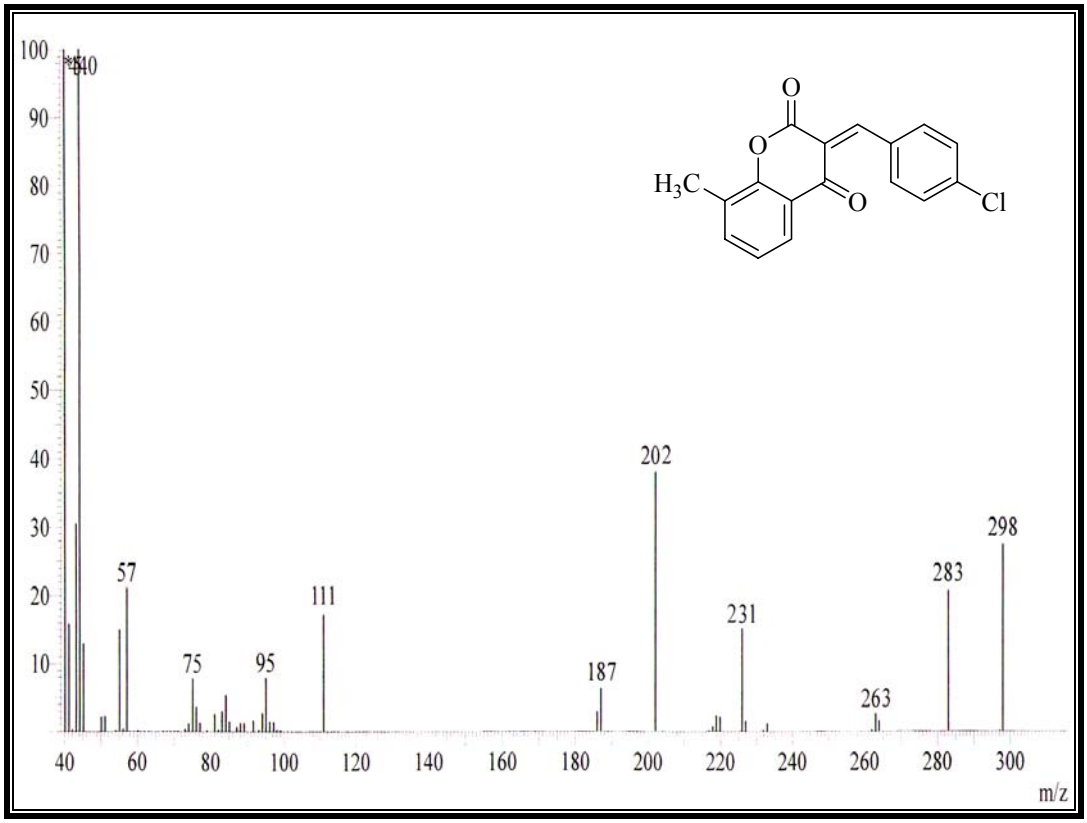


4.B.15.3 ^{1}H NMR Spectrum of SPC-02

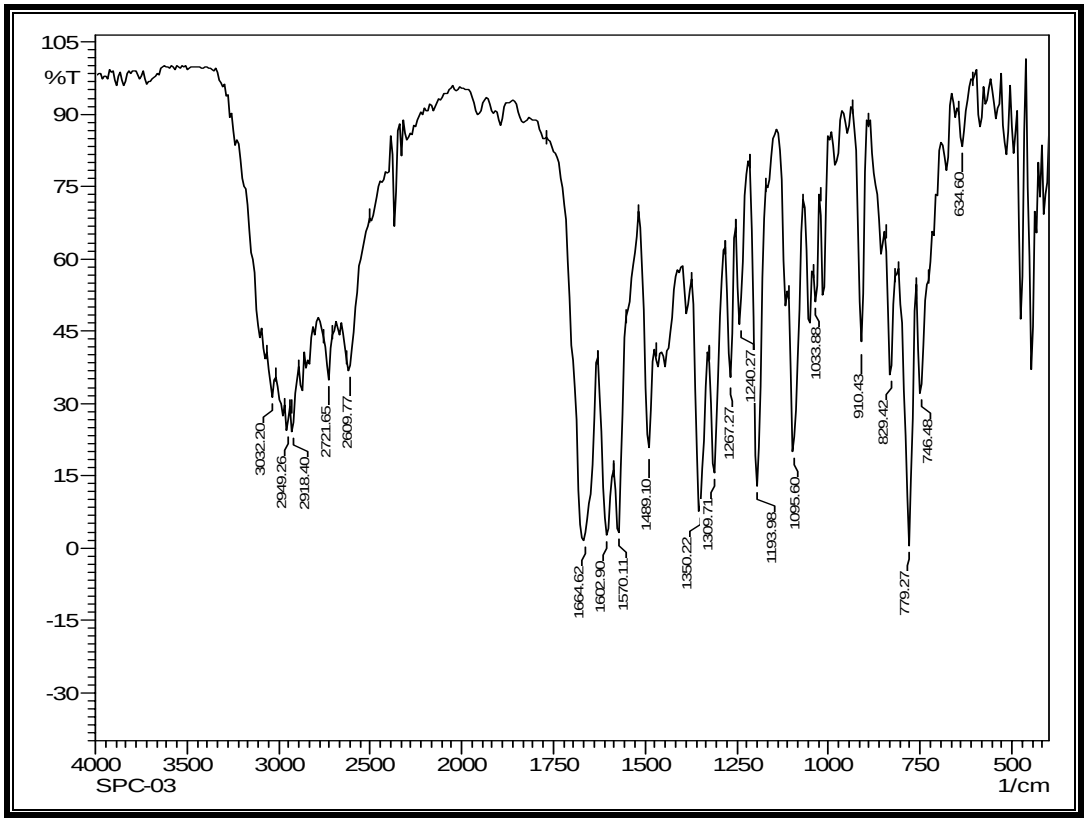
EXPANDED ^{1}H-NMR SPECTRUM OF SPC-02



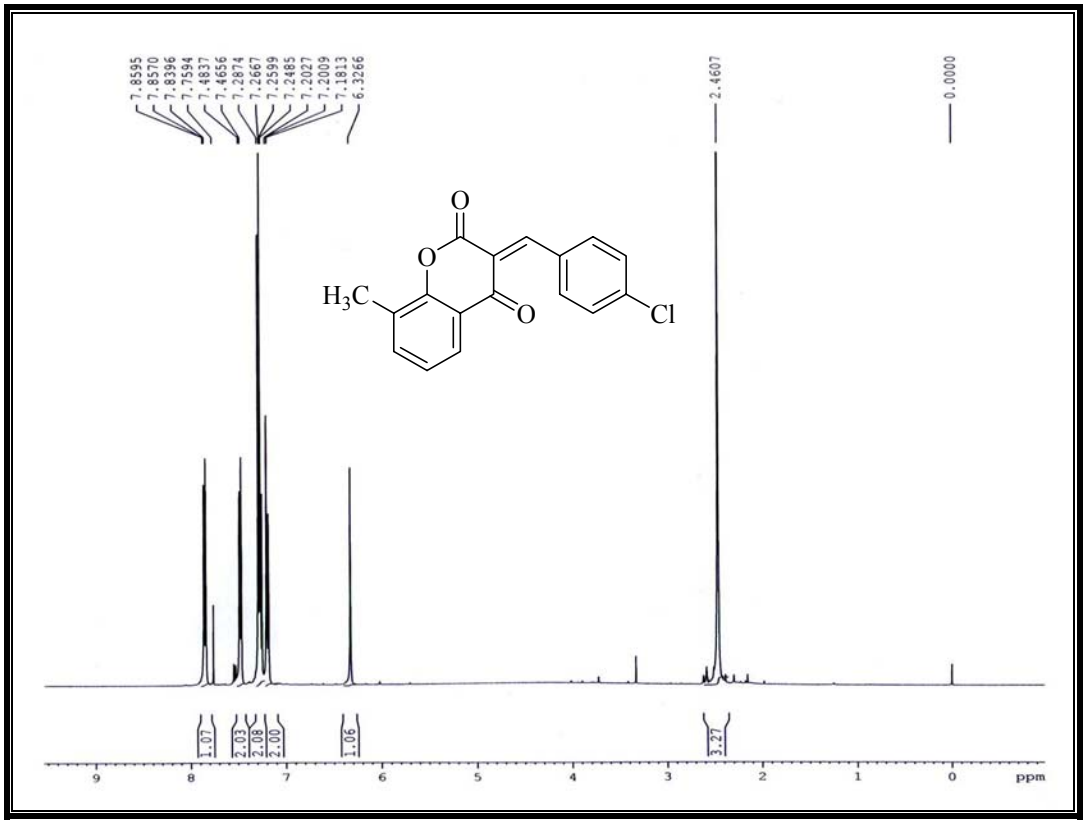
4.B.15.4 Mass Spectrum of SPC-03



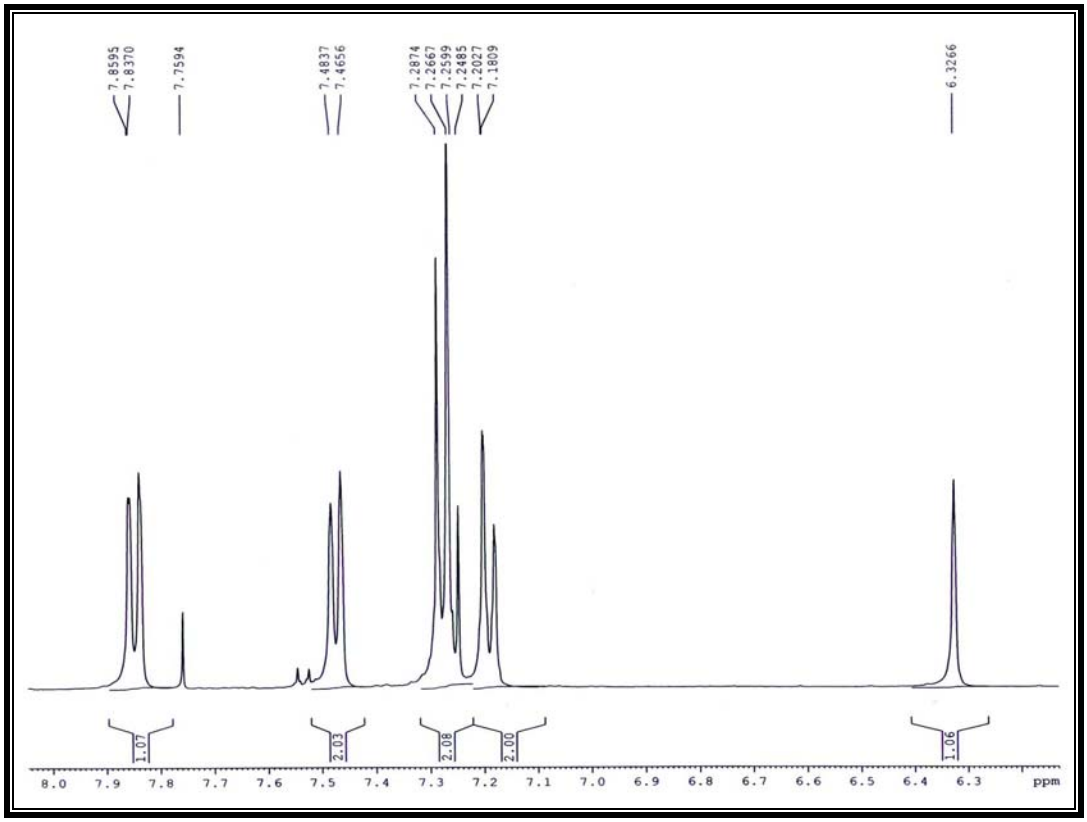
4.B.15.5 IR Spectrum of SPC-03



4.B.15.6 ^{1}H NMR Spectrum of SPC-03



EXPANDED ^{1}H-NMR SPECTRUM OF SPC-03



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CHAPTER - 5

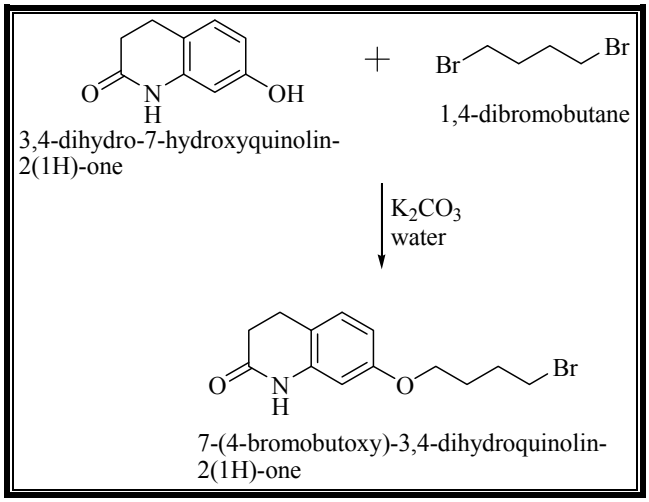
SECTION - A

STUDIES ON
7-(4-(SUBSTITUTED AMINO)
BUTOXY)-3,4-DIHYDRO
QUINOLIN-2-(1H)-ONE

5.A.1 INTRODUCTION

Quinolones are a very important family of antibacterial agents that are widely prescribed for the treatment of infections in humans. Since their discovery in the early 1960s, the quinolone group of antibacterial has generated considerable clinical and scientific interest. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones. The 4-pyridone-3-carboxylic acid associated with a 5, 6-fused aromatic ring is the common chemical feature of bactericidal quinolones. In the resulting bicyclic ring, the 1-, 5-, 6-, 7-and 8-positions are the major targets of chemical variation. Manipulations of the basic molecule, including replacing hydrogen with fluorine at position 6, substituting a cyclic amine residue at position 7 and adding new residues at position 1 of the quinolone ring, have led to improved breadth and potency of antibacterial activity and pharmacokinetics. One of the most significant developments has been the improved anti-Gram-positive activity of the newer compounds, such as moxifloxacin and garenoxacin. However, some of these structural changes have been found to correlate with specific adverse effects: the addition of fluorine or chlorine at position 8 being associated with photo reactivity, e.g. sparfloxacin; and the substitution of an amine or a methyl group at position 5 having a potential role in QTc prolongation, e.g. sparfloxacin and grepafloxacin. The clinical utility of this expanding class of antimicrobial agentsand the lower propensity for the development of resistance with the newer quinolones will need to be continually monitored in the changing therapeutic environment. Antibiotic drug choice will remain difficult in the presence of increasing resistance, but introduction of the new quinolones has created a new and exciting era in antimicrobial chemotherapy.

5.A.2 SYNTHESIS OF BROMOBUTOXY QUINOLONE



5.A.3 ARIPIPRAZOLE

Aripiprazole (Abilify, Abilify Discmelt) is an atypical antipsychotic and antidepressant used in the treatment of schizophrenia, bipolar disorder and clinical depression. It was approved by the Food and Drug Administration (FDA) for schizophrenia on November 15, 2002, for acute manic and mixed episodes associated with bipolar disorder on October 1, 2004and as an adjunct for major depressive disorder on November 20, 2007. Aripiprazole was developed by Otsuka in Japanand in the United States, Otsuka America markets it jointly with Bristol-Myers Squibb.

5.A.3.1 PHARMACEUTICAL INFORMATION

Drug Substance:

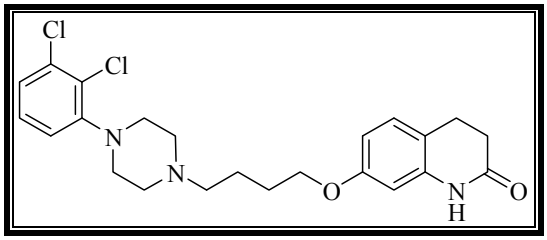
Proper name: Aripiprazole

Chemicalname:7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril.

Molecular formula: $C_{23}H_{27}Cl_2N_3O_2$

Molecular mass: 448.38

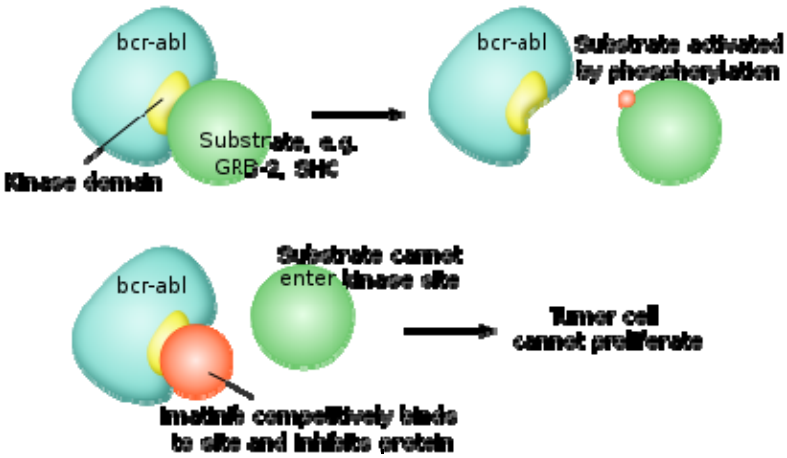
Structural formula:



Physicochemical properties: Aripiprazole is a white crystalline powder. Aripiprazole is practically insoluble in water. The pKa was determined to be 7.6 (in 20% ethanol solution).

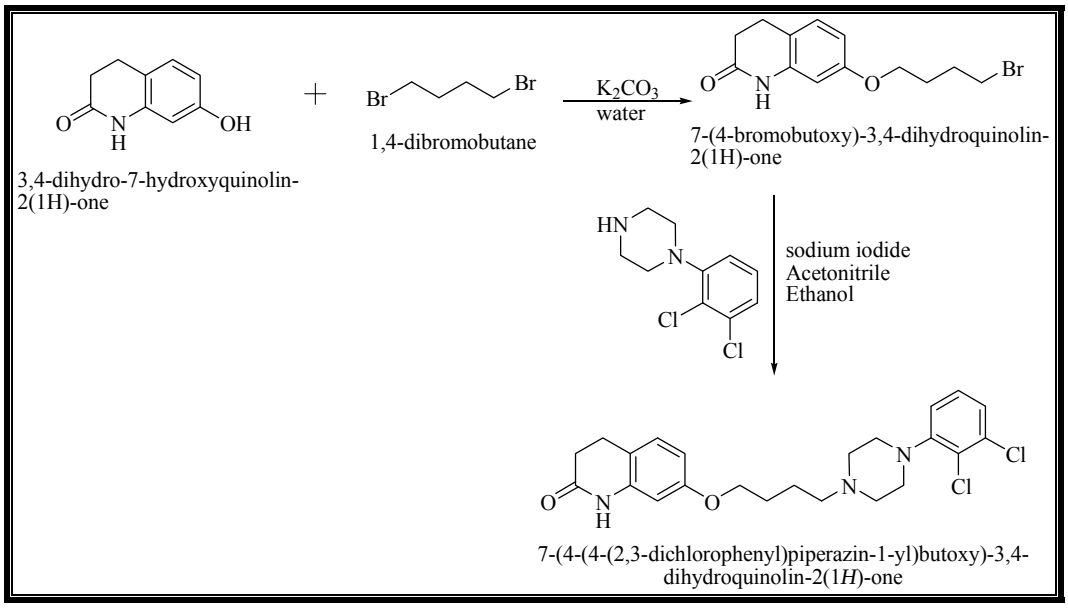
5.A.4 MECHANISM OF ACTION OF ARIPIPRAZOLE

The mechanism of action of aripiprazole, as with other drugs having efficacy in schizophrenia and bipolar disorder is unknown. However, it has been proposed that the efficacy of aripiprazole may be mediated through a combination of partial agonist activity at D2 and 5-HT1A receptors and antagonist activity at 5-HT2A receptors; however, the clinical relevance of these interactions has not been established. Actions at receptors other than D2, 5-HT1Aand 5-HT2A may explain some of the other clinical effects of aripiprazole (eg, the orthostatic hypotension observed with aripiprazole may be explained by its antagonist activity at adrenergic alpha1 receptors). The clinical relevance of these receptor interactions with aripiprazole is unknown.



5.A.5 SYNTHESIS OF ARIPIPRAZOLE

Representative procedure for the synthesis of 7-{4-[4-(2,3-dichlorophenyl) piperazin-1-yl]butoxy}-1,2,3,4-tetrahydro quinolin-2-one To a suspension of 7-(4-bromobutoxy)-1,2,3,4-tetrahydrochinolin-2-one (**1**) (29.8 g; 0.1 M) and 1-(2,3-dichlorophenyl)piprazine hydrochloride (**2**) (29.4 g; 0.1 M) in 300 mL of technical ethanol, powdered anhydrous sodium carbonate (23.3 g; 0.2 M) was added. The mixture was refluxed for 12 h. The resulting solid was filtered, taken up in technical ethanol (50 mL) and refluxed for 10 min. The insoluble inorganic residue was filtered off and the two obtained filtrates were combined, refluxed and then left at the room temperature for crystallization for 12 h. The crystalline aripiprazole (**3**) was filtered and dried to give 42.2 g (yield 85 %) of final product (HPLC purity 99.32 %).



Aripiprazole(7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro carbostyril) belongs to the group of new antipsychotics (like, for example, olanzapine, quetiapine, risperidone, amisulpride). It is used in the treatment of schizophrenia, bipolar mania and some dementia related psychosis symptoms[1]. The drug was invented initially by the Otsuka Pharmaceutical Co., Ltd., Tokyo [OPC-14597[2,3]] and co-marketed with Bristol-Myers Squibb [AbilifyTM, BMS-337039[4]].Aripiprazole can form several inclusion compounds containing polar and protic species.[5] In a search for optimal conditions to obtain crystals of aripiprazole solvate with ethanol suitable for

pharmaceutical technology, a series of experiments has been recently performed.[6, 7] In order to get the product of pharmaceutical quality it was necessary to optimize synthetic process, maximizing reaction yield and minimizing the level of potential impurities and unconsumed substrates. This lead to the application of theoretical methodology supporting the reaction response hypersurface.[8-10] Theoretical approach using statistical methods was shown to be very useful in analytical methods validation[11] as well as in prediction of biological activity.[12]

5.A.6 PHARMACOLOGY OF ARIPIPRAZOLE

Aripiprazole's mechanism of action is different from those of the other FDA-approved atypical antipsychotics (e.g., clozapine, olanzapine, quetiapine, ziprasidone and risperidone). Rather than antagonizing the D_2 receptor, aripiprazole acts as a D_2 partial agonist.[13, 14] Aripiprazole is also a partial agonist at the 5-HT_{1A} receptor and like the other atypical antipsychotics displays an antagonist profile at the 5-HT_{2A} receptor.[15,16] it also antagonizes the 5-$HT7_7$ receptor and acts as a partial agonist at the 5-HT_{2C} receptor, both with high affinity. The latter action may underlie the minimal weight gain seen in the course of therapy.[17] Aripiprazole has moderate affinity for histamine and α-adrenergic receptors and for the serotonin transporter and no appreciable affinity for cholinergic muscarinic receptors.[18] D_2 and D_3 receptor occupancy levels are high, with average levels ranging between ~71% at 2 mg/day to ~96% at 40 mg/day.[19],[20] most atypical antipsychotics bind preferentially to extrastriatal receptors, but aripiprazole appears to be less preferential in this regard, as binding rates are high throughout the brain.[21] Recently, it has been demonstrated that in 5-HT_7 receptor knockout mice, Aripiprazole does not reduce immobility time in the forced swim test (FST)and actually increases it.[22,23] This implicates 5-HT_7 antagonism as playing a major role in aripiprazole's antidepressant effects, similarly to amisulpride.[22,23,24]

5.A.7 BIOLOGICAL ASPECTS

Antidepressant drugs are medicines that relieve symptoms of depressive disorders. An antidepressant is a psychiatric medication used to alleviate mood disorders, such as major depression and dysthymia. Drugs including the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), selective serotonin reuptake inhibitors (SSRIs)and serotonin-nor epinephrine reuptake inhibitors (SNRIs) are most commonly associated with the term. These medications are among those most commonly prescribed by psychiatrists and other physicians and their effectiveness and adverse effects are the subject of many studies and competing claims. Many drugs produce an antidepressant effect, but restrictions on their use have caused controversy and off-label prescription

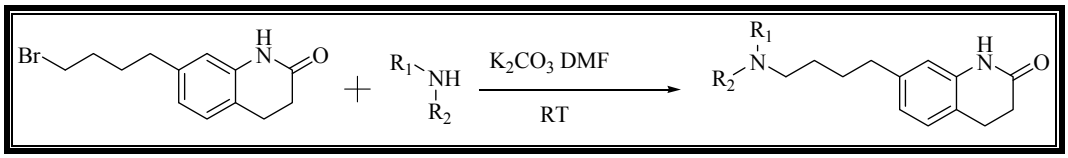
a risk, despite claims of superior efficacy. Most typical antidepressants have a delayed onset of action (2–6 weeks) and are usually administered for anywhere from months to years. Despite the name, antidepressants are often used to treat other conditions, such as anxiety disorders, obsessive compulsive disorder, eating disorders, chronic painand some hormone-mediated disorders such as dysmenorrhea. Alone or together with anticonvulsants (e.g., Tegretol or Depakote), these medications can be used to treat attention-deficit hyperactivity disorder (ADHD) and substance abuse by addressing underlying depression. Also, antidepressants have been used sometimes to treat snoring and migraines. Other medications that are not usually called antidepressants, including antipsychotics in low doses and benzodiazepines, may be used to manage depression, although benzodiazepines may cause physical dependence if treatment is not properly monitored by a doctor. Stopping benzodiazepine (or SSRI) treatment abruptly can cause unpleasant withdrawal symptoms. An extract of the herb St John's Wort is commonly used as an antidepressant, although it is labeled as a dietary supplement in some countries. The term *antidepressant* is sometimes applied to any therapy (e.g., psychotherapy, electro-convulsive therapy, acupuncture) or process (e.g., sleep disruption, increased light levels, regular exercise) found to improve a clinically depressed mood. Inert placebos can have significant antidepressant effectsand so to establish a substance as an "antidepressant" in a clinical trial it is necessary to show superior efficacy to placebo.

5.A.8 AIM OF CURRENT WORK

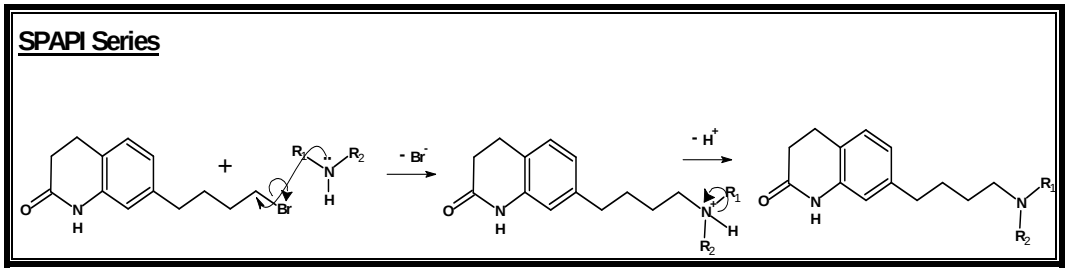
Owing to the above mentioned uses of Aripiprazole as the atypical antipsychotic drug, our main aim and plan was to synthesize some new chemical entities which are structural analogues of Aripiprazole which could also be screened to find out whether they would be able to mimic the biological activity of Aripiprazole.

5.A.9 REACTION SCHEMES

PREPARATION OF 7-(4-(SUBSTITUTED AMINO) BUTOXY)-3, 4-DIHYDRO QUINOLIN-2-(1H)-ONES



5.A.10 PLAUSIBLE REACTION MECHANISM



5.A.11 EXPERIMENTAL

5.A.11.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. Formation of all compounds was purified by using **flash chromatography**. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

5.A.11.2 GENERAL PROCEDURE FOR PREPARATION OF 7-(4-(SUBSTITUTED AMINO) BUTOXY)-3, 4-DIHYDRO QUINOLIN-2-(1H)-ONES

Bromobutoxy Quinolone (0.01 mole) was charged into 250 ml round bottom flask. 10 ml of dimethylformamide was added to dissolve it. Then add in to 0.03 mole of potassium carbonate as a catalyst. And add into Different secondary amines (0.01 mole) at RT. the reaction mixture was stirred at RT overnight. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane: ethyl acetate (3: 7) as a mobile phase. After the reaction to completed mixture poured into crushed ice, to get acquire the product. Finally, it was purified by Flash chromatography using hexane and ethyl acetate as eluents.

Similarly other compounds are also prepared.

5.A.12 PHYSICAL DATA

PHYSICAL DATA OF STUDIES ON 7-(4-(SUBSTITUTED AMINO) BUTOXY)-3, 4-DIHYDRO QUINOLIN-2-(1H)-ONES (SPAPI-01 TO SPAPI-12)

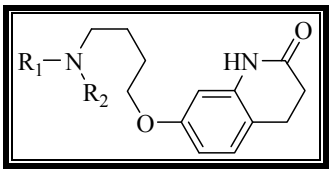
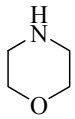
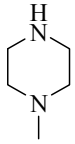
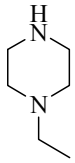
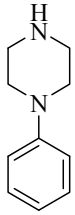
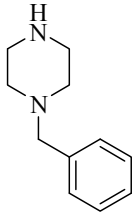
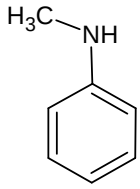
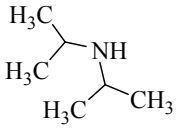
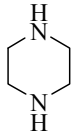
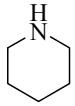
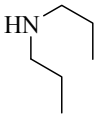
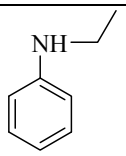


TABLE-5.A.1

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	BP ^{0}C	Rf Value
01	SPAPI-01		$C_{17}H_{24}N_2O_3$	304	184-186	0.41
02	SPAPI-02		$C_{18}H_{27}N_3O_2$	317	166-168	0.45
03	SPAPI-03		$C_{19}H_{29}N_3O_2$	331	156-158	0.42
04	SPAPI-04		$C_{23}H_{29}N_3O_2$	379	122-126	0.40
05	SPAPI-05		$C_{24}H_{31}N_3O_2$	394	202-204	0.51

06	SPAPI-06		$C_{20}H_{24}N_2O_2$	324	165-169	0.44
07	SPAPI-07		$C_{19}H_{30}N_2O_2$	318	174-176	0.46
08	SPAPI-08		$C_{17}H_{25}N_3O_2$	303	134-138	0.50
09	SPAPI-09		$C_{18}H_{26}N_2O_2$	302	164-166	0.39
10	SPAPI-10		$C_{19}H_{30}N_2O_2$	318	114-118	0.42
11	SPAPI-11		$C_{21}H_{26}N_2O_2$	338	186-188	0.46
12	SPAPI-12		$C_{17}H_{24}N_2O_2$	288	194-196	0.48

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (3: 7)

5.A.13 SPECTRAL DISCUSSION

5.A.13.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. SPAPI-01 and SPAPI-03 of Mass spectra are given on page no. 223 and 225.

5.A.13.2 IR SPECTRA

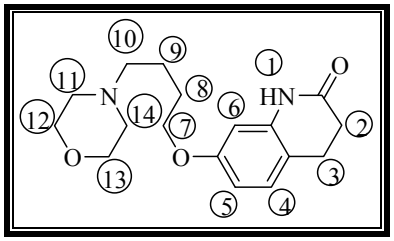
IR spectra of the synthesized compounds were recorded on **Shimadzu FT IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

-N-H stretching frequency was observed near 3400 cm^{-1} in case of SPAPI-01 to SPAPI-12. There are carbonyl groups present in all the compounds in the region of 1680 cm^{-1}. C-H stretching frequencies were observed between 2810 cm^{-1} and 3000 cm^{-1}, N-H bending frequency were observed near 1600 cm^{-1}, CN stretching frequencies were observed near 1200 cm^{-1}, -C-O-C- stretching observed between 1150 cm^{-1} and 1060 cm^{-1}. SPAPI-01 and SPAPI-03 of IR spectra are given on page no. 223 and 225.

5.A.13.3 ^{1}H NMR SPECTRA

^{1}H spectra of the synthesized compounds were recorded on **Bruker Avance II 400** spectrometer. Sample solutions were made in $CDCl_3$/DMSO-d_6 solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from proton NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify *o*, *m* and *p* coupling. ^{1}H NMR spectral interpretation can be discussed as under.

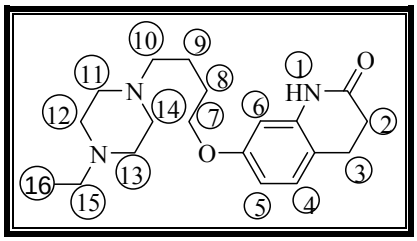
^{1}H NMR OF 7-(4-MORPHOLINOBUTOXY)-3,4-DIHYDROQUINOLIN-2(1H)-ONE (SPAPI-01)



1. The Proton no.1 of -NH group gave a characteristic singlet at 8.65 δ ppm.
2. Another four protons of piprazine methylene groups which attached with N atom of proton no. 11 and 14 and proton no. 10 of butyl chain which is attached with N atom and proton no. 2 of dihydroquinolone. All these eight protons are deshielded due to the nitrogen atmosphere. All these protons gave a multiplet at 2.41 δ ppm – 2.55 δ ppm.
3. Proton no. 3 of dihydroquinolone of two protons gave a multiplet at 2.82 δ ppm.
4. Another two protons of piprazine methylene group of proton no. 12 and 13 which becomes deshielded due to the oxygen atom. These entire four proton gave a multiplet at 3.67 δ ppm.
5. Proton no. 8 and 9 of butyl chain of four proton gave a multiplet at 1.62 δ ppm-1.72 δ ppm.
6. Proton no. 7 of butyl chain which is attached with oxygen atom and it gave a multiplet at 3.88 δ ppm.
7. Proton no. 4 and 6 of aromatic ring of of two proton gave a multiplet at 6.30 δ ppm-6.44 δ ppm.
8. Proton no. 5 of aromatic ring of proton gave a multiplet at 6.97 δ ppm.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPAPI-01 has been confirmed. Spectrum is given on page no. 224.

^{1}H NMR OF 7-(4-(4-ETHYLPIPERAZIN-1-YL)BUTOXY)-3,4-DIHYDROQUINOLIN-2(1H)-ONE (SPAPI-03)



1. The Proton no.1 of -NH group gave a characteristic singlet at 9.38 δ ppm.
2. Another eight protons of piprazine methylene groups which attached with N atom on same atmosphere of proton no. 11, 12, 13, 14, proton 10 of butyl chain and proton no.15 these all are attached with N atom, All these twelve protons are deshielded due to the nitrogen atmosphere. All these protons gave a multiplet at 2.85 δ ppm – 2.97 δ ppm.
3. Proton no. 8 of butyl chain of two protons gave a multiplet at 1.92 δ ppm.
4. Proton no. 9 of butyl chain of two protons gave a multiplet at 1.25 δ ppm.
5. Proton no. 2 and 3 of dihydroquinolone of four protons gave a multiplet at 2.54 δ ppm-2.60 δ ppm.
6. Proton no. 7 of butyl chain which is attached with oxygen atom and it gave a multiplet at 3.91 δ ppm – 3.98 δ ppm.
7. Proton no. 16 of methyl group gave a triplet at 0.87 δ ppm.
8. Proton no. 4 and 6 of aromatic ring of of two proton gave a multiplet at 6.42 δ ppm-6.49 δ ppm.
9. Proton no. 5 of aromatic ring of proton gave a doublet at 7.00 δ ppm-7.02 δ ppm. and *J* value of this proton are 8.0 Hz. So, it suggests ortho coupling.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SPAPI-03 has been confirmed. Spectrum is given on page no. 226.

5.A.13.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

5.A.14 ANALYTICAL DATA

***7-(4-morpholinobutoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-01)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3431 (-NH stretching), 3028 (-CH_3 asym str.), 2941 (-CH_2 asym. str.), 2862 (-CH_3 sym. Str.), 2767 (-CH_2 sym. Str.), 1678 (>C=O), 1624 (-NH bending), 1467 (-CH_3 asym bending deformation), 1379 (-CH_3 sym bending deformation), 1192 (-CN str.), 1111(-C-O-C- str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.72 (4H, m), 2.55 (8H, m), 2.82 (2H, m), 3.67 (4H, m), 3.88 (2H, m), 6.44 (2H, m), 6.97 (1H, m), 8.65 (1H, s), MS *m/z* = 304 (M^+); Anal. Calcd. for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20; O, 15.77. Found: C, 67.01; H, 7.93; N, 9.15; O, 15.72%.

***7-(4-(4-methylpiperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-02)*:**

Yield: 80%, IR (KBr, cm^{-1}): 3437 (N-H stretching), 3030 (-CH_3 asym str.), 2946 (-CH_2 asym. str.), 2864 (CH_3 sym. Str.), 2761 (CH_2 sym. Str.), 1672 (-C=O), 1622 (N-H bending), 1461 (-CH_3 asym bending deformation), 1371 (-CH_3 sym bending deformation), 1194 (-CN str.), 1116(-C-O-C- str.); MS *m/z* = 317 (M^+); Anal. Calcd. for $C_{18}H_{27}N_3O_2$: C, 68.11; H, 8.57; N, 13.24; O, 10.08. Found: C, 68.06; H, 8.54; N, 13.22; O, 10.03%.

***7-(4-(4-ethylpiperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one*(*SPAPI*-03):**

Yield: 82%, IR (KBr, cm^{-1}): 3439 (N-H stretching), 3057 (-CH_3 asym str.), 2953 (-CH_2 asym. str.), 2893 (CH_3 sym. Str.), 2858 (CH_2 sym. Str.), 1689 (-C=O), 1626 (N-H bending), 1448 (-CH_3 asym bending deformation), 1375 (-CH_3 sym bending deformation), 1170 (-CN str.), 1132 (-C-O-C- str.); ^{1}H NMR (400 MHz, DMSO): δ

(ppm) 0.87 (3H, s), 1.25 (2H, m), 1.92 (2H, m), 2.60 (4H, m), 2.97 (12H, m), 3.98 (2H, m), 6.49 (2H, m), 7.02 (1H, d, *J*=8.0 Hz), 9.38 (1H, s), MS *m/z* = 331 (M^+); Anal. Calcd. for $C_{19}H_{29}N_3O_2$: C, 68.85; H, 8.82; N, 12.68; O, 9.65. Found: C, 68.82; H, 8.80; N, 12.64; O, 9.61%.

***7-(4-(4-phenylpiperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one*(*SPAPI*-04):**

Yield: 74%, IR (KBr, cm^{-1}): 3440 (N-H stretching), 3006 (-CH_3 asym str.), 2938 (-CH_2 asym. str.), 2865 (CH_3 sym. Str.), 2761 (CH_2 sym. Str.), 1671 (-C=O), 1630 (N-H bending), 1461 (-CH_3 asym bending deformation), 1368 (-CH_3 sym bending deformation), 1188 (-CN str.), 1115(-C-O-C- str.); MS *m/z* = 379 (M^+); Anal. Calcd. for $C_{23}H_{29}N_3O_2$: C, 72.79; H, 7.70; N, 11.07; O, 8.43. Found: C, 72.74; H, 7.66; N, 11.05; O, 8.41%.

***7-(4-(4-benzylpiperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-05)*:**

Yield: 70%, IR (KBr, cm^{-1}): 3436 (N-H stretching), 3021 (-CH_3 asym str.), 2948 (-CH_2 asym. str.), 2864 (CH_3 sym. Str.), 2760 (CH_2 sym. Str.), 1671 (-C=O), 1621 (N-H bending), 1476 (-CH_3 asym bending deformation), 1371 (-CH_3 sym bending deformation), 1193 (-CN str.), 1110(-C-O-C- str.); MS *m/z* = 394 (M^+); Anal. Calcd. for $C_{24}H_{31}N_3O_2$: C, 73.25; H, 7.94; N, 10.68; O, 8.13. Found: C, 73.23; H, 7.91; N, 10.63; O, 8.10%.

***7-(4-(methyl(phenyl)amino)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-06)*:**

Yield: 68%, IR (KBr, cm^{-1}): 3421 (N-H stretching), 3022 (-CH_3 asym str.), 2931 (-CH_2 asym. str.), 2872 (CH_3 sym. Str.), 2867 (CH_2 sym. Str.), 1674 (-C=O), 1622 (N-H bending), 1465 (-CH_3 asym bending deformation), 1371 (-CH_3 sym bending deformation), 1182 (-CN str.), 1109 (-C-O-C- str.); MS *m/z* = 324 (M^+); Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64; O, 9.86. Found: C, 74.01; H, 7.43; N, 8.61; O, 9.83%.

***7-(4-(diisopropylamino)butoxy)-3,4-dihydroquinolin-2(1H)-one (SPAPI -07)*:**

Yield: 74%, IR (KBr, cm^{-1}): 3442 (N-H stretching), 3012 (-CH_3 asym str.), 2935 (-CH_2 asym. str.), 2866 (CH_3 sym. Str.), 2856 (CH_2 sym. Str.), 1688 (-C=O), 1620 (N-H bending), 1462 (-CH_3 asym bending deformation), 1376 (-CH_3 sym bending deformation), 1186 (-CN str.), 1117 (-C-O-C- str.); MS *m/z* = 318 (M^+); Anal. Calcd.

for $C_{19}H_{30}N_2O_2$: C, 71.66; H, 9.50; N, 8.80; O, 10.05. Found: C, 71.62; H, 9.48; N, 8.76; O, 10.03%.

***7-(4-(piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-08)*:**

Yield: 78%, IR (KBr, cm^{-1}): 3438 (N-H stretching), 3025 (-CH_3 asym str.), 2936 (-CH_2 asym. str.), 2856 (CH_3 sym. Str.), 2787 (CH_2 sym. Str.), 1677 (-C=O), 1622 (N-H bending), 1463 (-CH_3 asym bending deformation), 1371 (-CH_3 sym bending deformation), 1191 (-CN str.), 1114 (-C-O-C- str.); MS *m/z* = 303 (M^+); Anal. Calcd. for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85; O, 10.55. Found: C, 67.28; H, 8.30; N, 13.82; O, 10.53%.

***7-(4-(piperidin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-09)*:**

Yield: 66%, IR (KBr, cm^{-1}): 3447 (N-H stretching), 3018 (-CH_3 asym str.), 2940 (-CH_2 asym. str.), 2872 (CH_3 sym. Str.), 2867 (CH_2 sym. Str.), 1672 (-C=O), 1621 (N-H bending), 1469 (-CH_3 asym bending deformation), 1389 (-CH_3 sym bending deformation), 1190 (-CN str.), 1104 (-C-O-C- str.); MS *m/z* = 302 (M^+); Anal. Calcd. for $C_{18}H_{26}N_2O_2$: C, 71.49; H, 8.67; N, 9.26; O, 10.58. Found: C, 71.45; H, 8.65; N, 9.23; O, 10.56%.

***7-(4-(dipropylamino)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-10)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3445 (N-H stretching), 3011 (-CH_3 asym str.), 2966 (-CH_2 asym. str.), 2864 (CH_3 sym. Str.), 2854 (CH_2 sym. Str.), 1678 (-C=O), 1634 (N-H bending), 1468 (-CH_3 asym bending deformation), 1373 (-CH_3 sym bending deformation), 1182 (-CN str.), 1119 (-C-O-C- str.); MS *m/z* = 318 (M^+); Anal. Calcd. for $C_{19}H_{30}N_2O_2$: C, 71.66; H, 9.50; N, 8.80; O, 10.05. Found: C, 71.63; H, 9.44; N, 8.79; O, 10.03%.

***7-(4-(ethyl(phenyl)amino)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-11)*:**

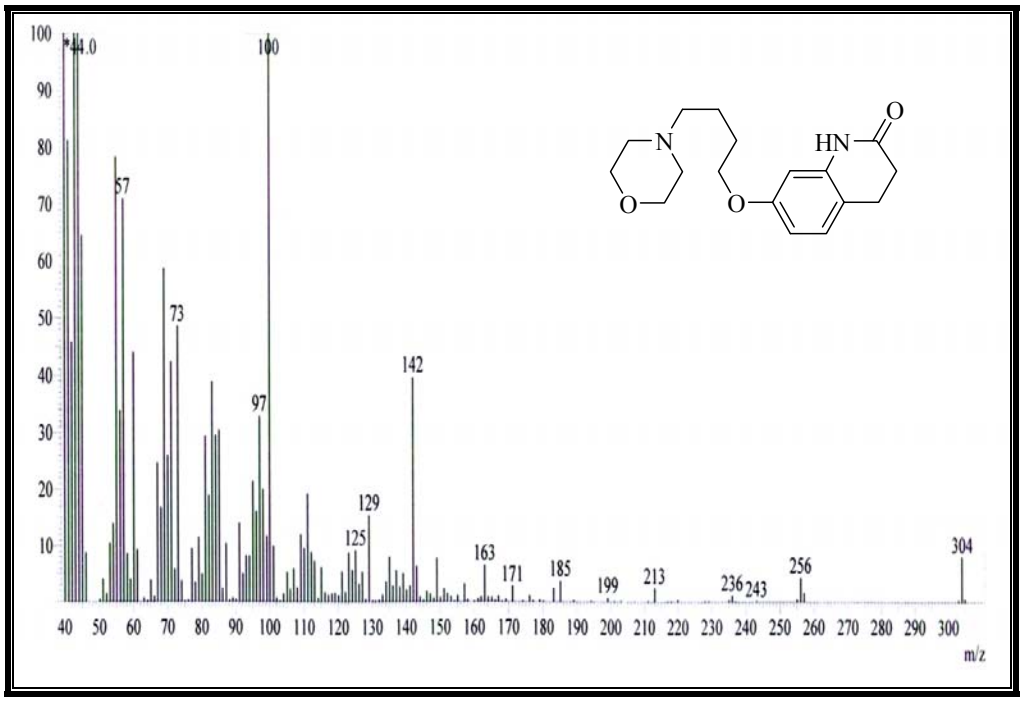
Yield: 76%, IR (KBr, cm^{-1}): 3427 (N-H stretching), 3022 (-CH_3 asym str.), 2936 (-CH_2 asym. str.), 2872 (CH_3 sym. Str.), 2860 (CH_2 sym. Str.), 1672 (-C=O), 1625 (N-H bending), 1462 (-CH_3 asym bending deformation), 1371 (-CH_3 sym bending deformation), 1187 (-CN str.), 1117 (-C-O-C- str.); MS *m/z* = 338 (M^+); Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28; O, 9.45. Found: C, 74.50; H, 7.72; N, 8.24; O, 9.42%.

***7-(4-(pyrrolidin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one(SPAPI-12)*:**

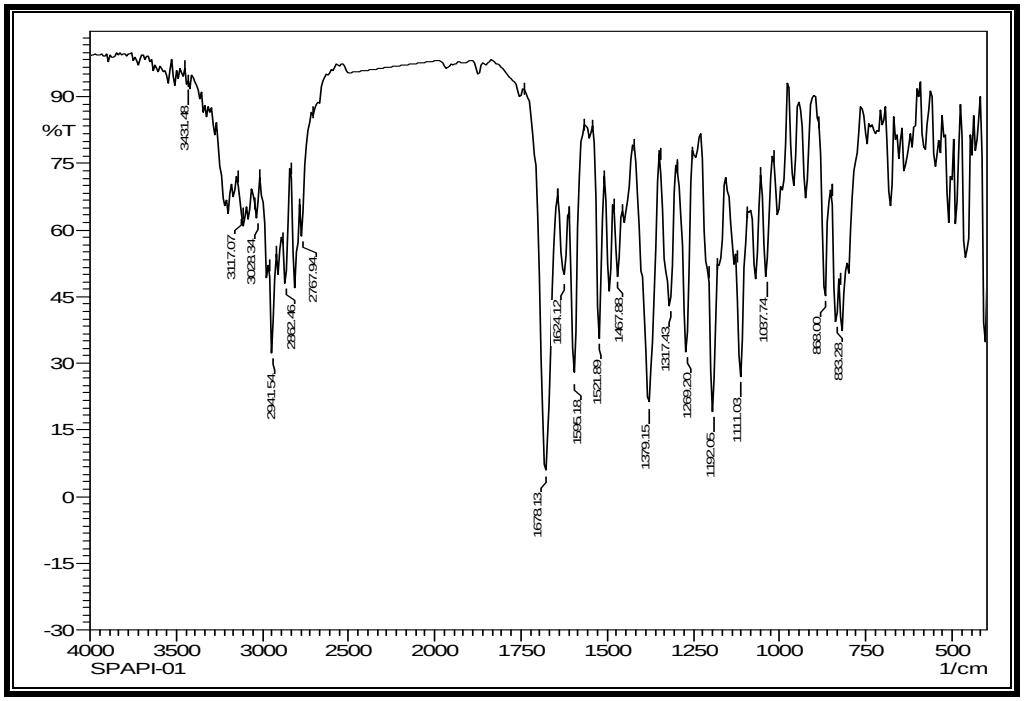
Yield: 70%, IR (KBr, cm^{-1}): 3451 (N-H stretching), 3010 (-CH_3 asym str.), 2924 (-CH_2 asym. str.), 2878 (CH_3 sym. Str.), 2844 (CH_2 sym. Str.), 1676 (-C=O), 1620 (N-H bending), 1463 (-CH_3 asym bending deformation), 1377 (-CH_3 sym bending deformation), 1184 (-CN str.), 1102 (-C-O-C- str.); MS *m/z* = 288 (M^+); Anal. Calcd. for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71; O, 11.10. Found: C, 70.77; H, 8.35; N, 9.70; O, 11.08%.

5.A.15 REPRESENTATIVE SPECTRA

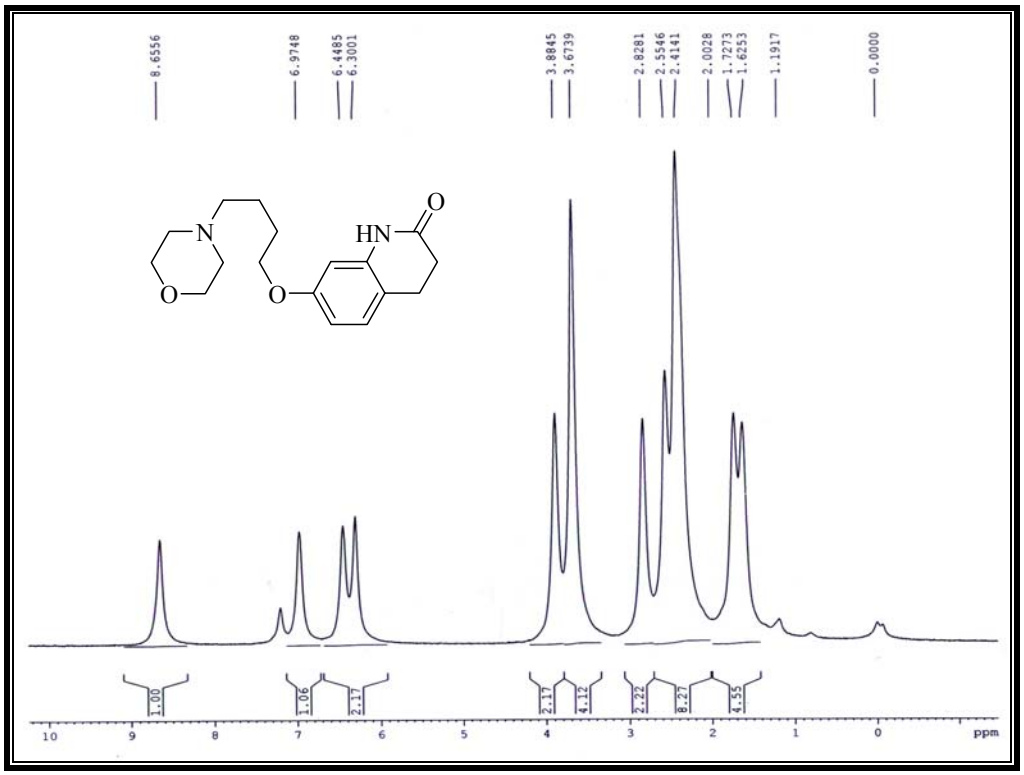
5.A.15.1 Mass Spectrum of SPAPI-01



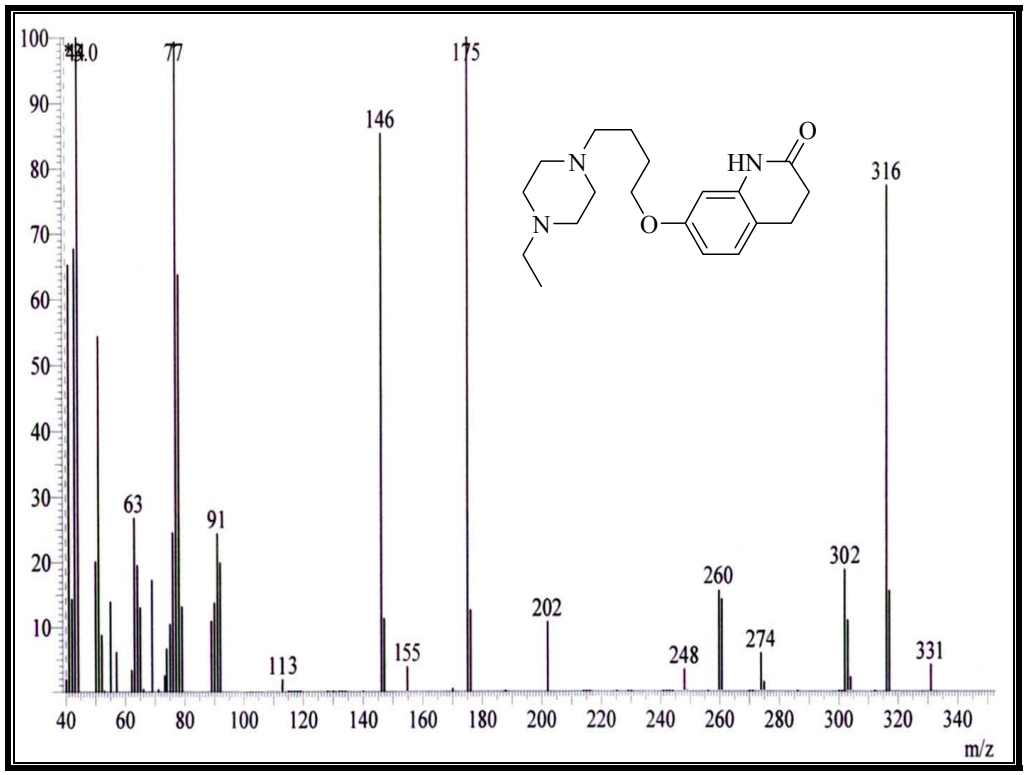
5.A.15.2 IR Spectrum of SPAPI-01



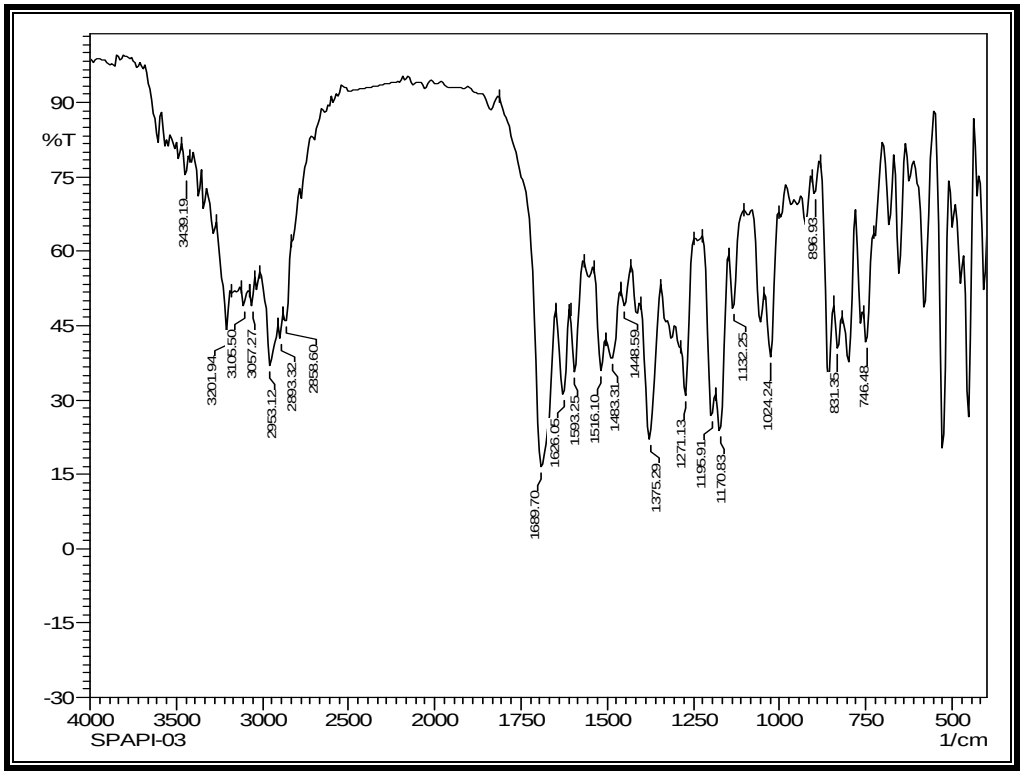
5.A.15.3 ^{1}H NMR Spectrum of SPAPI-01



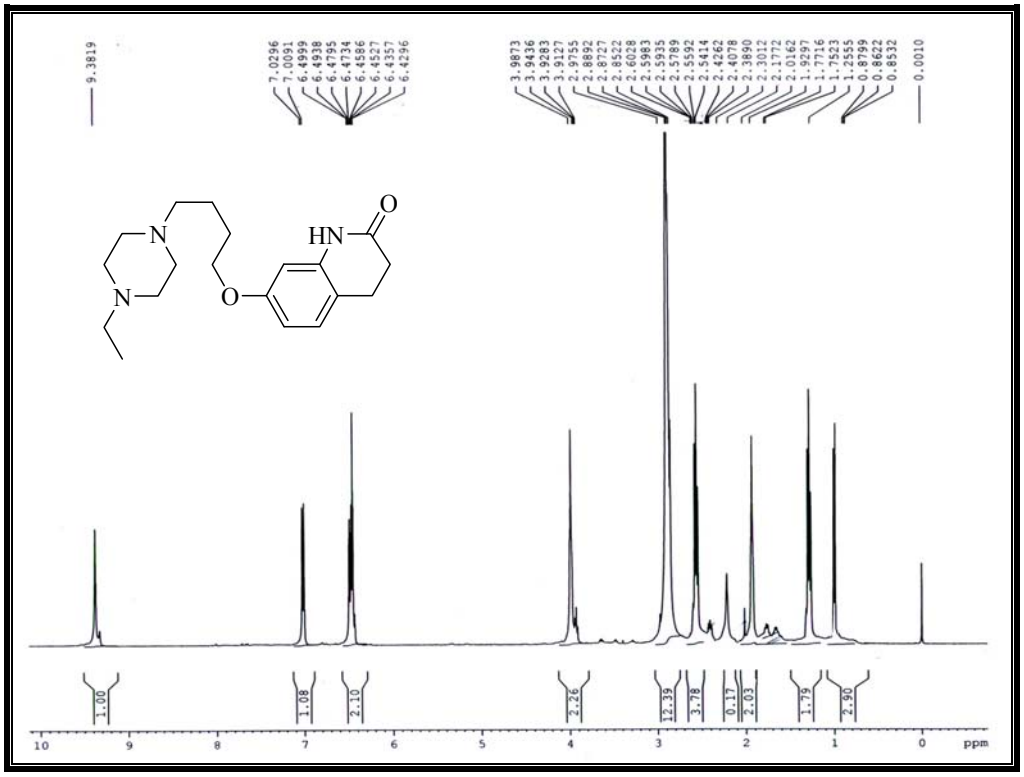
5.A.15.4 Mass Spectrum of SPAPI-03



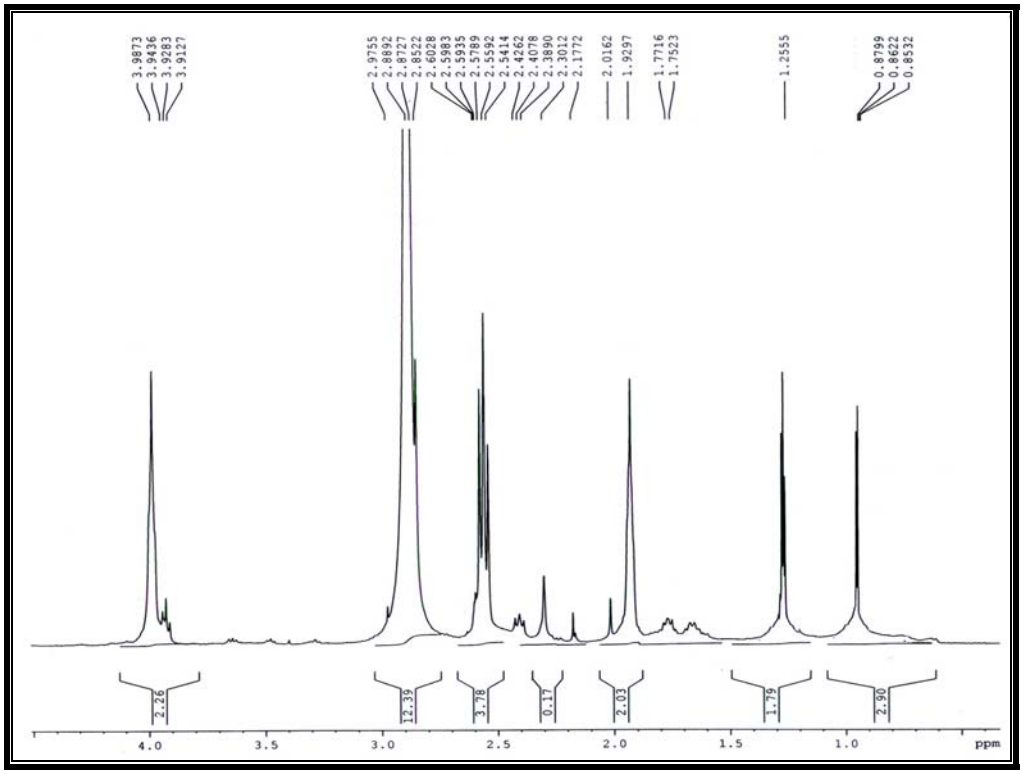
5.A.15.5 IR Spectrum of SPAPI-03



5.A.15.6 ^{1}H NMR Spectrum of SPAPI-03



EXPANDED ^{1}H-NMR SPECTRUM OF SPAPI-03



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CHAPTER - 5

SECTION - B

STUDIES ON MANNICH BASE LIKE 1H-INDOLE-2-CARBOXYLIC ACID

5.B.1 INDOLE SYSTEM

Indole (2, 3-benzopyrrole, ketole, 1-benzazole; C_8H_7N) is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The participation of the nitrogen lone electron pair in the aromatic ring means that indole is not a base, and it does not behave like a simple amine.

Indole is a solid at room temperature. Indole can be produced by bacteria as a degradation product of the amino acid tryptophan. It occurs naturally in human feces and has an intense fecal odor. At very low concentrations, however, it has a flowery smell, and is a constituent of many flower scents (such as orange blossoms) and perfumes. It also occurs in coal tar.

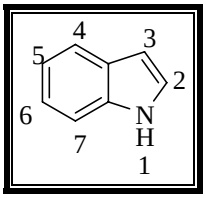


Fig. A.1

Many researchers have described synthesis of indole and its derivatives along with its applications in literature. [1-47]

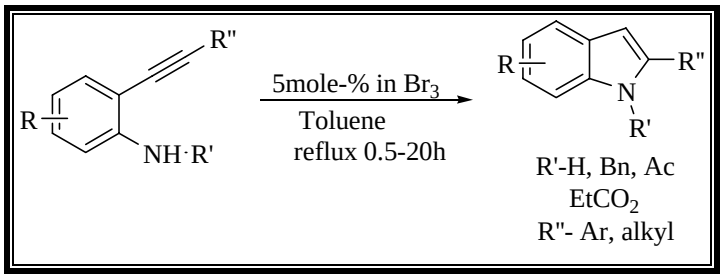
5.B.2 PHYSICAL PROPERTIES OF INDOLE

Indole is a white colored solid, melting at 52-54°C and boiling at 253-254 °C. 0.19 gm of indole is soluble in 100 ml of hot water. Indole is soluble in alcohol, ethyl acetate etc. Indole is having planar molecular shape, 1.22 g/cm^3 density and 2.11 D dipole moment in benzene. All indole derivatives show certain family resemblances to indole [4], but striking changes can be brought about by substitution of groups in the pyrrole ring. Thus, the fecal-like odor of skatole is the most pronounced of all the methyl indoles, less pronounced for the 2-methylindole and the 2, 3-dimethyl indole; 1-methylindole, on the other hand, resembles methyl aniline in odor. Introduction of

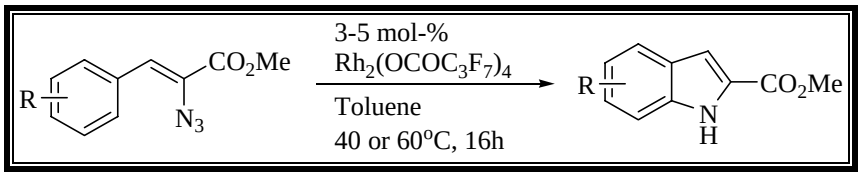
carboxyl groups or phenolic hydroxyl groups causes elimination of the odor, and the naphtha indoles are also without odor.

All the common indole derivatives, like indole, form well-defined crystalline picrates, yellow to red in color. Formation of picrates is usually a suitable procedure for identification and purification.

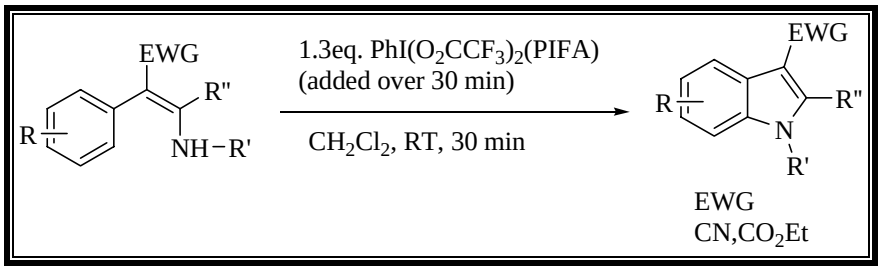
5.B.3 SYNTHESIS OF INDOLES



Indium-catalyzed cyclization of 2-ethynylanilines produced various oly functionalized indole derivatives in good yields for substrates having an alkyl or aryl group on the terminal alkyne. In contrast, substrates with a tri methyl silyl group or without substituent on the triple bond afforded poly substituted quinoline derivatives in good yields via an intermolecular dimerization.[48]

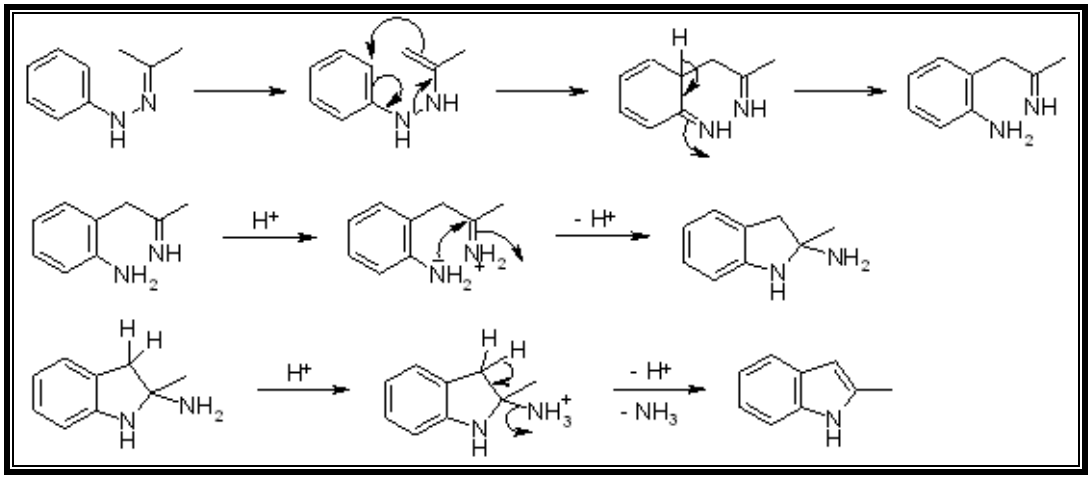


Rhodium (II) perfluorobutyrate-mediated decomposition of vinyl azides allows rapid access to a variety of complex, functionalized *N*-heterocycles in two steps from commercially available starting materials.[49]



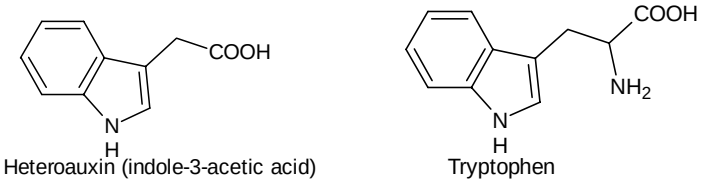
Various N-arylated and N-alkylated indoles and pyrrole-fused aromatic compounds were synthesized by a phenyliodine bis(trifluoroacetate) (PIFA)-mediated intramolecular cyclization.[50]

5.B.4 MECHANISM OF THE FISCHER INDOLE SYNTHESIS



5.B.5 SOME INDOLE NATURAL PRODUCTS

The indole nucleus is found to be present in a varied group of products of natural occurance in both the animal and vegetable kingdom. [51, 52, 53] Most of these product show marked physiological activity and some are extremely complex in nature.



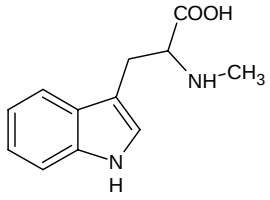
Heteroauxin (indole-3-acetic acid)

Fig. 2

Tryptophen

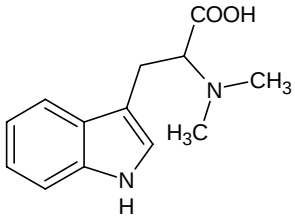
Fig. 3

Tryptophan is one of the essential amino acids, isolated from natural sources exists as the levo isomer.



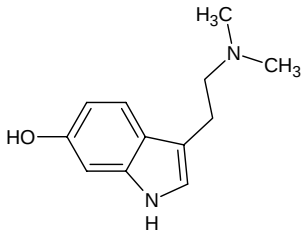
"Abrine" (d-(Indolyl-3) propionic acid)

Fig. 4



Hypaphorine

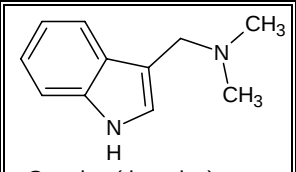
Fig. 5



Butofenine

Fig. 6

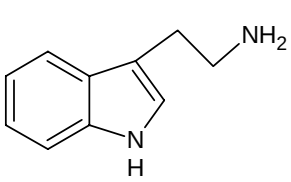
Bufotenine has a pronounced effect upon blood pressure.[54]



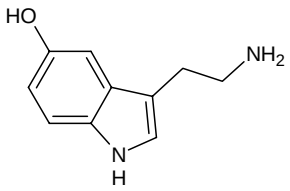
Gramine (donaxine)

Fig. 7

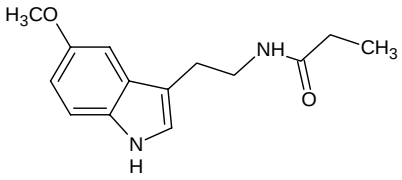
Indoles are probably the most widely distributed heterocyclic compounds in nature.



Triptamine
Fig. 8



Serotonin
Fig. 9

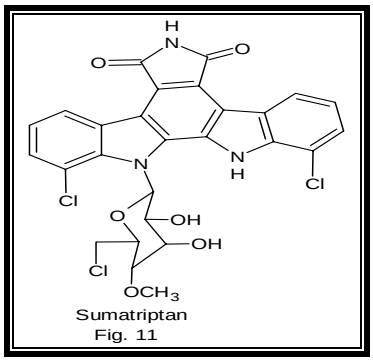


Melatonin
Fig. 10

5.B.6 BIOLOGICAL IMPORTANCE

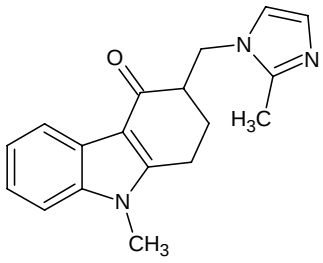
The Indole derivatives display a wide range of biological activities. This is exemplified by the amino acid tryptophan, the hormones serotonin and melatonin, the anti-inflammatory drug indo methacin, the psychotropic drug LSD and the anti-tumor agent vinblastine.[55]

In animals serotonin is a very important neurotransmitter in the central nervous system and also plays a vital role in the cardiovascular and gastrointestinal system. Many indole derivatives are used as pharmaceuticals and highly selective medicines which are in current use.



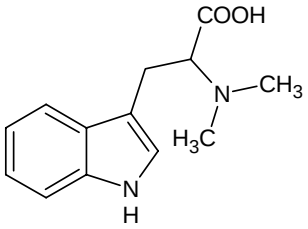
Sumatriptan
Fig. 11

Sumatriptan is used for the treatment of migraine.



Ondansetron

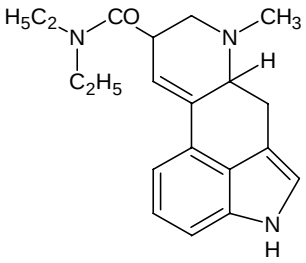
Fig. 12



Hypaphorine

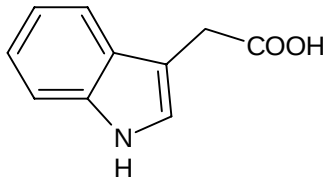
Fig. 13

Ondansetron is used for the suppression of the nausea and vomiting caused by the cancer chemotherapy and radiotherapy. Indoles are also good plant growth hormones.



Lysergic acid diethylamide

Fig. 14



Plant growth hormone

Fig. 15

5.B.7 INDOLES AS ANTICANCER AGENTS

Interest in the synthesis of novel indole-based heterocycles has increased in recent years due the prevalence of indoles in biologically-active natural products such as a fumitremorgin class and the significance of the indole moiety in clinical chemotherapeutics like ellipticine. Many MDR cell lines which are found to be resistant to many present pharmacological agents, proved as non-resistant to many of synthetic indole derivatives. Therefore indole derivatives have gained much attention in the field of discovering potent and effective anticancer agents.

5.B.8 MANNICH REACTION ON INDOLE 2- CARBOXYLIC ACID

Abonia *et. al.*[56] in their effort to synthesize pyrroloquinolines, synthesized 1-(benzotriazol-1(2)-ylmethyl)indolines for which they carried out Mannich reaction on 2-methyl indoline using benzotriazole as a secondary amine, formaldehyde and diethyl ether as a solvent and stirred for 30 minutes at room temperature. This Mannich base was reacted with un activated and electron-rich alkenes in the presence of p-toluenesulfonic acid catalyst to give pyrroloquinolines but the pharmacological importance of the synthesized molecules was not reported.

5.B.9 AIM OF CURRENT WORK

Since last few years, our group is involved in the synthesis of nitrogen containing heterocycles *viz*. pyrrole, indole, 2-methyl indole, dihydro pyridine, dihydropyrimidine, 4-hydroxy quinolones etc. Where, pyrrole, indole,[57a] dihydropyridine[57b], dihydropyrimidine[57c], 4-hydroxy quinolone[57d] and 2-methyl indole showed good anti tubercular[57e], anti diabetic, anti cancer[57f] and multi drug resistance reversal[57g] activity. Looking to the interesting biological profile showed by indole, 2-methyl indole and indole 2-Carboxylic acid from the literature survey and development of a simple preparation method for 2-methyl indole by our group we decided to prepare indole 2-Carboxylic acid and to explore the chemistry involving indole 2-Carboxylic acid moiety.

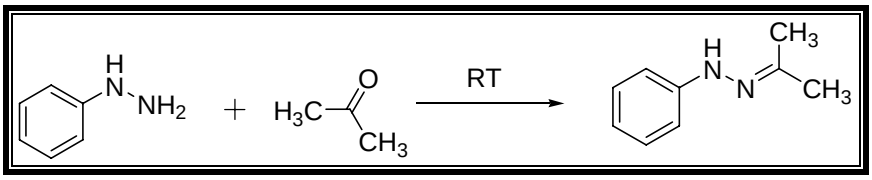
Recently [57h] Mannich reaction has been carried out on N_1 position in indole 2-Carboxylic acid using formaldehyde. Looking to the reactivity of N_1 position for Mannich reaction, the secondary nitrogen is more active than C_3 while in 2-methyl indole, Mannich reaction goes on both N_1 and C_3 positions depending upon the reaction condition.

Mannich bases can be synthesized by Mannich reaction on nitrogen of secondary amine having hydrogen atom with pronounced activity using simplified methodology and easy work up and this inspired us to develop some new *N*-substituted indole 2-Carboxylic acid derivatives by Mannich reaction. Literature also revealed that secondary amines *viz.* morpholine, piperidine, pyrrolidine, piperazine derivatives and other secondary amines like dimethylamine, diethylamine etc. and secondary aromatic amines have not been used yet that is why we used secondary amines to acquire Mannich bases having desired scaffolds. These interesting Mannich bases derived from indole 2-Carboxylic acid are not only structurally novel but the biological evaluation is reported here for the first time. Biological importance of such an important scaffold is the rational behind the current work done in this chapter.

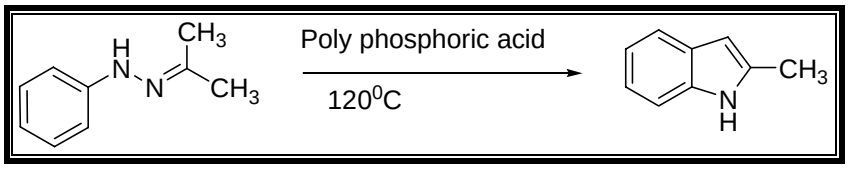
5.B.10 REACTION SCHEMES

5.B.10.1 PREPARATION OF INDOLE 2-CARBOXYLIC ACID

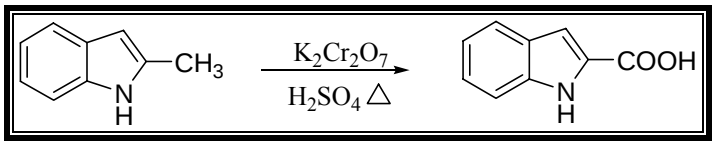
STEP – 1



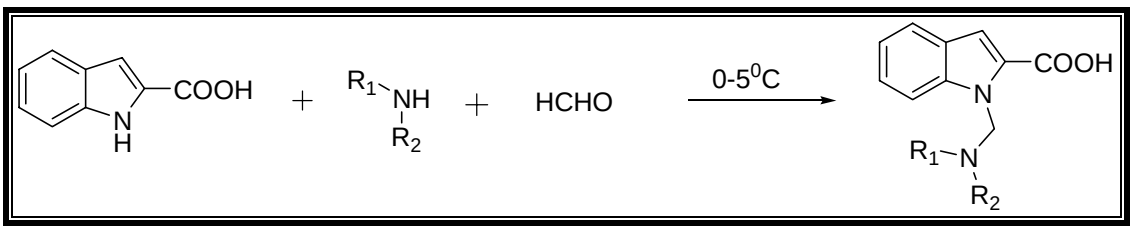
STEP – 2



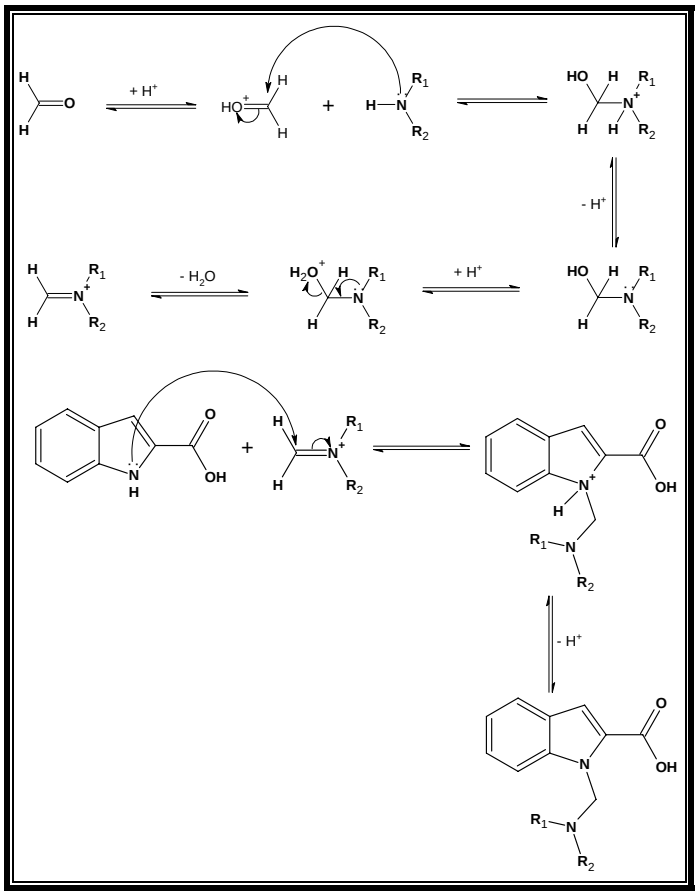
STEP-3



5.B.10.2 PREPARATION OF 1-(SUBSTITUTED METHYL)-1H-INDOLE-2-CARBOXYLIC ACID N-MANNICH BASES



5.B.11 PLAUSIBLE REACTION MECHANISM



5.B.12 EXPERIMENTAL

5.B.12.1 MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV. IR spectra were recorded in **Shimadzu FT-IR-8400** instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR was determined in DMSO-d_6 solution on a **Bruker Ac 400 MHz spectrometer**. Elemental analysis of the all the synthesized compounds was carried out on Elemental **Vario EL III Carlo Erba 1108** model and the results are in agreements with the structures assigned.

5.B.12.2 PREPARATION OF INDOLE 2-CARBOXYLIC ACID

STEP – 1 PREPARATION OF ACETONE PHENYL HYDRAZONE

25 ml of phenyl hydrazine was added drop wise to a magnetically stirred solution of 20 ml of acetone. After the completion of the addition, 5 ml of acetone was added to the reaction mixture and the reaction mixture was heated on the water bath to remove the excess of the acetone. Afterwards the reaction mixture was cooled to room temperature and it was made anhydrous by means of anhydrous sodium sulphate or anhydrous calcium chloride. The solution was filtered to give the dark yellow solution of phenyl hydrazone. Yield - 80 %, BP - 140-142°C (141-142°C[a])

STEP – 2 PREPARATION OF 2-METHYL INDOLE

30 gm of acetone phenyl hydrazone was added drop wise to a beaker containing 75 gm of poly phosphoric acid with constant stirring. The reaction mixture was heated on water bath for 2-3 hours, where the orange colored solution became dark red-brown. After that the temperature of the reaction mixture was raised to

[a] H. M. Kissman, D. W. Farnsworth and B. Witkop; *J. Am. Chem. Soc.*, **1952**, *74*, 3948.

120°C and then it was cooled to room temperature. After that 400 ml of distilled water was added to the reaction mixture to decompose the polyphosphoric acid, the whole content was steam distilled to acquire the 2-Methyl Indole as white colored shining crystals. Yield - 79 %, MP - 58-59°C (56-57°C[b])

STEP-3 PREPARATION OF INDOLE 2-CARBOXYLIC ACID

Take 2-methyl indole (0.01 mole) into 100 ml RBF then add into 10 ml of water and 5 gm of $K_2Cr_2O_7$ then add 10 ml of H_2SO_4 drop wise with stirring in approx 20-25min. after addition allow the reaction mixture to cool down at rt. then check the TLC using hexane : ethyl acetate (9: 1) as a mobile phase. then reaction mixture Poured into crushed ice filter the separated product. Transfer the solid material in to beaker and add 5%H_2SO_4.digest the content on water bath for 20-25 min. allow the reaction mixture to cool at RT. filter the separated product wash with water. Dissolve the solid material into 5%NaOH solution. Shake well and filter it, wash with water. Collect the filtrate and acidify with 15% H_2SO_4 solution. so indole 2-carboxylic acid precipitate out by acid base treatment. filter it and wash with water. Crystalline with suitable organic solvent.

5.B.12.3 PREPARATION OF 1-(SUBSTITUTED METHYL)-1H-INDOLE-2-CARBOXYLIC ACIDS (SP-20 TO SP-32)

GENERAL METHOD

It was prepared according to the method described by Abonia et. Al[56]. indole 2-Carboxylic acid (0.01 mole) was charged into 50 ml erlenmeyer flask and formaldehyde (0.015 mole) (37-41% w/w solution) and 10 ml diethyl ether were added into above flask and the mixture was magnetically stirred for some time at room temperature under acidic condition. and an appropriate secondary amine (0.01 mole) was added drop wise into above reaction mixture. Stirring was continued for further half an hour at room temperature. The progress and the completion of the reaction were checked by silica gel-G F_{254} thin layer chromatography using hexane : ethyl acetate (9: 1) as a mobile phase. After the reaction to be completed, the reaction

[b] C. F. H. Allen and J. Vanallan; *Organic Syntheses*, **1955**, *Coll. Vol. 3*, p. 597.

mixture was extracted using ethyl acetate (30 ml×3). The combined organic layer was washed using water (20 ml × 2). The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to acquire the product. Similarly other compounds are also prepared.

Physical data of the synthesized end products are summarized in the Table No. 5.B.1.

5.B.13 PHYSICAL DATA

PHYSICAL DATA OF 1-(SUBSTITUTED METHYL)-1H-INDOLE-2-CARBOXYLIC ACIDS (SP-20 TO SP-32)

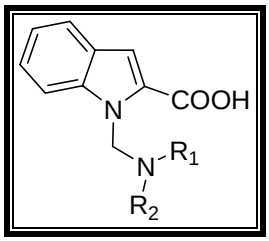
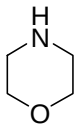
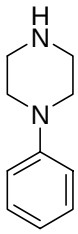
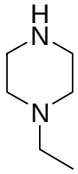
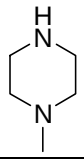
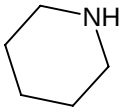
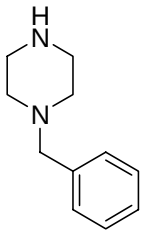
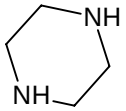
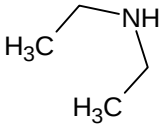
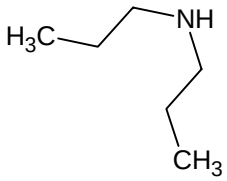
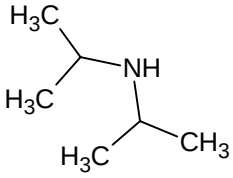
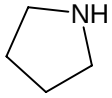
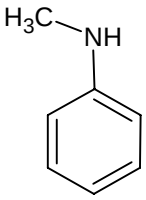


TABLE-5.B.1

Sr. No.	Code Name & No.	Substitutions	Molecular Formula	Molecular Weight	MP ^{0}C	Rf Value
01	SP-20		$C_{14}H_{16}N_2O_3$	260	184-186	0.41
02	SP-21		$C_{20}H_{21}N_3O_2$	335	166-168	0.45
03	SP-23		$C_{16}H_{21}N_3O_2$	287	156-158	0.42
04	SP-24		$C_{15}H_{19}N_3O_2$	273	122-126	0.40
05	SP-25		$C_{15}H_{18}N_2O_2$	258	202-204	0.51
06	SP-26		$C_{21}H_{23}N_3O_2$	349	165-169	0.44

07	SP-27		$C_{14}H_{17}N_3O_2$	259	174-176	0.46
08	SP-28		$C_{14}H_{18}N_2O_2$	246	134-138	0.50
09	SP-29		$C_{16}H_{22}N_2O_2$	274	164-166	0.39
10	SP-30		$C_{16}H_{22}N_2O_2$	274	114-118	0.42
11	SP-31		$C_{14}H_{16}N_2O_2$	244	186-188	0.46
12	SP-32		$C_{17}H_{16}N_2O_2$	280	194-196	0.48

R_f value was calculated using solvent system, Hexane : Ethyl Acetate (9: 1)

5.B.14 SPECTRAL DISCUSSION

5.B.14.1 MASS SPECTRA

Mass spectra of the synthesized compounds were recorded on **Shimadzu GC-MS QP-2010** model using direct injection probe technique. The molecular ion peak was found in agreement with molecular weight of the respective compound. SP-24 and SP-25 of Mass spectra are given on page no. 251 and 253.

5.B.14.2 IR SPECTRA

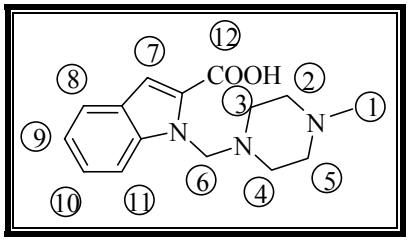
IR spectra of the synthesized compounds were recorded on **Shimadzu FT-IR 8400** spectrophotometer using Diffused Reflectance Attachment (DRA) system using Potassium Bromide.

In case of SP-20 to SP-32, there is no characteristic peak obtained in the spectra. In case of SP-21 to SP-26 secondary amine of piperazine gave stretching frequency in the region of 3310 to 3530 cm^{-1} and bending vibrations in the region of 1550 to 1650 cm^{-1}. Aliphatic C-N vibrations are found near 1220 cm^{-1}. SP-24 and SP-25 of IR spectra are given on page no. 251 and 253.

5.B.14.3 ^{1}H NMR SPECTRA

^{1}H NMR spectra of the synthesized compounds were recorded on **Bruker Avance II 400 spectrometer**. Making a solution of samples in DMSO-d_6 solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule. *J* values were calculated to identify *o, m* and *p* coupling. In some cases, aromatic protons were obtained as multiplet. ^{1}H NMR spectral interpretation can be discussed as under.

^{1}H NMR OF 1-((4-METHYLPIPERAZIN-1-YL)METHYL)-1H-INDOLE-2-CARBOXYLIC ACID (SP-24)

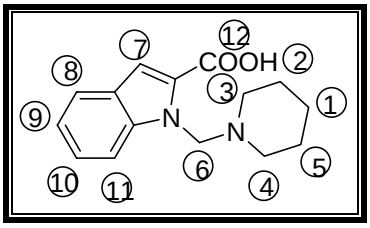


1. The most deshielded proton no. 12 of hydroxyl group gave a characteristic singlet at 11.28 δ ppm and as it is bonded to the electronegative oxygen atom the singlet is shifted to downfield significantly.
2. The proton no.1 of methyl group gave a characteristic singlet at 2.40 δ ppm it became deshielded due to attached with nitrogen atom.
3. The proton no. 2, 3, 4 and 5 it clearly shows 8H gave a multiplet at 3.14 δ ppm – 3.47 δ ppm. All eight protons became deshielded due to attached with nitrogen atom on both side.
4. The proton no. 6 of methylene proton gave a characteristic singlet at 4.21 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
5. The proton no. 7 of aromatic region gave a characteristic singlet at 7.70 δ ppm.
6. The proton no. 8 of aromatic protons gave triplet at 7.08 δ ppm -7.11 δ ppm.
7. The Proton no. 11 of aromatic gave a triplet at 7.21 δ ppm-7.24 δ ppm.
8. The Proton no. 9 of aromatic ring gave a doublet at 7.49 δ ppm -7.51 δ ppm and *J* value of proton no. 9 is 8.0 Hz it clearly suggesting a ortho-coupled with another proton.
9. The Proton no. 10 of aromatic ring gave a doublet at 7.55 δ ppm -7.57 δ ppm and *J* value of proton no. 10 is 8.0 Hz it clearly suggesting a ortho-coupled with another proton.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed

structure for compound no. SP-24 has been confirmed. Spectrum is given on page no. 252.

^{1}H NMR OF 1-((PIPERIDIN-1-YL) METHYL)-1H-INDOLE-2-CARBOXYLIC ACID (SP-25)



1. The proton no.1, 2 and 5 of piprazine six protons gave a multiplet at 1.78 δ ppm -1.87 δ ppm. it clearly shows in expanded spectra.
2. The proton no. 3 and 4 of piprazine four proton which is attached with nitrogen atom gave a multiplet at 2.59 δ ppm-3.42 δ ppm. it became a deshielded due to the nitrogen atom.
3. The proton no. 6 of methylene group gave a characteristic singlet at 4.25 δ ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure.
4. The proton no. 7 of aromatic region gave a characteristic singlet at 7.68 δ ppm.
5. The proton no. 8 of aromatic proton gave triplet at 7.07 δ ppm -7.11 δ ppm.
6. The proton no. 11 of aromatic proton gave triplet at 7.19 δ ppm -7.23 δ ppm.
7. The Proton no. 9 and 10 of two aromatic protons gave a triplet at 7.50 δ ppm -7.54 δ ppm.
8. The most deshielded proton no.12 of hydroxyl group gave a characteristic singlet at 11.17 δ ppm and as it is bonded to the electronegative oxygen atom the singlet is shifted to downfield significantly.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the *J* values for each of the above proton, the proposed structure for compound no. SP-25 has been confirmed. Spectrum is given on page no. 254.

5.B.14.4 ELEMENTAL ANALYSIS

Elemental analysis of the synthesized compounds was carried out on **Vario EL Carlo Erba 1108** which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

5.B.15 ANALYTICAL DATA

***1-(morpholinomethyl)-1H-indole-2-carboxylic acid (SP-20)*:**

Yield: 72%, IR (KBr, cm^{-1}): 3240 (-OH bonded), 2990 ($–CH_3$ stretching), 2780 ($-CH_2$ stretching), 1692 (-C=O stretching), 1547, 1468, (Ar-C-C- skleton), 1151 (C-N stretching), 748 (-C-H oop bending); MS *m/z* = 260 (M^+); Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76; O, 18.44. Found: C, 64.52; H, 6.18; N, 10.70; O, 18.41%.

***1-((4-phenylpiperazin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-21)*:**

Yield: 80%, IR (KBr, cm^{-1}): 3520 (secondary amine of piprazine stretching), 3254 (-OH bonded), 2987 ($–CH_3$ stretching), 2758 ($-CH_2$ stretching), 1694 (-C=O stretching), 1545, 1478, (Ar-C-C skleton), 1182 (C-N stretching), 746 (-C-H oop bending); MS *m/z* = 335 (M^+); Anal. Calcd. for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53; O, 9.54. Found: C, 71.61; H, 6.30; N, 12.51; O, 9.52%.

***1-((4-ethylpiperazin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-23)*:**

Yield: 91%, IR (KBr, cm^{-1}): 3478 (secondary amine of piprazine stretching), 3242 (-OH bonded), 2977 ($–CH_3$ stretching), 2774 ($-CH_2$ stretching), 1694 (-C=O stretching), 1535, 1459, (Ar-C-C skleton), 1188 (C-N stretching), 739 (-C-H oop bending); MS *m/z* = 287 (M^+); Anal. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62; O, 11.14. Found: C, 66.77; H, 7.31; N, 14.61; O, 11.11%.

***1-((4-methylpiperazin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-24)*:**

Yield: 94%, IR (KBr, cm^{-1}): 3524 (secondary amine of piprazine stretching), 3250 (-OH bonded), 3005 (–CH_3 stretching), 2766 (-CH_2 stretching), 1680 (-C=O stretching), 1535, 1469, (Ar. C-C skleton), 860 (-C-H oop bending); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.40 (3H, s), 3.47 (8H, m), 4.21 (2H, s), 7.11 (1H, t), 7.24 (1H, t), 7.51 (1H, d, *J*=8.0 Hz), 7.57 (1H, d, *J*=8.0 Hz), 7.70 (1H, s), 11.28 (1H, s); MS *m/z* = 273 (M^+); Anal. Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37; O, 11.71. Found: C, 65.90; H, 7.00; N, 15.34; O, 11.66%.

***1-((piperidin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-25)*:**

Yield: 90%, IR (KBr, cm^{-1}): 3435 (secondary amine of piprazine stretching), 3190 (-OH bonded), 2966 (–CH_3 stretching), 2796 (-CH_2 stretching), 1790 (-C=O stretching), 1533, 1475 (Ar-C-C skleton), 887 (-C-H oop bending); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.87 (6H, m), 2.70 (2H, m), 3.42 (2H, m), 4.25 (2H, s), 7.11 (1H, t), 7.23 (1H, t), 7.54 (2H, t), 7.68 (1H, s), 11.17 (1H, s); MS *m/z* = 258 (M^+); Anal. Calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84; O, 12.39. Found: C, 69.71; H, 7.01; N, 10.80; O, 12.30%.

***1-((4-benzylpiperazin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-26)*:**

Yield: 84%, IR (KBr, cm^{-1}): 3466 (secondary amine of piprazine stretching), 3238 (-OH bonded), 2987 (–CH_3 stretching), 2778 (-CH_2 stretching), 1698 (-C=O stretching), 1535, 1469, (Ar-C-C skleton), 1166 (C-N stretching), 745 (-C-H oop bending); MS *m/z* = 349 (M^+); Anal. Calcd. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03; O, 9.16;. Found: C, 72.11; H, 6.61; N, 12.02; O, 9.10%.

***1-((piperazin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-27)*:**

Yield: 86%, IR (KBr, cm^{-1}): 3262 (-OH bonded), 2992 (–CH_3 stretching), 2794 (-CH_2 stretching), 1691 (-C=O stretching), 1535, 1477, (Ar-C-C skleton), 1201 (C-N stretching), 750 (-C-H oop bending); MS *m/z* = 259 (M^+); Anal. Calcd. for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20; O, 12.34. Found: C, 64.82; H, 6.61; N, 16.20; O, 12.26%.

***1-((diethylamino)methyl)-1H-indole-2-carboxylic acid (SP-28)*:**

Yield: 81%, IR (KBr, cm^{-1}): 3241 (-OH bonded), 2955 (–CH_3 stretching), 2776 (-CH_2 stretching), 1680 (-C=O stretching), 1545, 1469, (Ar-C-C skleton), 1146 (C-N stretching), 738 (-C-H oop bending); MS *m/z* = 246 (M^+); Anal. Calcd. for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; O, 12.99. Found: C, 68.22; H, 7.30; N, 11.30; O, 12.92%.

***1-((diisopropylamino)methyl)-1H-indole-2-carboxylic acid (SP-29)*:**

Yield: 90%, IR (KBr, cm^{-1}): 3262 (-OH bonded), 2988 (–CH_3 stretching), 2788 (-CH_2 stretching), 1684 (-C=O stretching), 1535, 1477, (Ar-C-C skleton), 1174 (C-N stretching), 751 (-C-H oop bending); MS *m/z* = 274 (M^+); Anal. Calcd. for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21; O, 11.66. Found: C, 70.00; H, 8.02; N, 10.21; O, 11.63%.

***1-((dipropylamino)methyl)-1H-indole-2-carboxylic acid (SP-30)*:**

Yield: 84%, IR (KBr, cm^{-1}): 3244 (-OH bonded), 2991 (–CH_3 stretching), 2766 (-CH_2 stretching), 1694 (-C=O stretching), 1537, 1469, (Ar-C-C skleton), 1147 (C-N stretching), 753 (-C-H oop bending); MS *m/z* = 274 (M^+); Anal. Calcd. for $C_{16}H_{22}N_2O_2$. C, 70.04; H, 8.08; N, 10.21; O, 11.66. Found: C, 70.01; H, 8.02; N, 10.17; O, 11.63%.

***1-((pyrrolidin-1-yl)methyl)-1H-indole-2-carboxylic acid (SP-31)*:**

Yield: 90%, IR (KBr, cm^{-1}): 3258 (-OH bonded), 2997 (–CH_3 stretching), 2769 (-CH_2 stretching), 1694 (-C=O stretching), 1535, 1469, (Ar-C-C skleton), 1161 (C-N stretching), 748 (-C-H oop bending); MS *m/z* = 244 (M^+); Anal. Calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; O, 13.10. Found: C, 68.74; H, 6.61; N, 11.46; O, 13.15%.

***1-((methyl (phenyl) amino) methyl)-1H-indole-2-carboxylic acid (SP-32)*:**

Yield: 84%, IR (KBr, cm^{-1}): 3254 (-OH bonded), 2997 (–CH_3 stretching), 2781 (-CH_2 stretching), 1689 (-C=O stretching), 1535, 1469, (Ar-C-C skleton), 1144 (C-N stretching), 734 (-C-H oop bending); MS *m/z* = 280 (M^+); Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99; O, 11.42. Found: C, 72.80; H, 5.71; N, 9.88; O, 11.40%.

5.B.16 RESULTS AND DISCUSSION

In Section-A, many mimics of Aripiprazole were prepared as a 'Core' moiety.

In Section-B, known methods were adopted for the preparation of 2-methyl indole as well as indole 2-carboxylic acid with slight modifications in the previously reported methods.

Earlier reported method for the preparation of *N*-Mannich bases of indole 2-carboxylic acid where they used benzotriazole was used as secondary amine and formaldehyde (37-41% w/w solution). They mixed all the three reagents in diethylether and stirred the reaction mixture at the room temperature and obtained solid product. Thus it may be concluded that the physical state of the product may depend upon the secondary amine used.

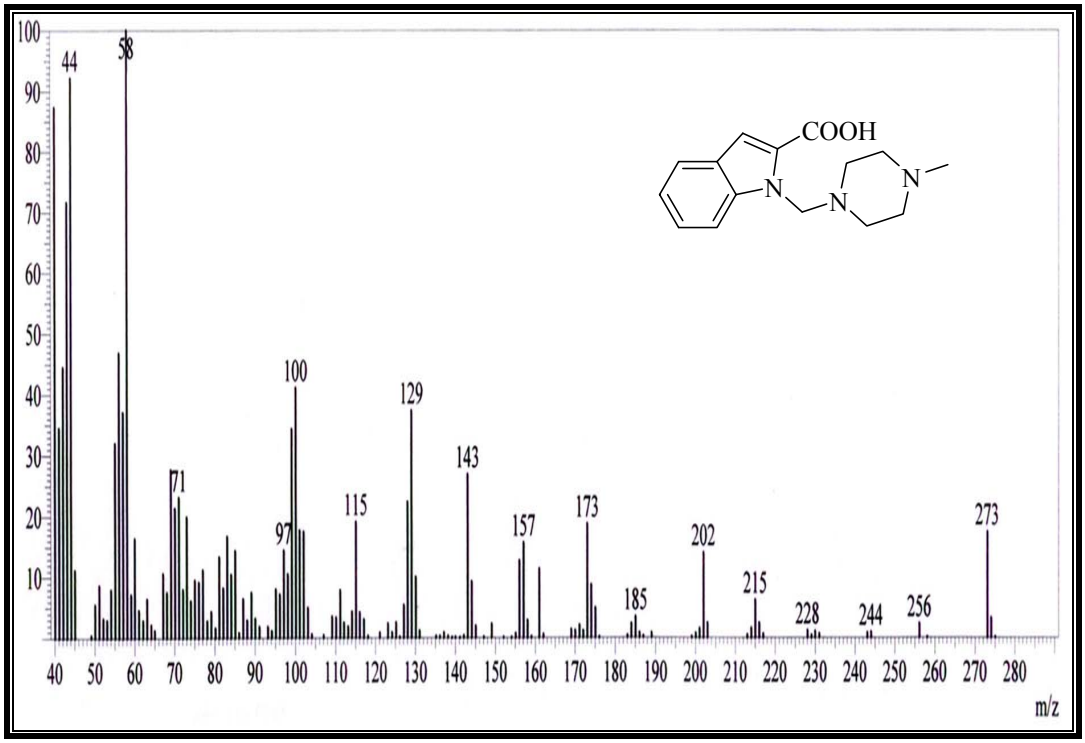
5.B.17 CONCLUSION

In Section-A, New chemical entities were prepared to obtain a small library of Aripiprazole mimics.

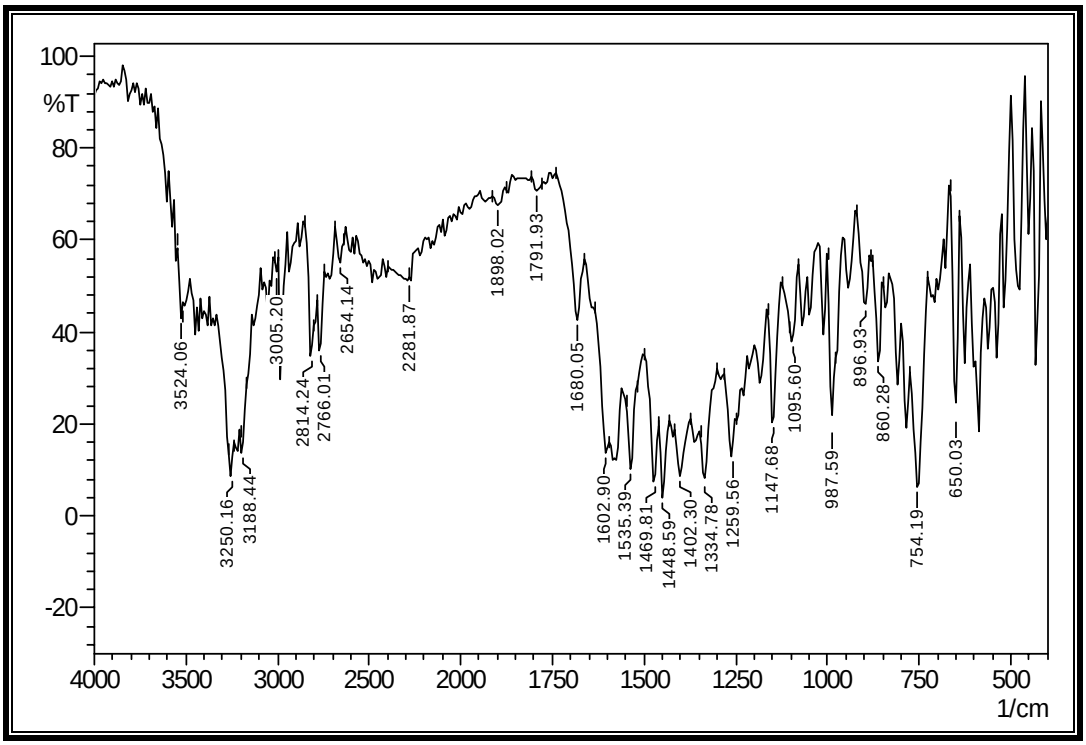
While in Section B, the Mannich base of indole 2-carboxylic acid were prepared to obtain several drug like important molecules having various secondary amines namely, morpholine, piprazine, N-methyl piprazine, N-ethyl piprazine, etc.

5.B.18 REPRESENTATIVE SPECTRA

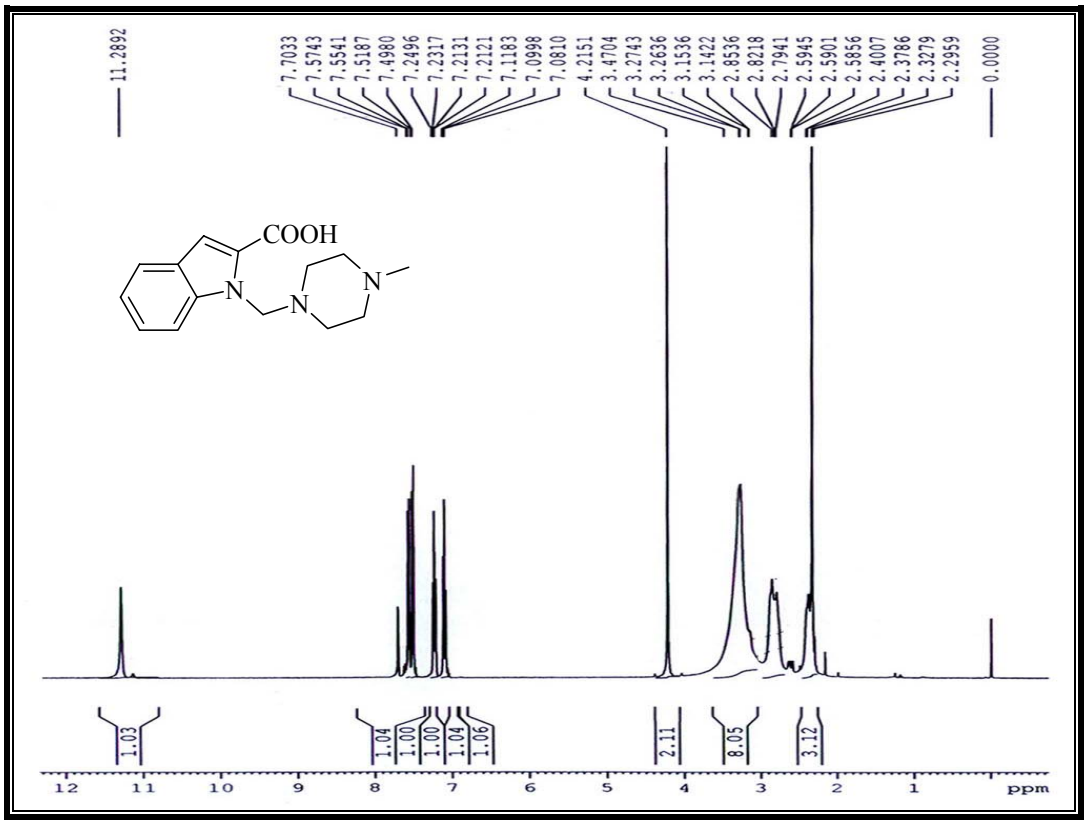
5.B.18.1 Mass Spectrum of SP-024



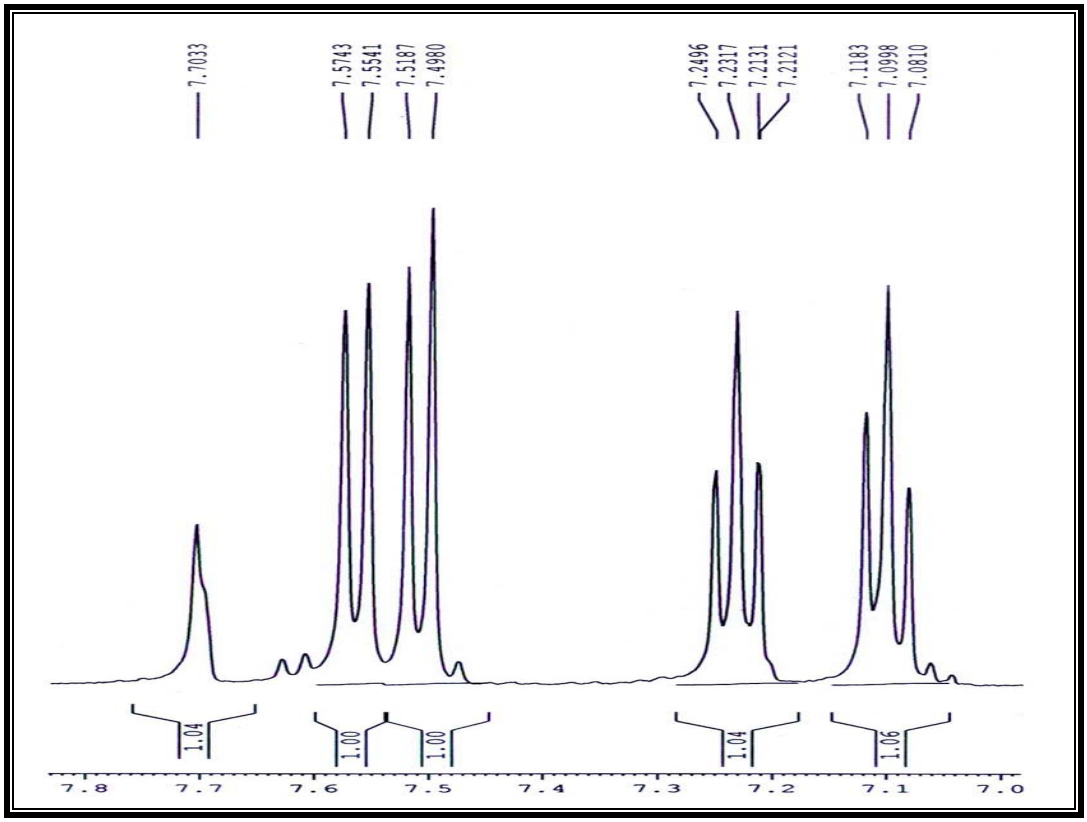
5.B.18.2 IR Spectrum of SP-024



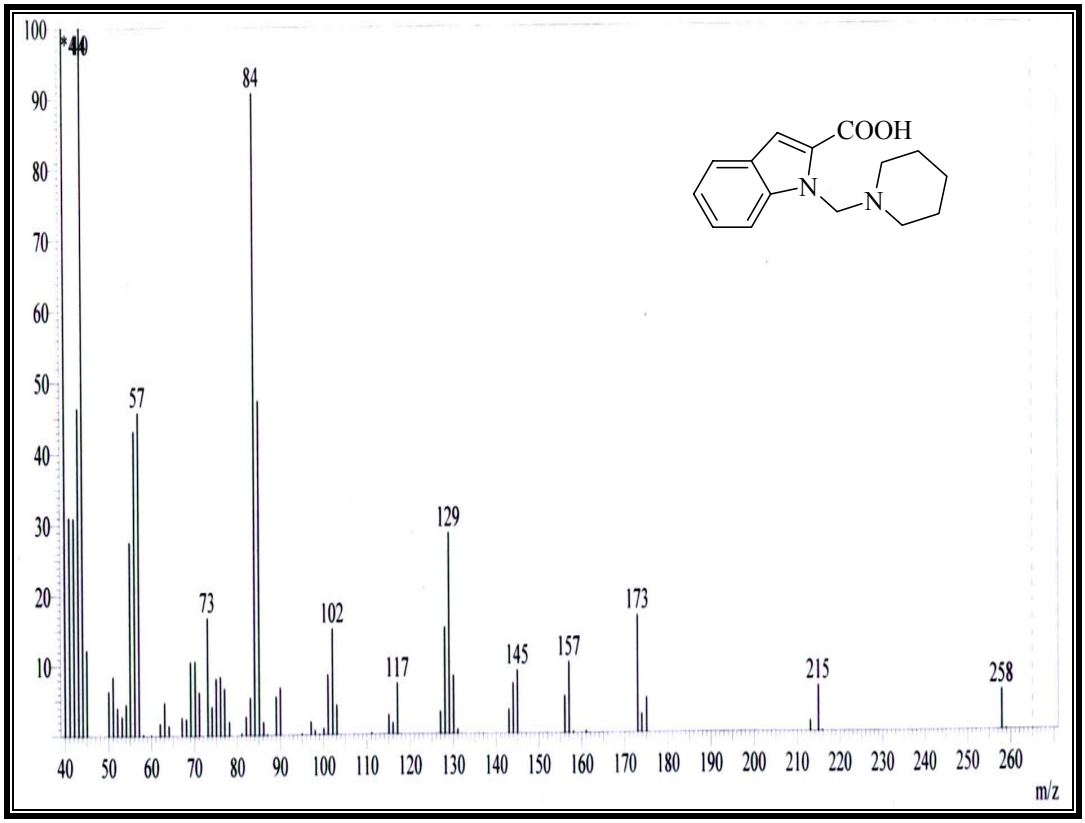
5.B.18.3 ^{1}H NMR Spectrum of SP-024



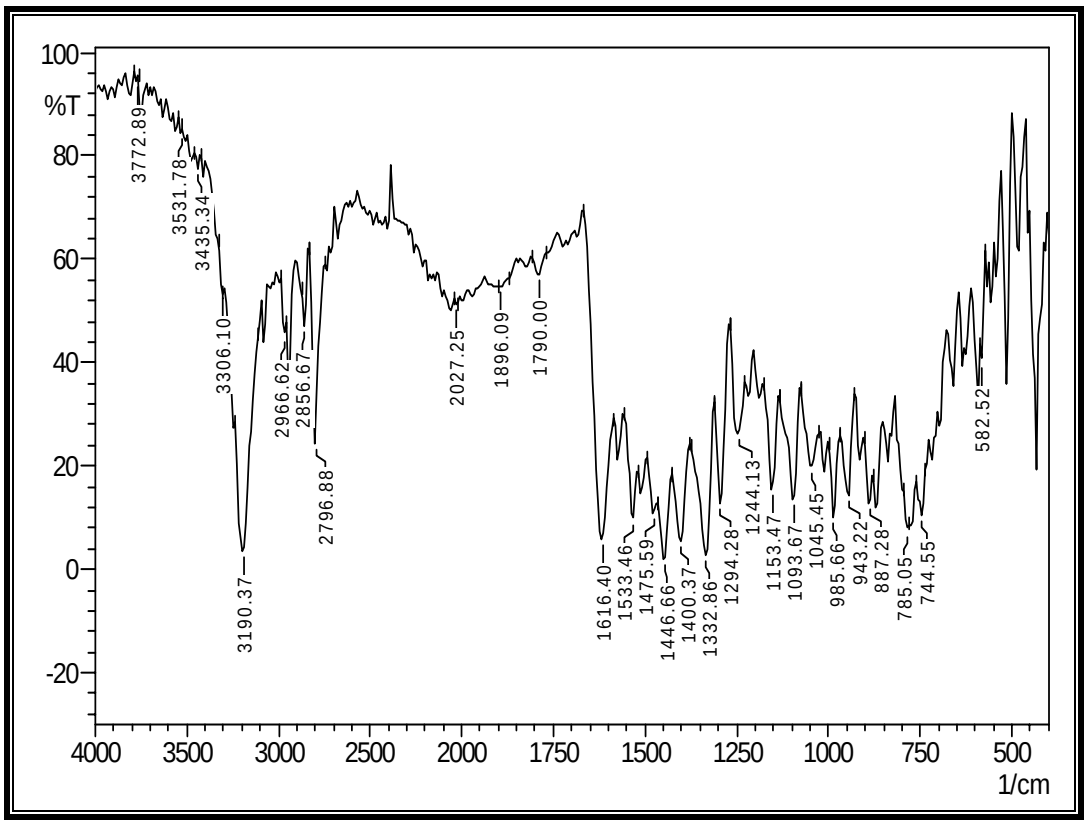
EXPANDED ^{1}H-NMR SPECTRUM OF SP-024



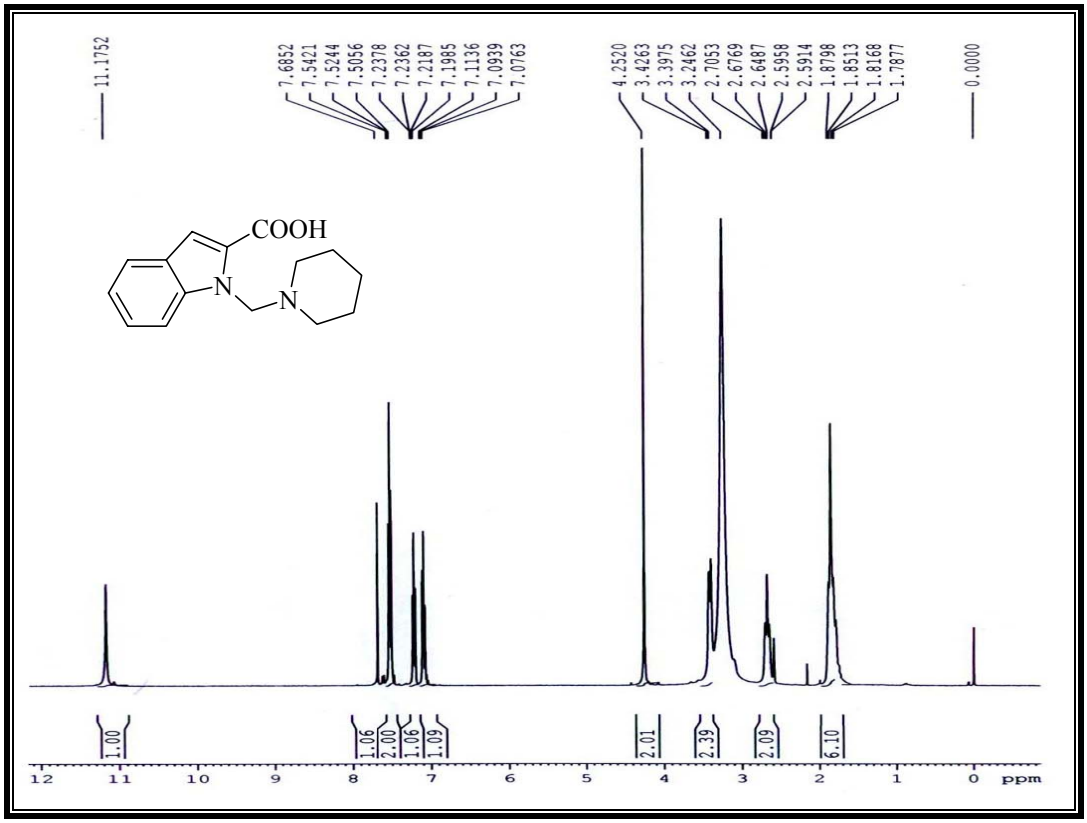
5.B.18.4 Mass Spectrum of SP-025



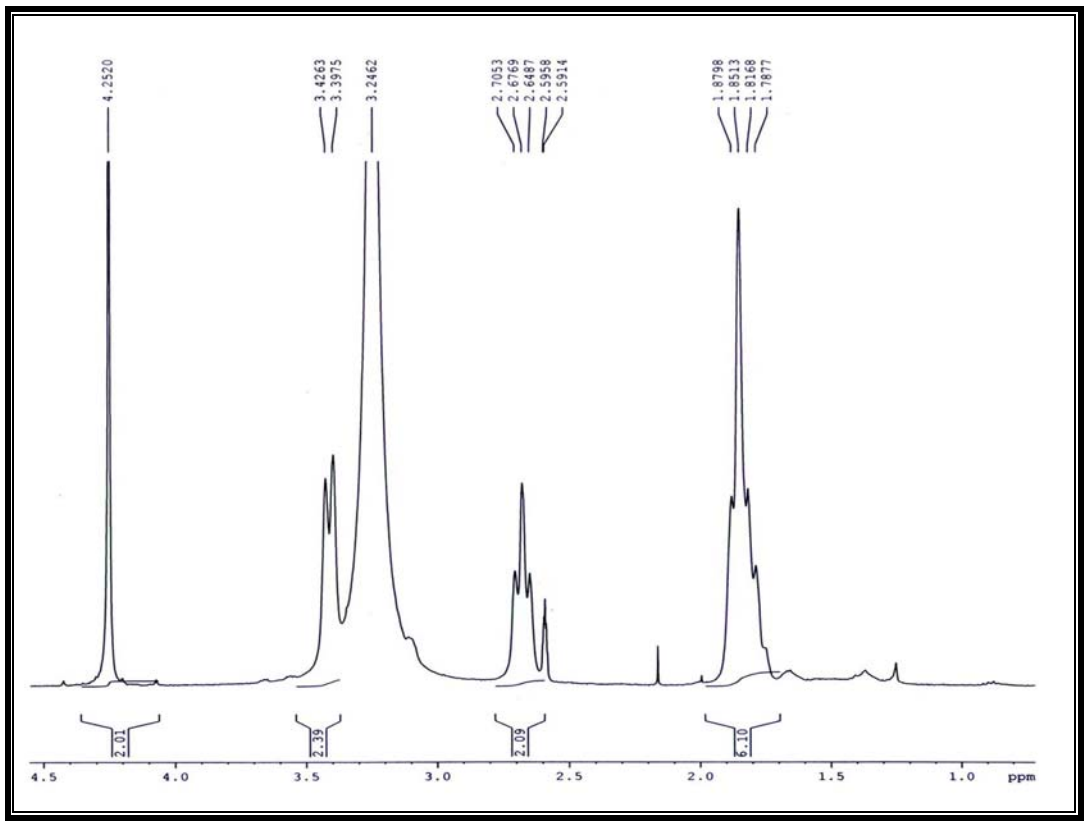
5.B.18.5 IR Spectrum of SP-025



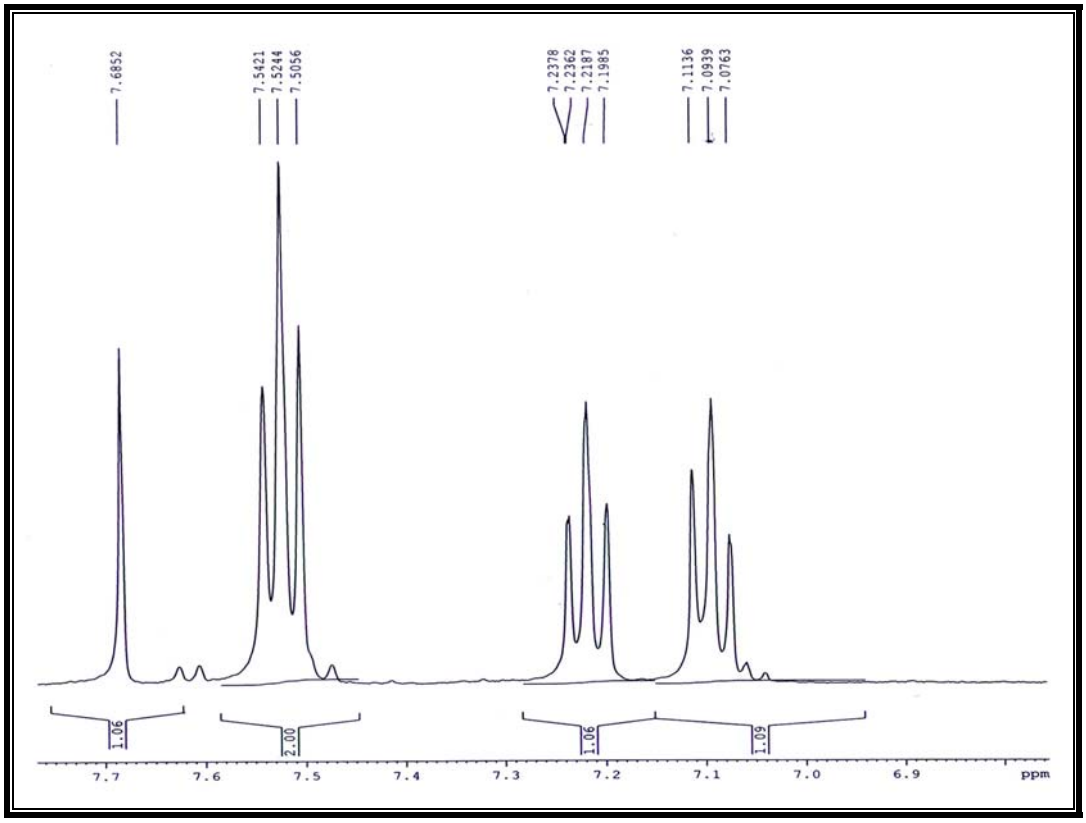
5.B.18.6 ^{1}H NMR Spectrum of SP-025



EXPANDED ^{1}H-NMR SPECTRUM OF SP-025



EXPANDED ^{1}H-NMR SPECTRUM OF SP-025



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PART-II

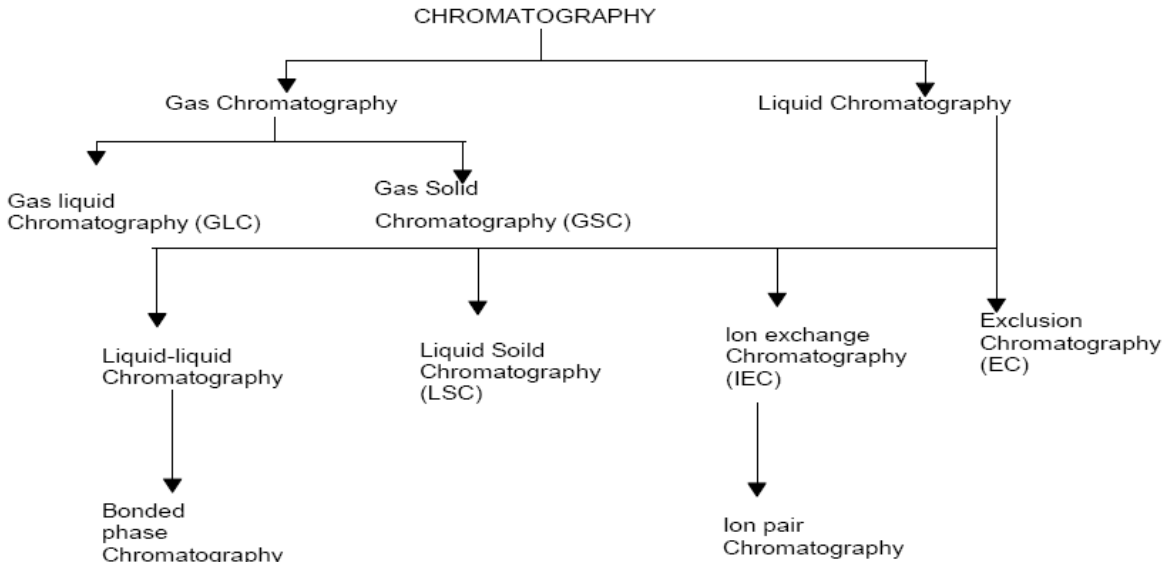
CHAPTER - 6

PURITY DETERMINATION OF SYNTHESIZED NEW CHEMICAL ENTITIES BY HPLC

6.1 INTRODUCTION TO CHROMATOGRAPHY

In 1906, the Russian botanist M.S. Tswett reported separation of different colored constituents of extract of green leaves into a series of colored bands by allowing a solvent to percolate through column bed of powdered calcium carbonate[1]. He termed this technique as 'chromatography' from the Greek words meaning 'color (chroma)' and 'writing (graphy)'. As a matter of above fact, chromatography owes its origin to the efforts of him. Tswett's this technique was virtually unnoticed in the literature until the early 1940s, when the well-known paper of Martin and Synge was published[2]. They reported the discovery of liquid-liquid partition chromatography, both on columns and on paper. They also provided a theoretical frame work for the basic chromatographic process and they received the Nobel Prize in chemistry in 1952 for their work. The next major step that led to progress in this field was the development of gas-liquid chromatography by James and Martin[3]. The success of modern chromatography is greatly due to the excellent extensive treatment of chromatographic theory by Giddings in 1965 through his book entitled Dynamics of Chromatography[4]. Afterwards, a number of well-known scientists whose contributions are too numerous to be recounted here and their work has led to the development of modern liquid chromatography, which is often called high-pressure or high performance liquid chromatography. This technique is also called as HPLC, or simply LC.

Classification of Chromatographic Methods



6.2 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY[5, 6]

Based on the preceding discussion, chromatography can be simply defined as: 'Chromatography is the technique in which the components of a mixture are separated based upon the rates at which they are distributed through two phases, one of which does not move (stationary phase) and the other that moves (mobile phase) .' When mobile phase is liquid, this technique is known as 'liquid chromatography.' Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between two non miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column. It is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereo chemical interaction. Early liquid chromatography is carried out in long glass columns with wide diameter. Now days with the help of advent of latest technology, the particle diameters were reduced as small as to below 10 μm with replacement of glass columns to steel ones. The flow rate of the mobile phase was improved by applying high pressure to the column using pumps and hence the performance was improved. This development led to be mostly called as 'high-performance liquid chromatography' or 'high-pressure liquid chromatography' (HPLC).

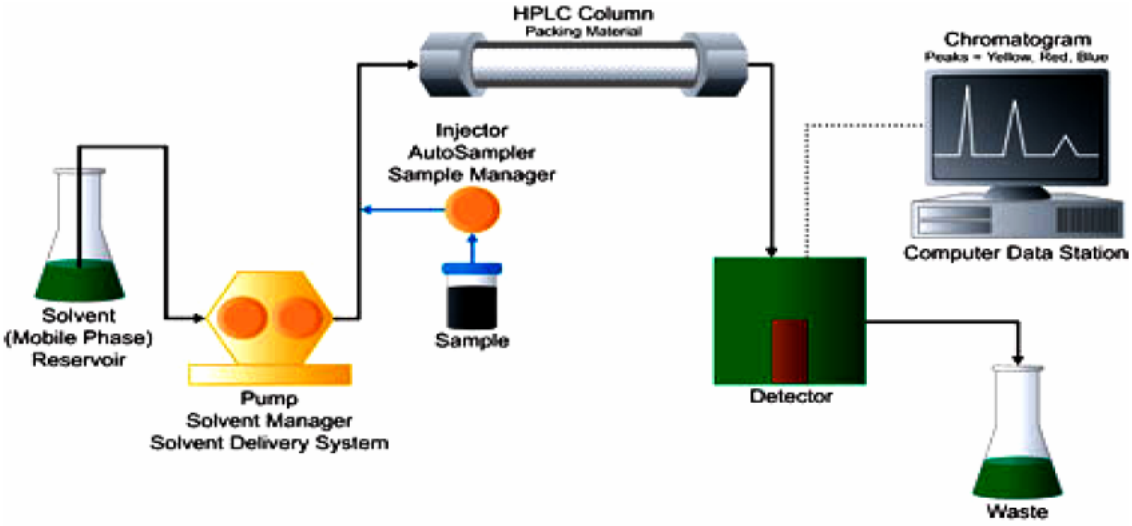
HPLC is most widely used analytical separation technique that offers major improvements over the old chromatography. The technique is more popular because it is non-destructive and may be applied to thermally liable compounds (unlike GC). HPLC is ideally suitable for the separation of macromolecules and ionic species of biomedical interest, liable natural products and diverse less stable and/or high molecular weight compounds. The majority of difficult separations are often more readily attained by HPLC because both phases used in HPLC participate in the chromatographic process (as opposed to only one in GC) to increase more selective interactions with the sample molecule. Short, small-bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. A large variety of unique column packing (stationary phase) provide a wide range of selectivity to separation through HPLC. HPLC also offers wide choice of detection methods as numbers of unique detectors

are available.

INSTRUMENTATION OF HPLC

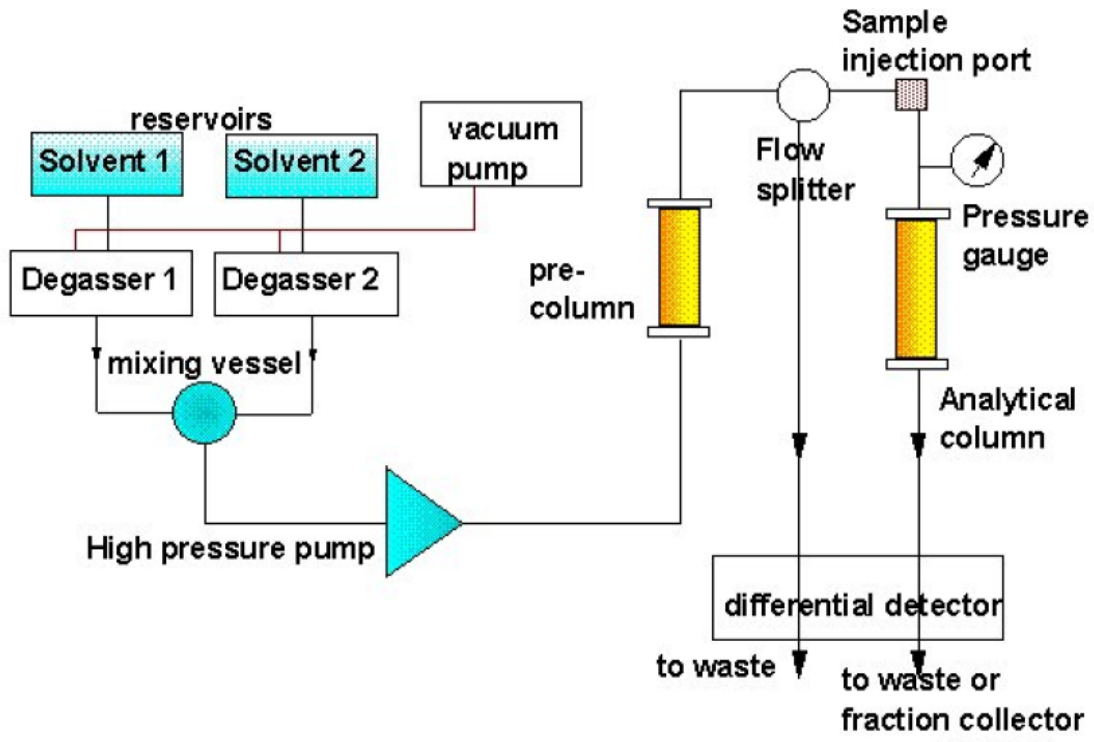
A schematic instrumentation of HPLC is given through figure-1 as under:

Figure-1: A schematic instrumentation of HPLC equipment



A liquid chromatography consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column (a column temperature controller may be also used) to attain retention, a detector to detect analyze response and a data collection device such as a computer, integrator, or recorder. Further, in some cases, degasser with vacuum pump and pre-column facility can implement in the modern HPLC; represented as figure-2:

Figure-2: Designing of HPLC path



A brief introduction of HPLC components is given as under

Pumping Systems

HPLC pumping system delivers measured amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Operating pressures up to 5000 psi or higher, with delivery rates up to about 10 ml/minute is typical. Pumps used for quantitative analysis should be constructed of materials that inert to corrosive mobile phase components and be capable of delivering the mobile phase at a constant rate with minimal fluctuations over extended periods of time. Modern systems consist of microprocessor controlled metering pumps that can be programmed to deliver either constant (isocratic) flow of mobile phase or vary the ratio of mobile phase components, as is required for gradient run. Advanced pumping system is equipped with a degasser to remove dissolved air and other gases from the solvent through solvent delivery system.

Injectors

After dissolution in mobile phase or suitable diluent, compounds to be chromatographed are injected into the mobile phase, either manually by syringe/loop injectors or automatically by auto sampler. Autosampler consist of a carousel or rack to hold sample vials with tops that have a pierce able septum or stopper and an injection device to transfer sample from the vials to a loop from which it is loaded into the chromatograph. Autosampler can be programmed to control sample volume, the number of injections, the interval between injections and rinse cycles.

Columns[7-15]

The column is usually made up of stainless steel to withst and high pressure. Columns used for analytical separations usually have 10-30 cm length and 4-10 mm inside diameter containing stationary phase having particle diameter of 3-10 μm. Particles may range up to 50 μm or more for preparative columns. Stationary phases for modern, reverse-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Small particles thinly coated with organic phase provide for low mass transfer resistance and, hence, rapid transfer of compounds between the stationary and mobile phases. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60° C. Unmodified silica, porous graphite or polar chemically modified silica, e.g. cyano propyl or diol, used as the stationary phase for normal-phase liquid chromatography. Most of separations for reversed-phase liquid chromatography are based upon partition mechanisms that utilize chemically modified silica as the stationary phase and polar solvents as the mobile phase. The surface of the support, e.g. the silanol groups of silica, is reacted with various saline reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system.

Detectors[16-21]

UV/Vis spectrophotometers, including diode array detectors, are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors, radioactivity detectors or other special detectors may also be used.

Detector consists of a flow-through cell mounted at the end of the column. A beam of UV radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes. Fixed (mercury lamp), variable (deuterium or high-pressure xenon lamp) and multi-wavelength detectors are widely available. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi wavelength detectors measure absorbance at two or more wavelengths simultaneously. In diode array multi-wavelength detectors, continuous radiation is passed through the sample cell and then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at multiple, selectable wavelengths, spectra of the eluting peaks and also peak purity.

Data Collection Devices

Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification and method variables. They are also used to program the liquid chromatograph, controlling most of variables and providing for long periods of unattended operation.

Mode of HPLC

Various modes of HPLC utilized to separate compounds are classified as follows:

1) Adsorption chromatography
2) Normal-phase chromatography
3) Reversed-phase chromatography
4) Ion-pair chromatography
5) Ion-exchange chromatography
6) Size exclusion chromatography

Adsorption chromatography

Adsorption chromatography uses polar stationary phases with relatively non polar mobile phases. Separations in adsorption chromatography result to a great extent from the interaction of sample polar functional groups with discrete adsorption sites

on the stationary phase. Adsorption chromatography is usually considered appropriate for the separation of nonionic molecules that are soluble in organic solvents.

Normal-phase chromatography

In HPLC, if stationary phase is more polar than the mobile phase, it is termed as normal-phase liquid chromatography. Polar bonded phases that have a diol, cyano, diethyl amino, amino, or diamino functional groups are used as stationary phase in normal-phase chromatography. Due to lower affinity of non polar compounds to the stationary phases used, non polar compounds are elute first while polar compounds are retained for longer time. Normal-phase chromatography is widely applied for chiral separations.

Reversed-phase chromatography

In HPLC, if stationary phase is less polar than the mobile phase, it is termed as reversed-phase liquid chromatography. In this technique, C-18, C-8, Phenyl and cyano-propyl functional groups that chemically bonded to micro porous silica particles are used as stationary phase. Retention in reversed phase chromatography occurs by nonspecific hydrophobic interactions of the solute with stationary phase. The ubiquitous application of reversed-phase chromatography arise from the fact that practically all organic molecules have hydrophobic regions in their structures and effectively interact with the stationary phase. It is estimated that over 65% (possibly as high as 90%) of all HPLC separations are executed in the reversed-phase mode. The rationale for this includes the simplicity, versatility and scope of the reversed-phase method.[22]

Ion-pair chromatography

Ionic or partially ionic compounds can be chromatographed on reversed phase columns by using ion-pairing reagents. These reagents are typically long chain alkyl anions or cations that, when used in dilute concentrations, can increase the retention of analyte ions. C-5 to C-10 alkyl sulfonates are commonly used for cationic compounds while C-5 to C-8 alkyl ammonium salts are generally used in the cases of anionic solutes.

Ion-exchange chromatography

Ion-exchange chromatography is an adaptable technique used primarily for the separation of ionic or easily ionizable species. The stationary phase is characterized by the presence of charged centers having exchangeable counter ions. Both anions and cations can be separated by choosing the suitable ion-exchange medium. Ion-exchange chromatography employs the dynamic interactions between charged solute ions and stationary phases that have oppositely charged groups.

Size exclusion chromatography

Size exclusion chromatography separates molecules according to their molecular mass. In Size exclusion chromatography, column is filled with material having precisely controlled pore sizes and the sample is simply screened or filtered according to its solvated molecular size. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

6.4 AIM OF CURRENT WORK

The prime and specific objective of the work is to develop simple and Rapid Liquid Chromatographic Method of Newly Synthesized Compounds Like, Benzofuran derivatives, Valproic acid derivatives, Quinolone derivatives, Coumarin derivatives and Indole derivatives for the quantification and Purity Determination by High-performance liquid chromatographic method.

6.5 EXPERIMENTAL

Instrument:

Liquid chromatography was performed using Waters equipments with TM-600 quaternary pump, Waters 2489 UV-Visible detector, Waters 600 Controller, waters inline degasser AF and manual injector with 20 µl loop. The equipment was connected to multi-instrument data acquisition and data processing system (Empower 2.1 software).

Reagents:

Methanol (HPLC grade)

3.2 pH Phosphate Buffer

Water (Milli-Q)

Blank :

Diluent was used as blank.

Sample Preparation:

Weigh accurately 10 mg of different newly synthesized compounds in to 100 ml volumetric flask. Add 60 ml of diluent into the flask and sonicate of 30 minutes with normal hand-shaking. Cool the flask to room temperature and dilute to volume with diluent. Filter 10 ml of this solution through 0.45 µm nylon syringe filter. The concentration obtained is 100 µg/ml.

All samples were prepared as per above mentioned sample preparation method.

6.5 CHROMATOGRAMS

Chromatographic condition:

Sample Code : SBF-01

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

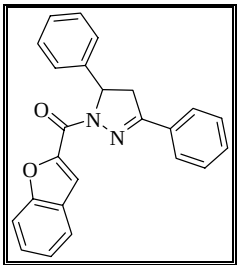
Column Temperature: ambient

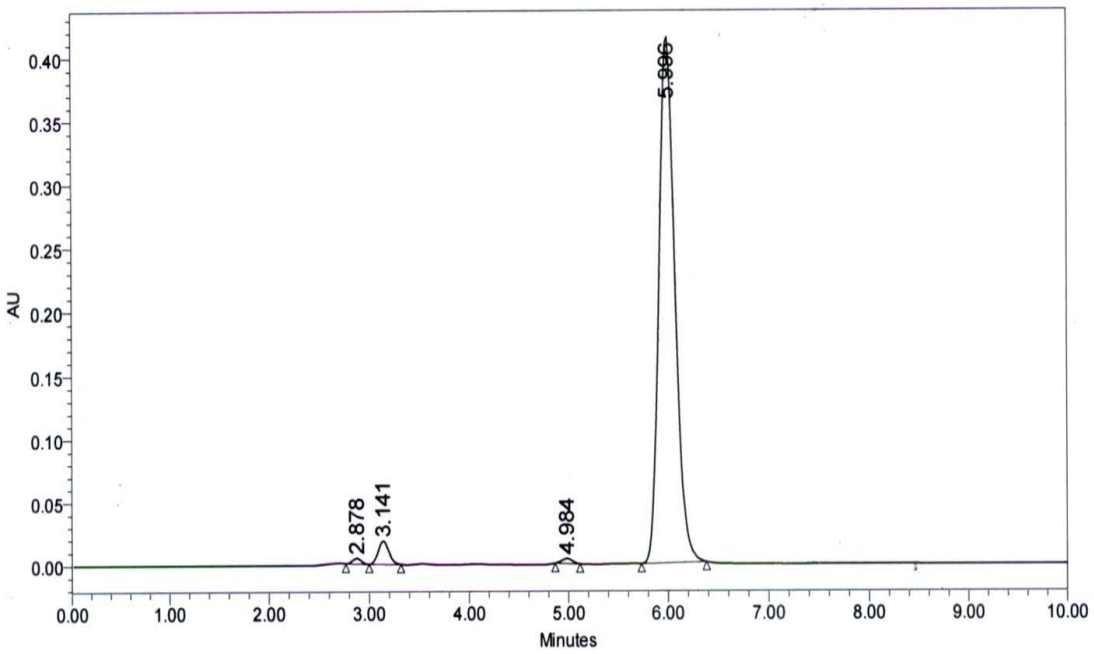
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	2.878	27439	0.57	4530	3.207502e+003	5.196422e+003
2	3.141	136771	2.86	18215	2.155656e+003	3.656276e+003
3	4.984	30762	0.64	4034	6.585745e+003	9.835649e+003
4	5.996	4580365	95.92	414955	3.318592e+003	6.025082e+003

Chromatographic condition:

Sample Code : SBF-02

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

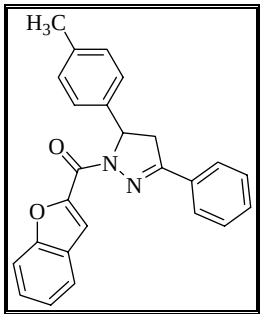
Column Temperature: ambient

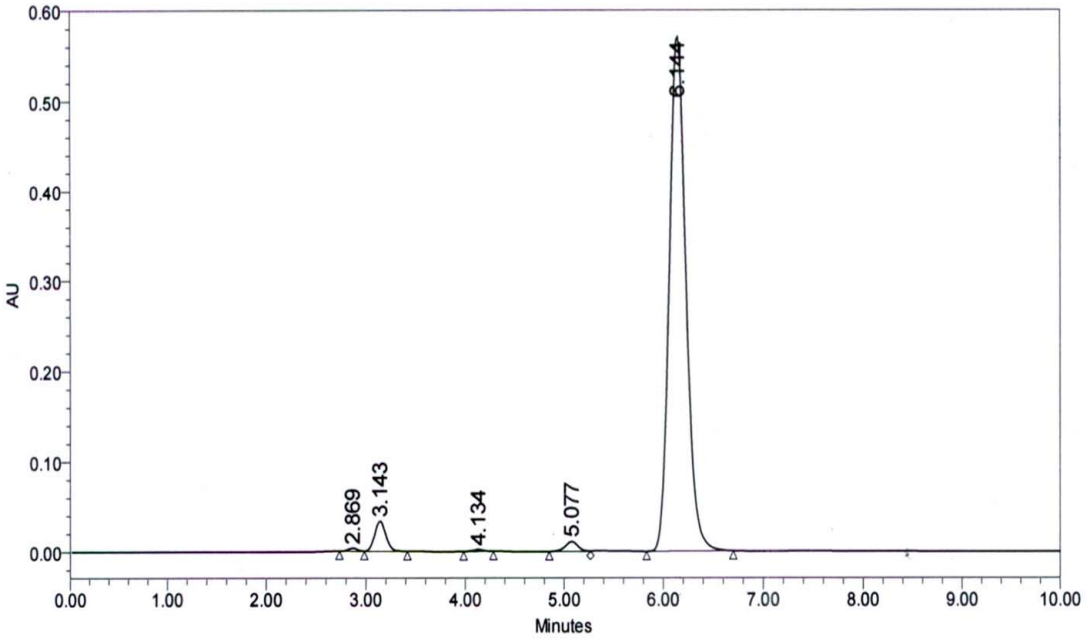
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	2.869	20313	0.29	3268	2.910641e+003	5.098848e+003
2	3.143	263830	3.76	33296	1.785640e+003	3.247636e+003
3	4.134	16180	0.23	2075	3.786193e+003	6.647771e+003
4	5.077	98203	1.40	11068	3.579392e+003	7.105856e+003
5	6.144	6613636	94.32	572402	3.125847e+003	5.719751e+003

Chromatographic condition:

Sample Code : SPSC-01

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

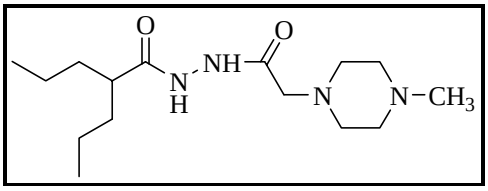
Column Temperature: ambient

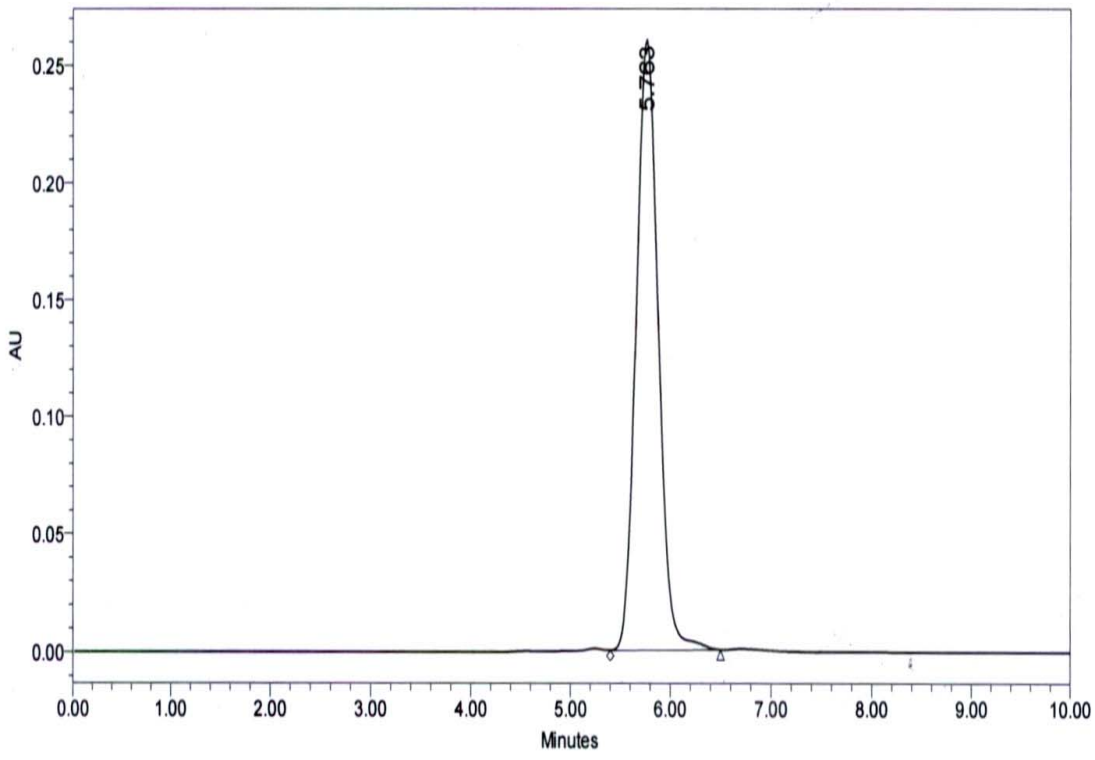
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	5.763	3986762	100.00	260232	1.731422e+003	3.071914e+003

Chromatographic condition:

Sample Code : SPSC-02

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

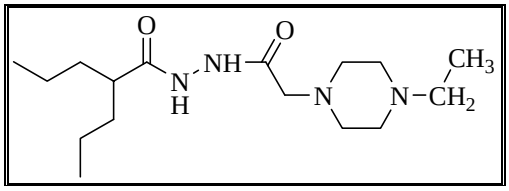
Column Temperature: ambient

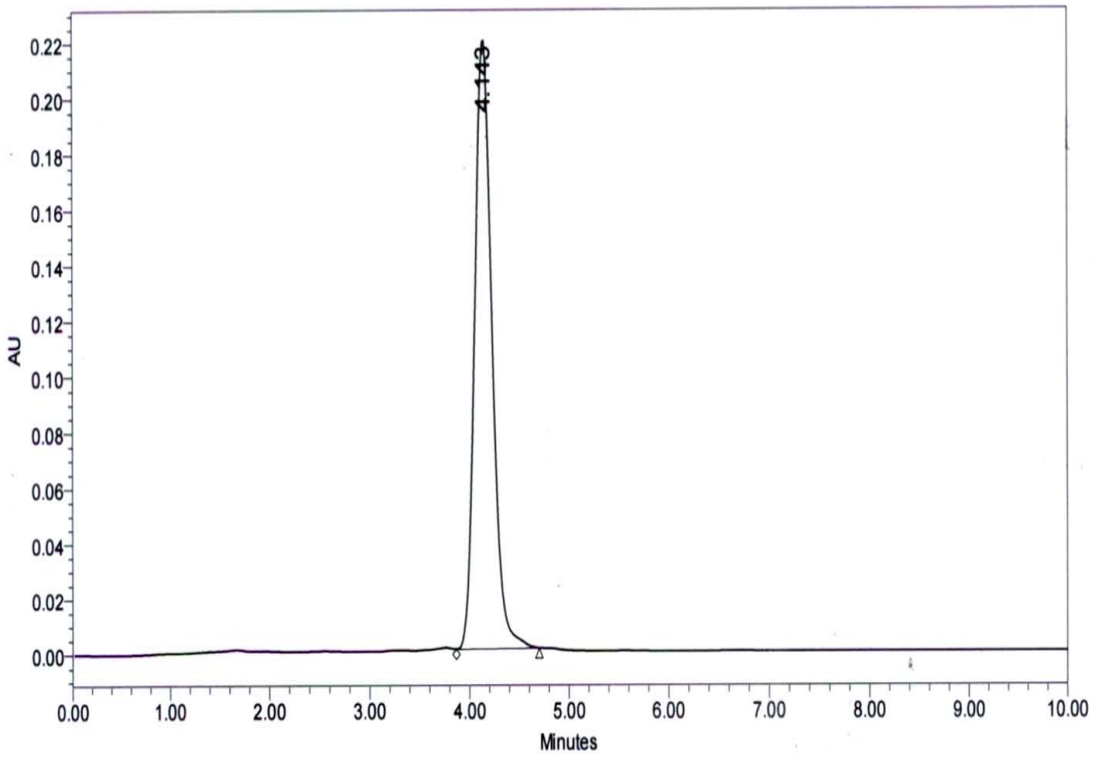
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	4.143	2650708	100.00	219682	1.332910e+003	2.461242e+003

Chromatographic condition:

Sample Code : SPA-01

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

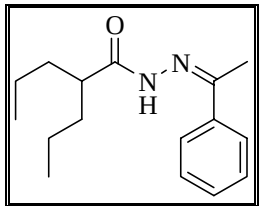
Column Temperature: ambient

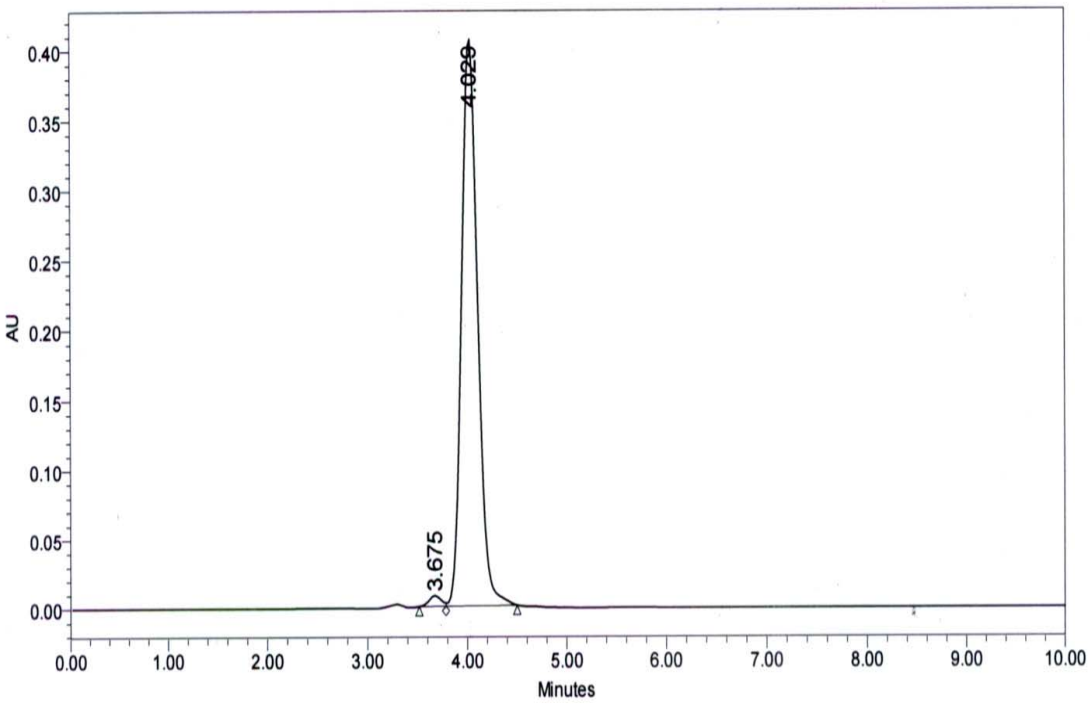
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.675	65630	1.42	7583		
2	4.029	4564966	98.58	406364	1.510587e+003	2.778509e+003

Chromatographic condition:

Sample Code : SPA-02

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

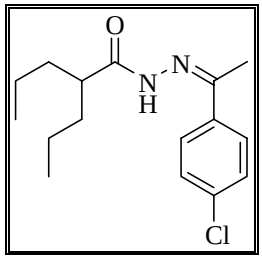
Column Temperature: ambient

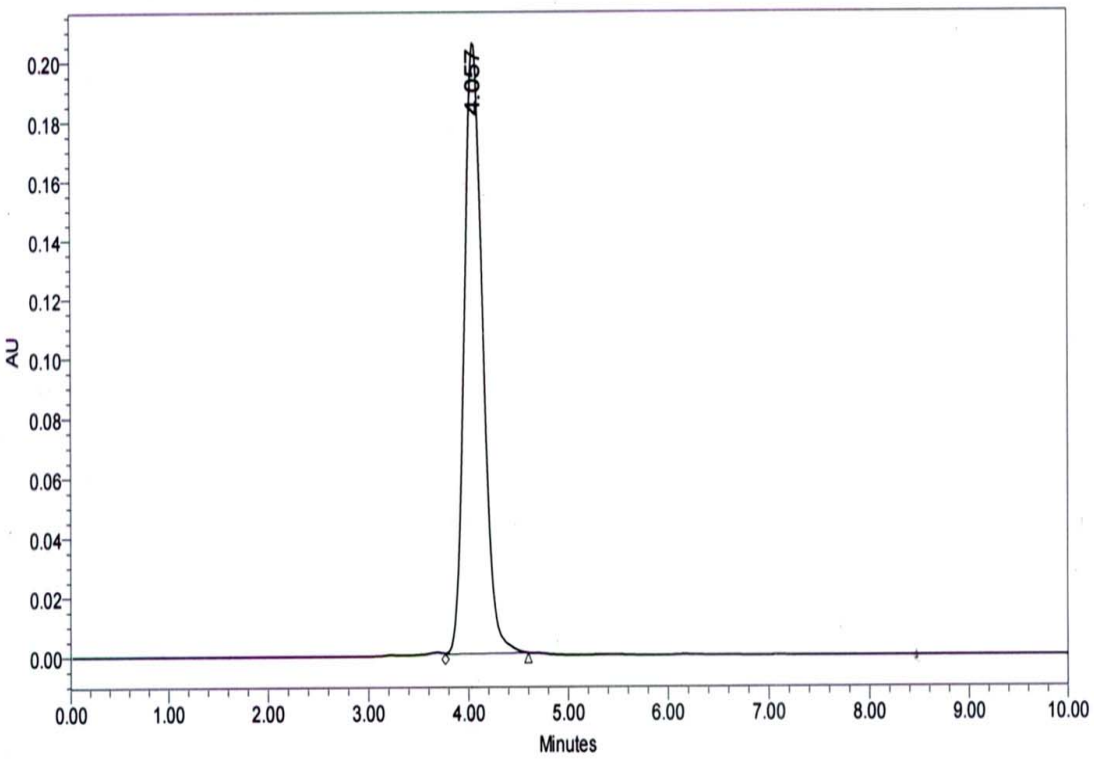
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	4.057	2625727	100.00	205843	1.179542e+003	2.138216e+003

Chromatographic condition:

Sample Code : SPAM-01

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

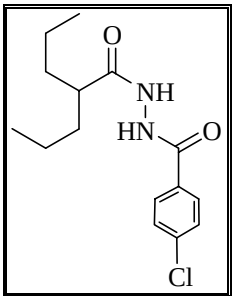
Column Temperature: ambient

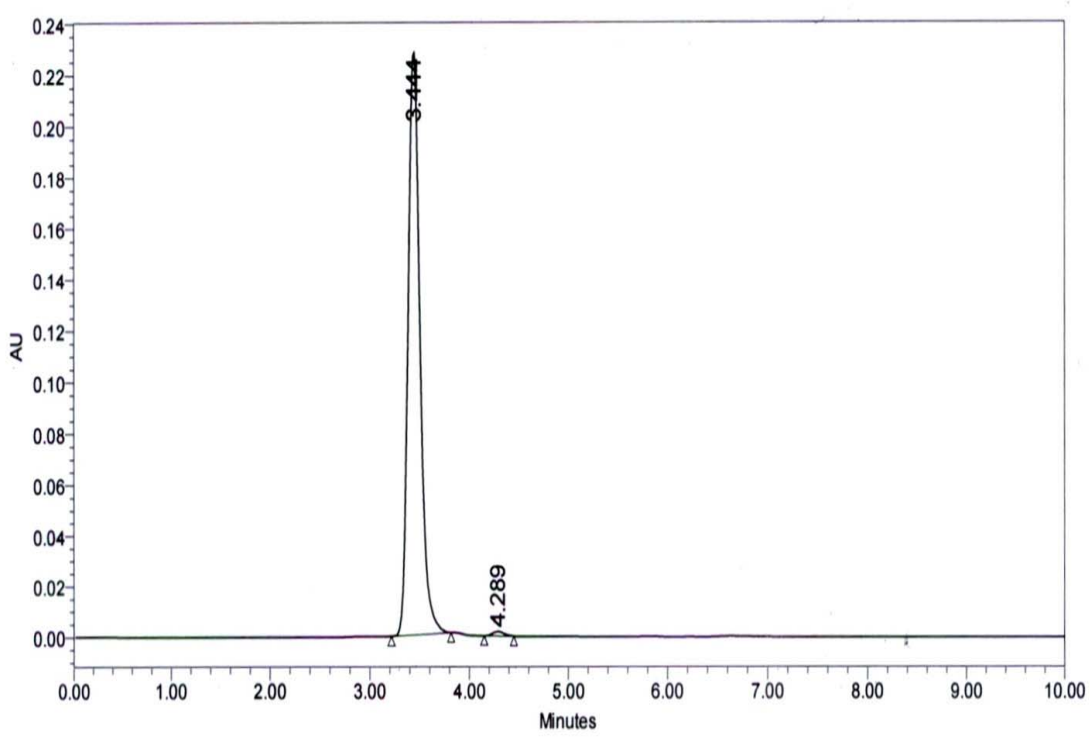
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.444	1902647	99.27	228925	1.798321e+003	3.431032e+003
2	4.289	14050	0.73	1724	3.756800e+003	5.855262e+003

Chromatographic condition:

Sample Code : SPAM-02

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

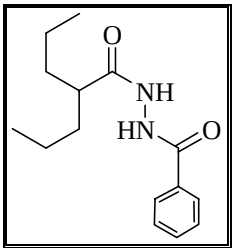
Column Temperature: ambient

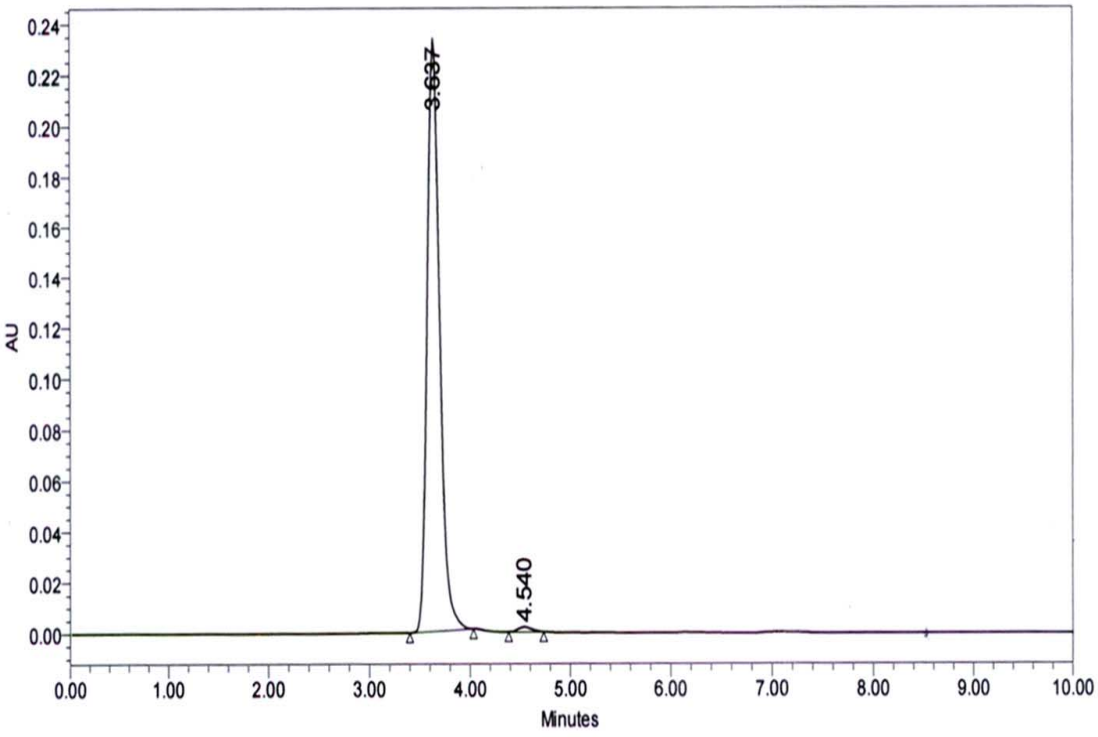
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.637	2017846	99.09	233518	1.881008e+003	3.555614e+003
2	4.540	18486	0.91	2004	3.080779e+003	4.676253e+003

Chromatographic condition:

Sample Code : SP-40

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

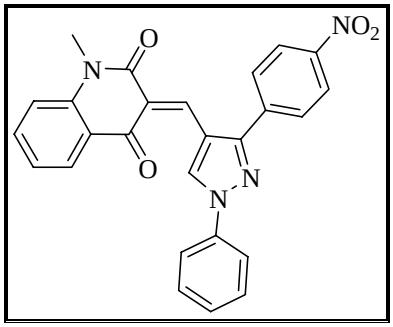
Column Temperature: ambient

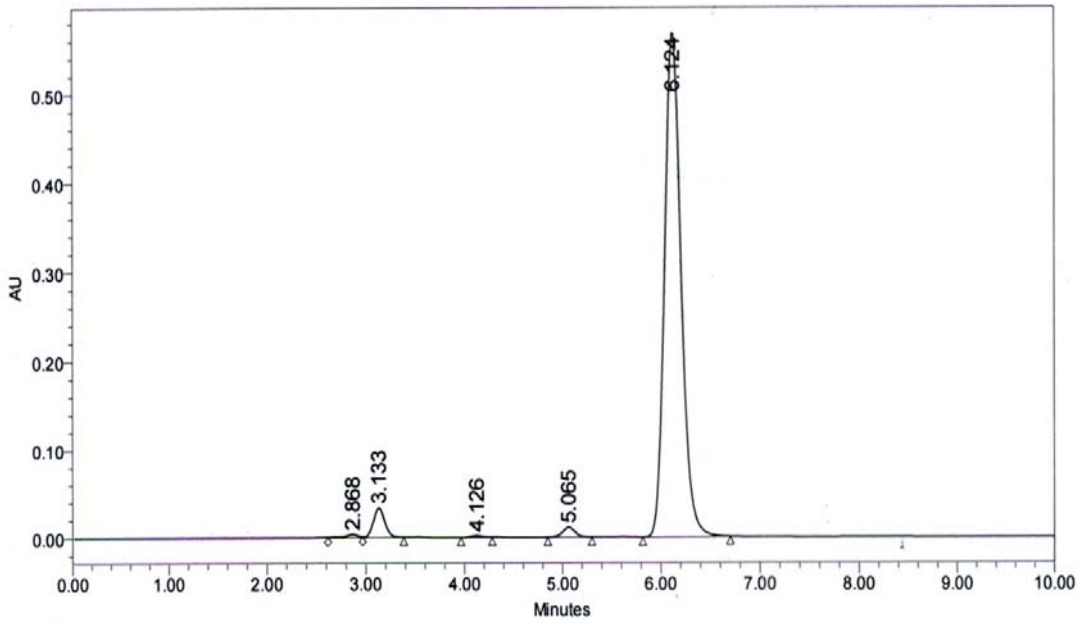
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	2.868	33868	0.48	3820		
2	3.133	267398	3.82	33592	1.731813e+003	3.241540e+003
3	4.126	15830	0.23	2032	3.779955e+003	6.634723e+003
4	5.065	100108	1.43	11204	3.446782e+003	6.826382e+003
5	6.124	6576222	94.03	571058	3.116324e+003	5.696866e+003

Chromatographic condition:

Sample Code : SP-41

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

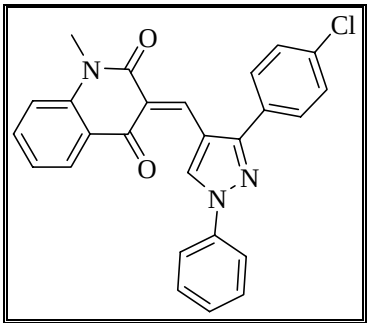
Column Temperature: ambient

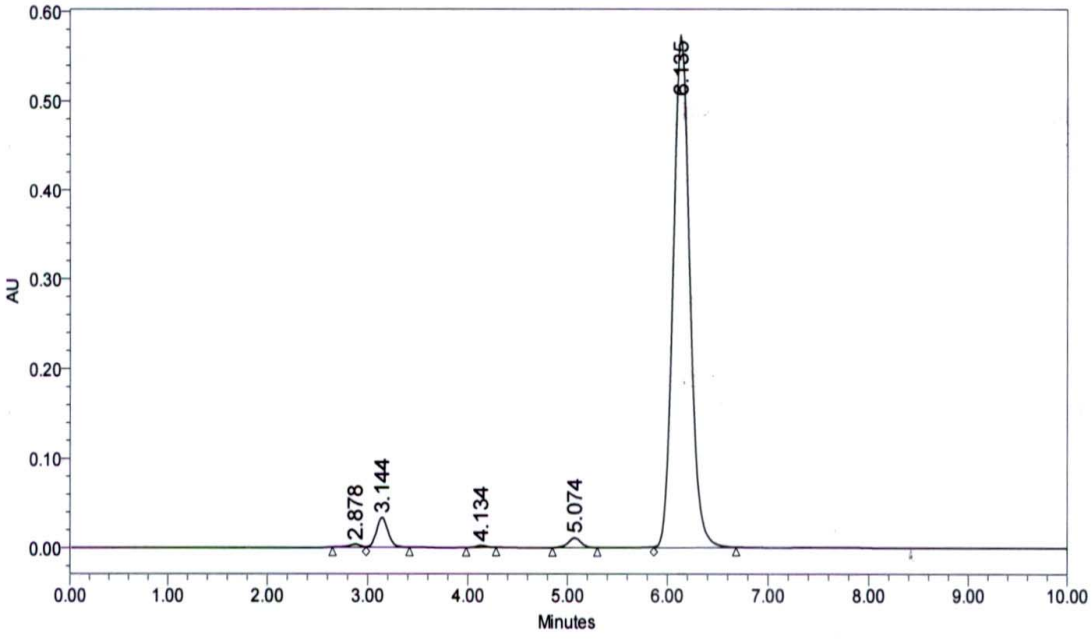
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	2.878	27114	0.39	3465		
2	3.144	266082	3.80	33193	1.710540e+003	3.210099e+003
3	4.134	15825	0.23	2036	3.797319e+003	6.630978e+003
4	5.074	100795	1.44	11221	3.417181e+003	6.696604e+003
5	6.135	6589782	94.15	573046	3.144778e+003	5.783252e+003

Chromatographic condition:

Sample Code : SPC-02

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

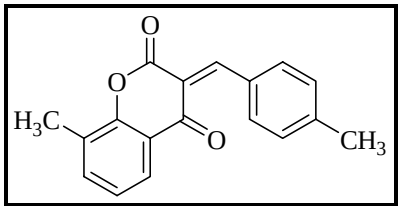
Column Temperature: ambient

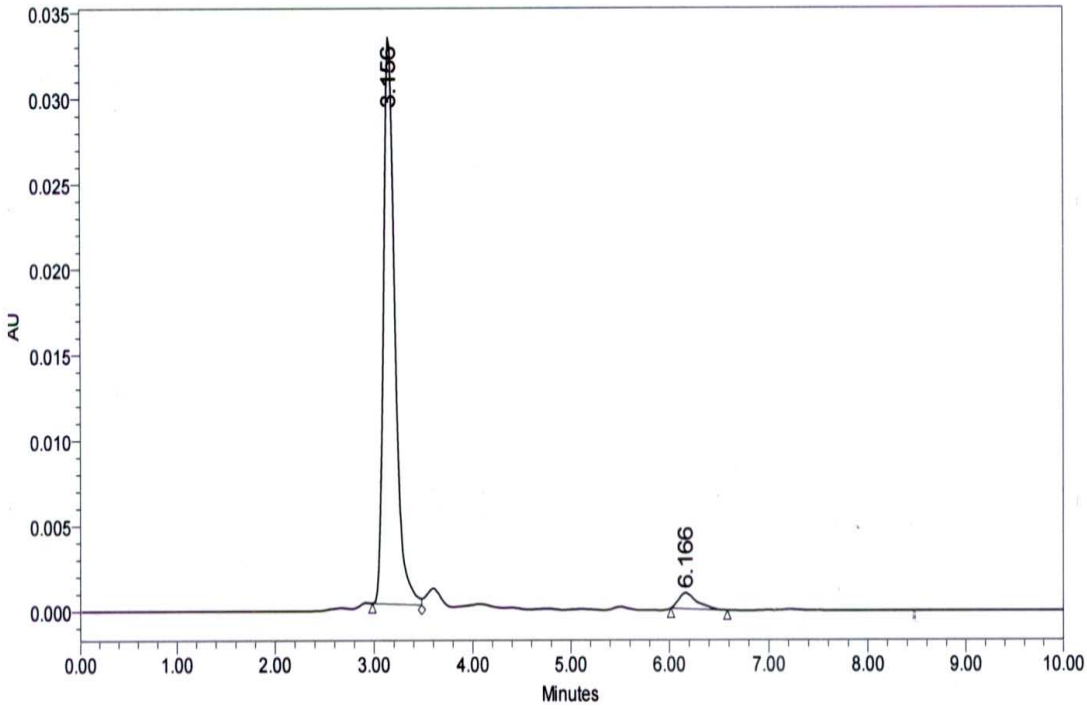
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.156	263959	95.69	33242	1.454318e+003	2.906445e+003
2	6.166	11902	4.31	925	2.608750e+003	2.747368e+003

Chromatographic condition:

Sample Code : SPC-03

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

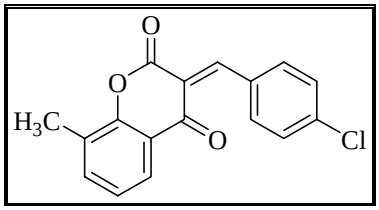
Column Temperature: ambient

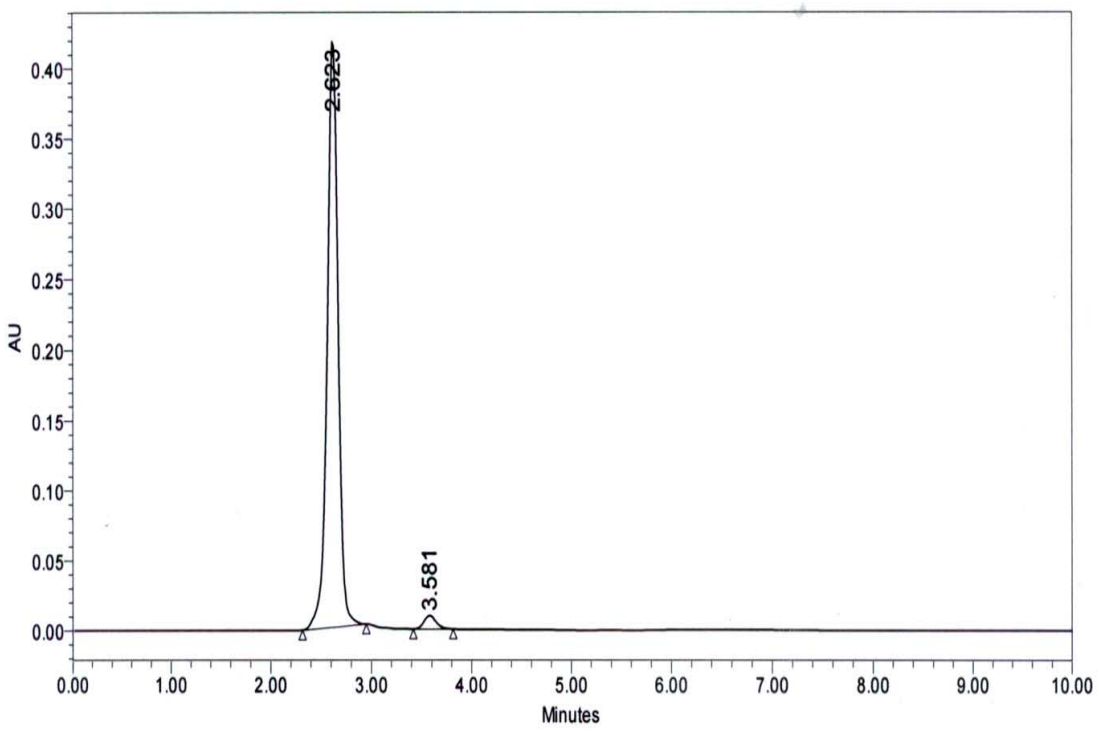
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	2.623	3141056	97.44	418699	1.169832e+003	2.812356e+003
2	3.581	82387	2.56	9595	2.064750e+003	3.435304e+003

Chromatographic condition:

Sample Code : SPAPI-01

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

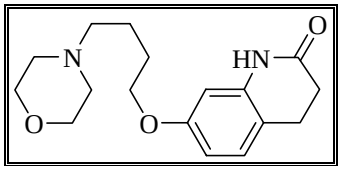
Column Temperature: ambient

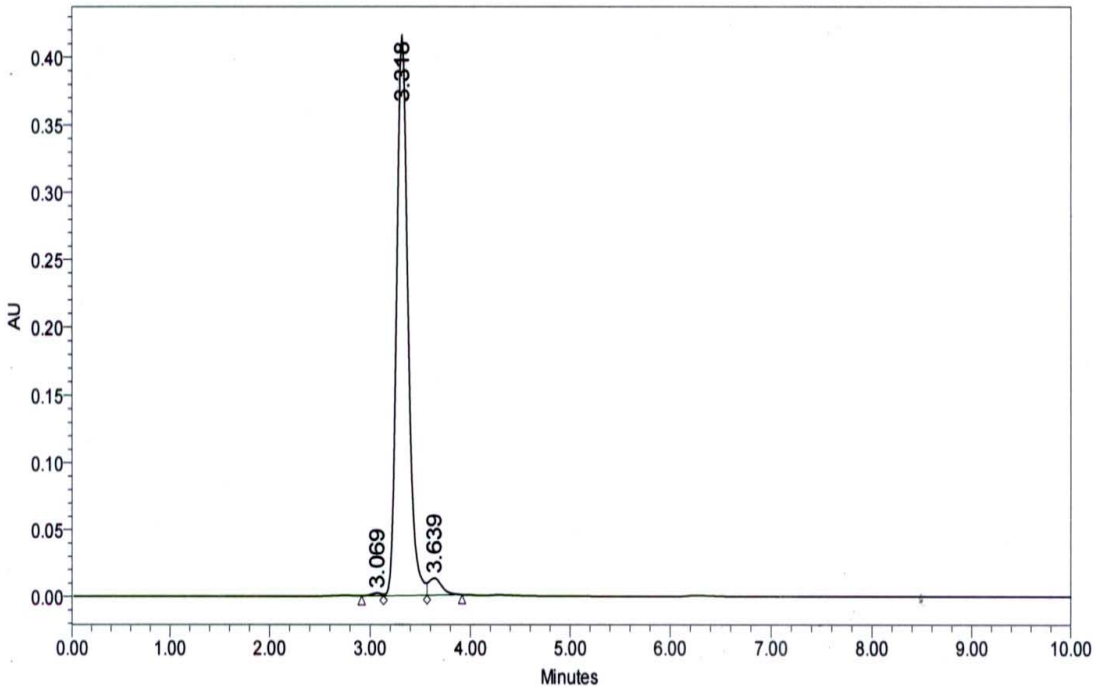
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.069	13370	0.39	1924		
2	3.318	3260163	96.21	414572	1.805227e+003	3.417149e+003
3	3.639	115125	3.40	12486		

Chromatographic condition:

Sample Code : SPAPI-03

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

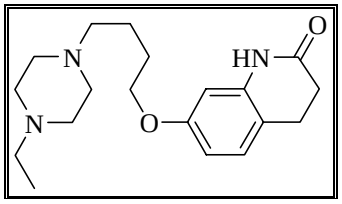
Column Temperature: ambient

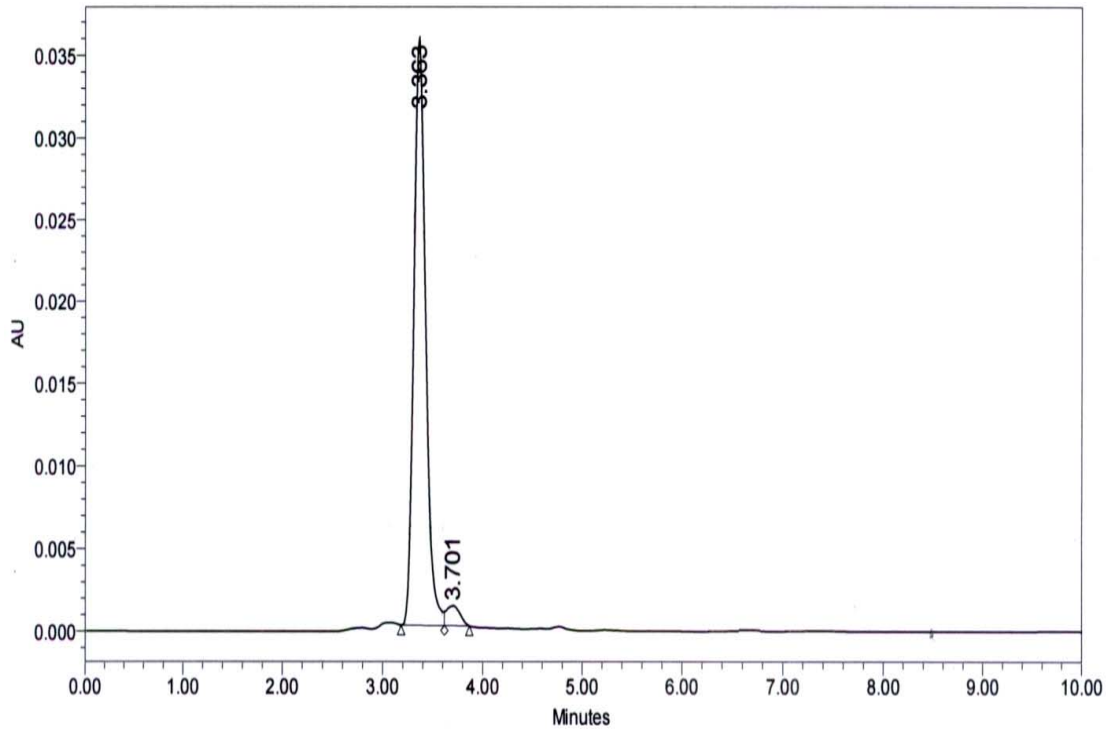
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.363	292079	96.28	35728	1.756336e+003	3.270066e+003
2	3.701	11273	3.72	1210		

Chromatographic condition:

Sample Code : SP-24

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

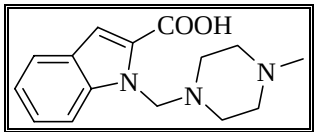
Column Temperature: ambient

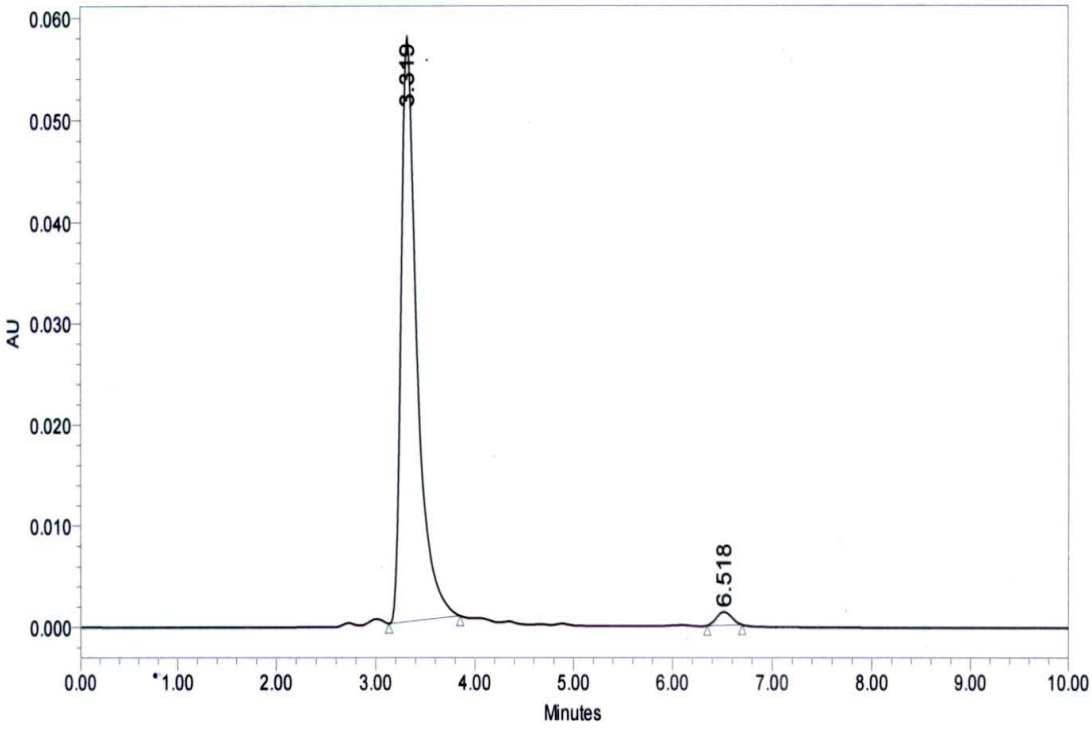
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.319	641061	97.90	57623	6.960941e+002	1.130792e+003
2	6.518	13754	2.10	1305	5.888252e+003	8.796421e+003

Chromatographic condition:

Sample Code : SP-25

Mobile phase :- 3.2 pH Phosphate Buffer : Methanol (10 : 90)

Column :- YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron)

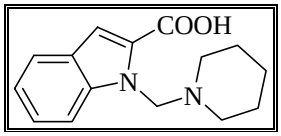
Column Temperature: ambient

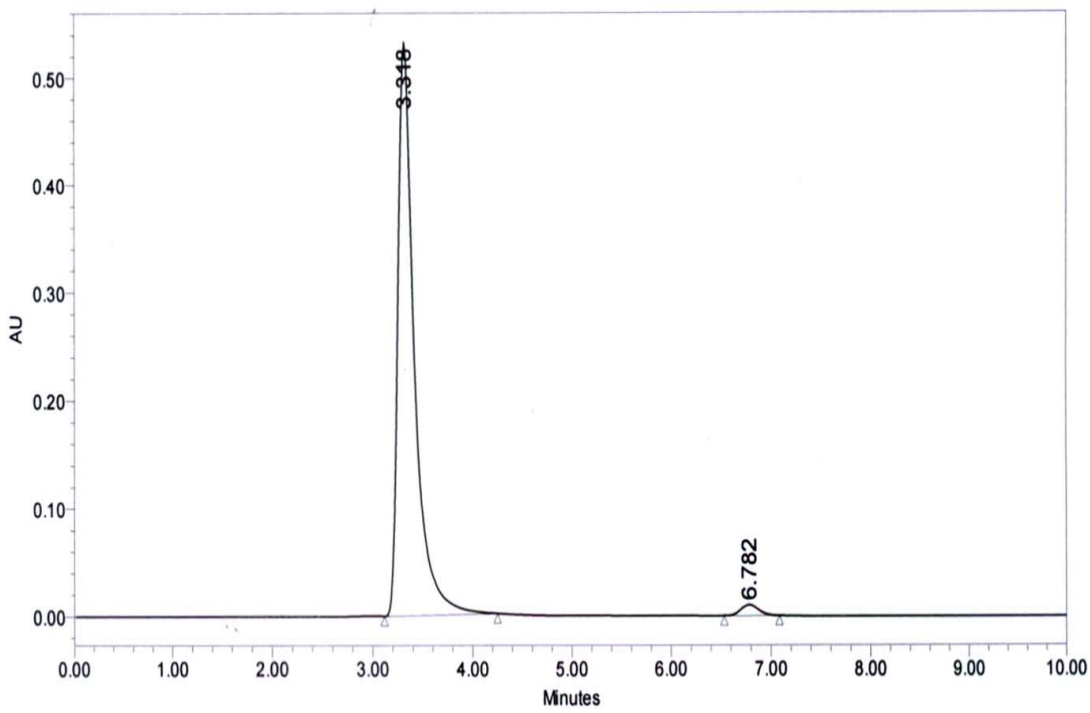
Flow rate :- 1.0 ml/min

Detection :- 300 nm

Injection volume :- 20 µl

Diluent :- Mobile phase





	RT	Area	% Area	Height	USP Plate Count	Asym
1	3.318	6041058	97.99	531948	6.463952e+002	1.119366e+003
2	6.782	124084	2.01	10067	3.531016e+003	6.049654e+003

6.6 RESULTS AND DISCUSSION

Benzofuran derivatives, Valproic acid derivatives, Quinolone derivatives, Coumarin derivatives and Indole derivatives have been analyzed on HPLC (Waters 600). Method developed for the all compound was quite, simple and rapid. Chromatography for all compounds found good with adequate sensitivity and Purity. For all compounds, Chromatographic method was carried out on Column-YMC Pack HPLC column (C-8, 150 mm x 4.6mm id x 5micron), Mobile phase - 3.2 pH Phosphate Buffer : Methanol (10 : 90), Column Temperature - ambient, Flow rate - 1.0 ml/min, Injection volume - 20 µl, Detection- 300 nm.

6.7 CONCLUSION

The Above Chromatographic data and observation during experiment suggest that the proposed analytical method is highly appropriate for the said objective, which further confirm by the purity results of newly synthesized bioactive molecules are between 94% – 100 %, which was subsequently bear out by other analytical data like NMR, Mass, Infra Red and Elemental analysis mentioned in the relevant chapter.

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PART-III

CHAPTER - 7

BIOLOGICAL ACTIVITY STUDY OF NEWLY SYNTHESIZED COMPOUNDS

7.1 MULTIDRUG RESISTANCE (MDR)

The membrane efflux protein P-glycoprotein, a member of the ATP binding cassette (ABC) family, recognizes and transports structurally, chemically, and pharmacologically diverse hydrophobic compounds, giving rise to a phenomenon known as multidrug resistance (MDR).

7. 2 NOVEL MDR MODIFIERS AND REVERSING AGENTS

7.2.1 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-7.1: 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

7.2.2 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]

7.2.3 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]

7.2.4 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]

7.3 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]

7.3.1 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.

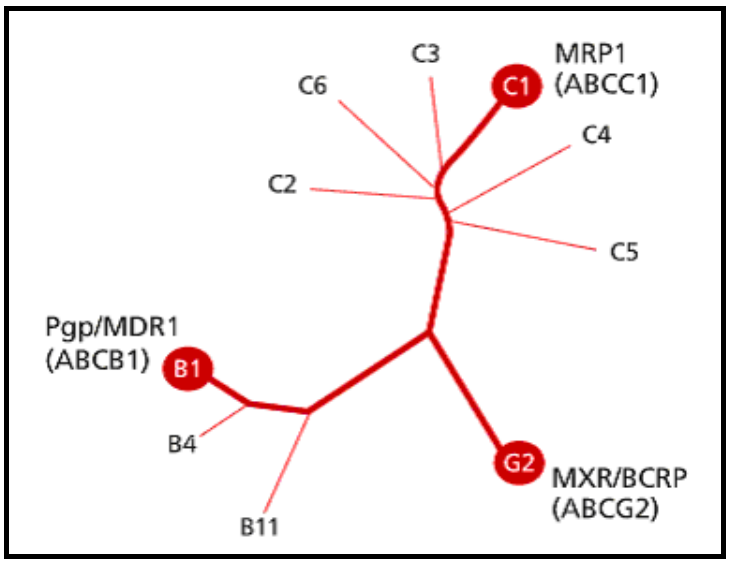


Figure-1. Phylogenic tree of the MDR- related ABC transporters. The thick lines represent the proteins definitively involved in multi-drug resistance. (Reproduced from reference-4)

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 (TMH0), 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]

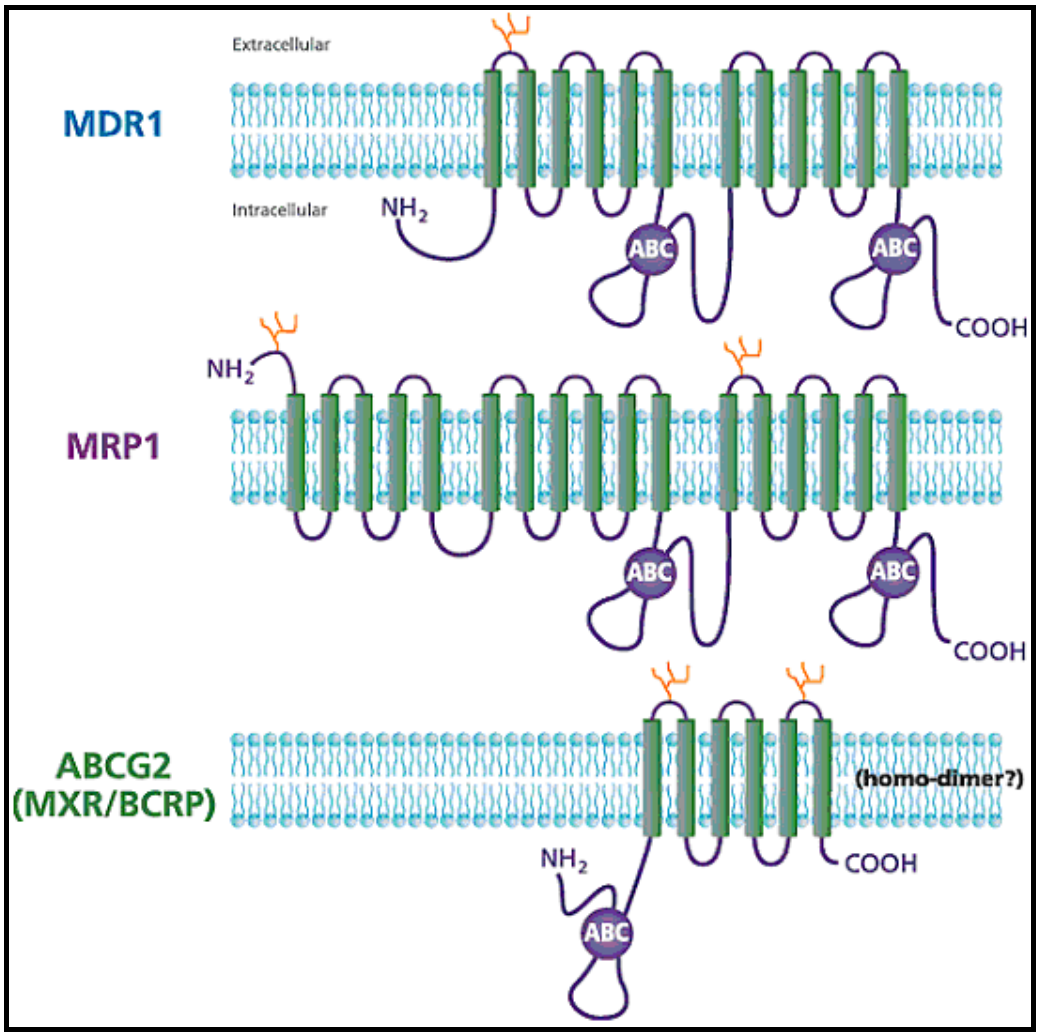


Figure-2. Membrane topology models for the MDR-related ABC transporters. Green Bars represents predicted transmembrane helices, the purple circles represents the ABC domains, the gold tree are glycosylation sites at the extra cellular surface. (Reproduced from reference-4)

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.

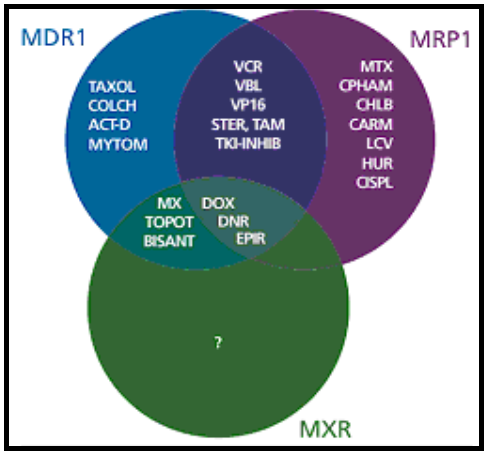
7.3.2 Substrate Specificity of MDR-ABC Transporter

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.

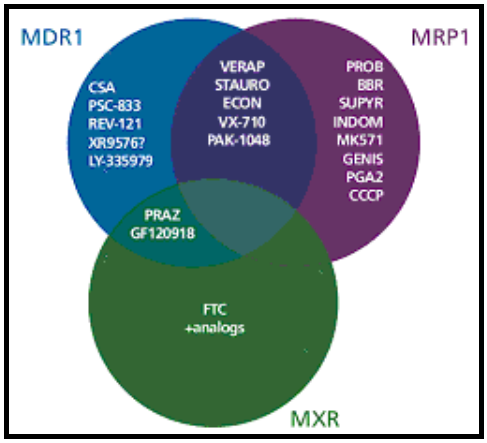
Pgp is a transporter for large hydrophobic, either uncharged or slightly positively charged compounds, while the MRP family primarily transports hydrophobic anionic conjugates and extrudes hydrophobic uncharged anticancer drugs. The MRP1-related uncharged drug transport is quite an enigma, and is somehow 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]

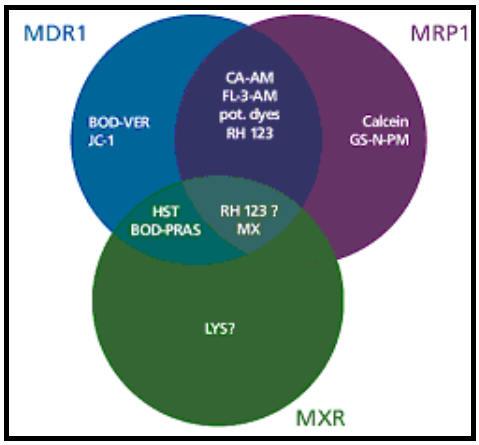
In order to put the MDR substrates in their medical and pharmacological context, we present some of the key molecules in separate figures. Figure 3A shows anticancer drugs, which are, unfortunately for the patients, also MDR substrates. Figure 3B shows the chemical MDR modulators used experimentally 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.

7.3.3 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]

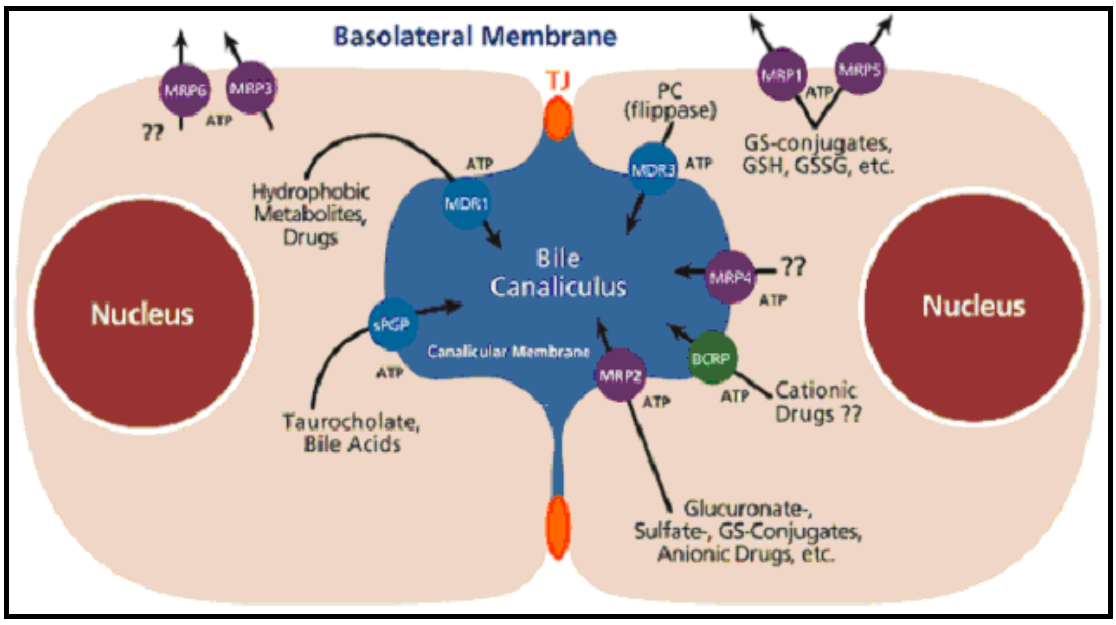


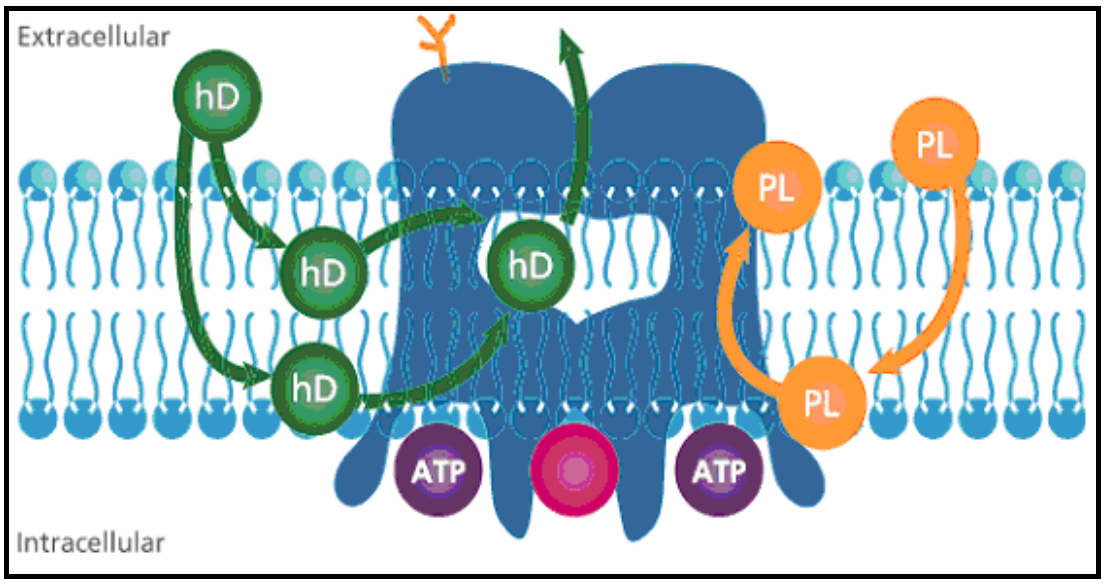
Figure-4. Multi-drug transporters in the human liver hepatocytes. Abbreviations: TJ, tight junction. (Reproduced from reference- 4)

7.3.4 Molecular Mechanism of the Multidrug Pumps

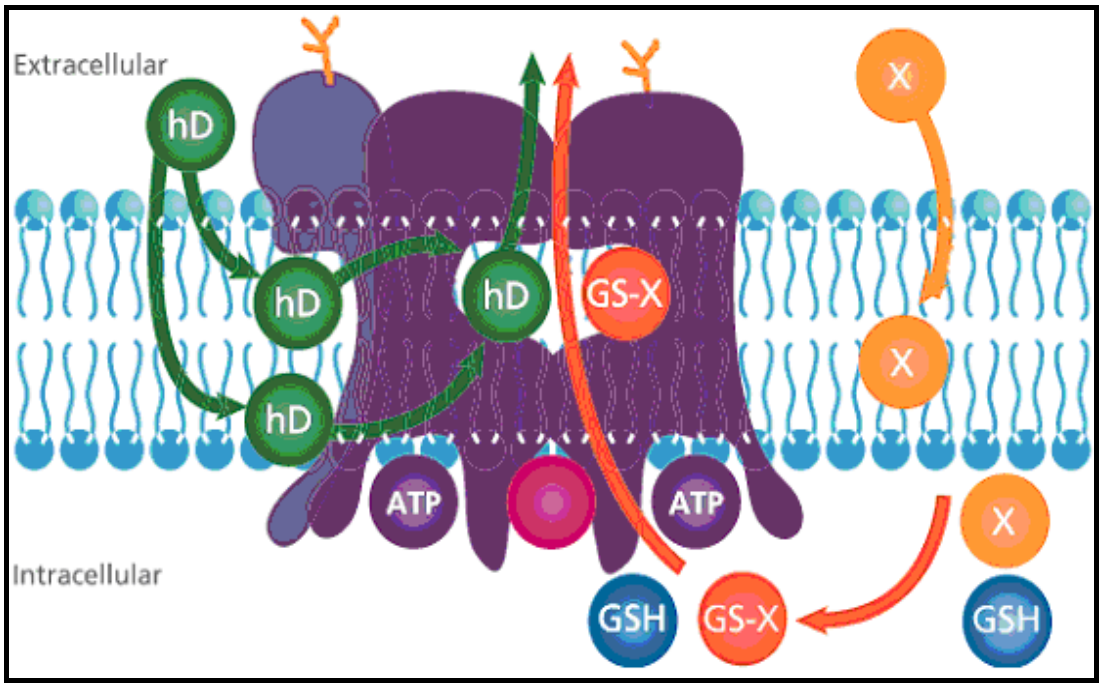
Drug transport by MDR proteins requires the energy of ATP-hydrolysis, controlled by drug interaction, and closely coupled to the actual drug translocation. Interaction with the drug-substrate significantly enhances the basal ATPase activity of the multidrug transporters, that is, the transported drug-substrates 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).

7.4 PROTOCOL FOR THE MDR SCREENING

Cell cultures

L5178Y mouse T-cell lymphoma cells (ECACC cat. no. 87111908, U.S. FDA, Silver Spring, MD, USA) were transfected with pHa MDR1/A retrovirus, as described previously.[60, 61] The MDR1-expressing cell line was selected by culturing the infected cells with 60 ng/ml colchicine to maintain the expression of the MDR phenotype. L5178Y (parental, PAR) mouse T-cell lymphoma cells and the human *mdr1*-transfected subline (MDR) were cultured at 37°C in McCoy's 5A medium supplemented with 10% heat-inactivated horse serum, L-glutamine and antibiotics. The mouse lymphoma cell lines were maintained in a 5% CO_2 atmosphere at 37°C.

A 2780cis cell line (ECACC cat. no. 93112517, Salisbury, UK) has been developed by chronic exposure of the parental cisplatin-sensitive A2780 human ovary cancer cell line to increasing concentrations of cisplatin[62,63]. This cell line was cultured in RPMI 1640 medium supplemented with 10 % heat-inactivated foetal bovine serum, L-glutamine and antibiotics. In order to retain resistance, cisplatin has to be added to the medium every 2-3 passages in 1 μM final concentration. The A2780cis cell line was maintained in a 5% CO_2 atmosphere at 37°C.

Assay for antiproliferative effect

The effects of increasing concentrations of the drugs on cell growth were tested in 96-well flat-bottomed microtitre plates in one parallel. The compounds were diluted in two-steps from a starting concentration of 50 μg/ml in a final volume of 150 μl, and DMSO was used as a control. A total of 6×10^3 cells in 50 μl of medium were then added to each well, with the exception of the medium control wells. The culture plates were further incubated at 37°C for 72 h, at the end of which 15 μl of MTT solution (thiazolyl blue solved in PBS to a final concentration of 5 mg/ml) were added to each well. After further incubation at 37°C for 4 h, 100 μl of sodium dodecyl sulphate (SDS) solution (10%) were measured into each well and the plates were further incubated at 37°C overnight. The cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with a Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Inhibition of cell growth was determined as a percentage according to the formula:

$$100 - \left[\frac{OD\,treated\,cells - OD\,medium\,control}{OD\,cell\,control - OD\,medium\,control} \times 100 \right]$$

Assay for reversal of MDR in tumor cells

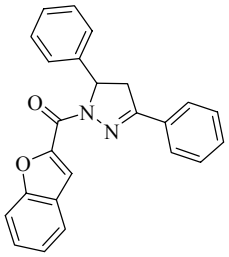
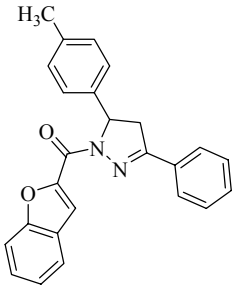
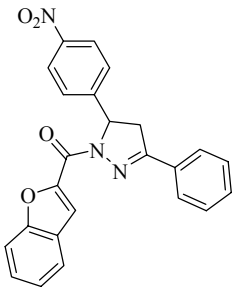
The cells were adjusted to a density of 2×10^6 cells/ml, resuspended in serum-free McCoy's 5A medium and distributed in 0.5 ml aliquots into Eppendorf centrifuge tubes. The tested compounds were added at different final concentrations (2.0 and 20 μg/ml), and the samples were incubated for 10 min at room temperature. Indicator rhodamine 123 (R123) (Sigma-Aldrich Kft, Budapest, Hungary) was added to each sample to a final concentration of 10 μg/ml and the cells were incubated for a further 20 min at 37°C, washed twice and resuspended in 0.5 ml phosphate-buffered saline (PBS) for analysis. The fluorescence of the cell population was measured with a FACStar Plus flow cytometer (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA). Verapamil (EGIS Pharmaceuticals PLC, Budapest, Hungary) was used as a positive control in the R123 exclusion experiments at final concentration of 22 μM. The percentage mean fluorescence intensity was calculated for the treated MDR/PAR and A2780cis cell lines as compared with the untreated cells. Fluorescent activity ratio (FAR) was calculated *via* the following equation, on the basis of the measured fluorescence values:

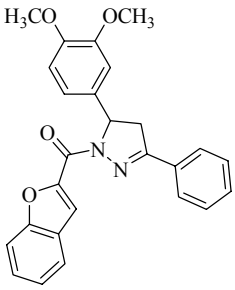
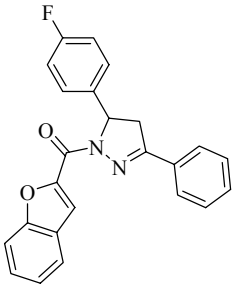
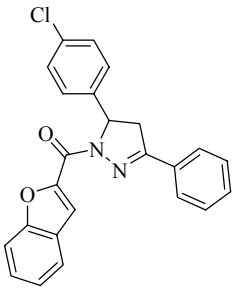
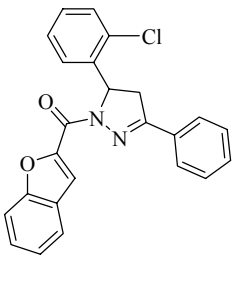
$$FAR = \frac{MDR\,treated \,/\, MDR\,control}{parental\ \ treated \,/\ \ parental\,control}$$

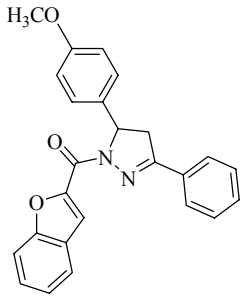
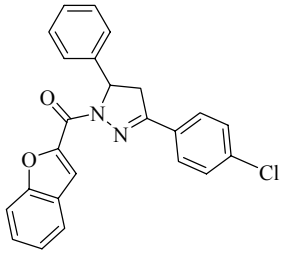
The results presented are obtained from a representative flow cytometric experiment in which 1×10^5 individual cells of the population were evaluated for the amount of R123 retained are first presented by the FACS Star Plus flow cytometer as histograms and the data were converted to FAR units that define fluorescence intensity, standard deviation, peak channel in the total and in the gated populations.

7.5 MDR REVERSAL ACTIVITY STUDY

TABLE-7.2 - FLUORESCENT ACTIVITIES OF NEWLY SYNTHESIZED COMPOUNDS

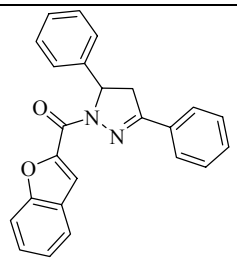
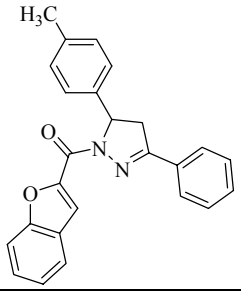
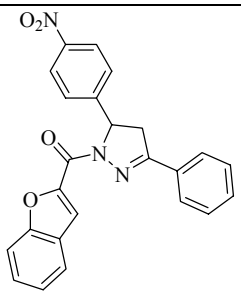
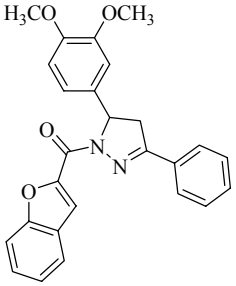
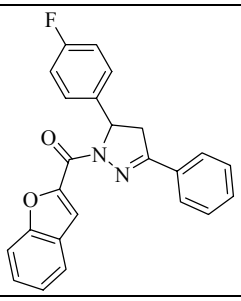
		Samples	final cc.	dye	FSC	SSC	FL-1	FAR *	Peak Ch
1		PAR	-	R123	477,39	162,62	1039,43	-	1027
2		PAR	-	R123	471,28	165,01	1029,18	-	1197
3		MDR	-	R123	521,70	190,17	40,75	-	40
		MDR mean			493,72	R123	**32,34**	-	30
4		Verapamil 1 mg/ml (2,2 mM)	5,2 µM	R123	516,66	188,56	236,90	7.33	149
5		SBF-01 (0.5mg/ml)	2 µg/ml	R123	522,30	194,59	352,14	10.89	289
6			20 µg/ml	R123	521,94	194,16	608,08	18.80	557
7		SBF-02 (0.5mg/ml)	2 µg/ml	R123	504,28	187,59	147,16	4.55	79
8			20 µg/ml	R123	518,36	198,85	245,41	7.59	201
9		SBF-03 (0.5mg/ml)	2 µg/ml	R123	506,78	186,23	536,56	16.59	429
10			20 µg/ml	R123	511,44	153,98	491,02	15.18	414

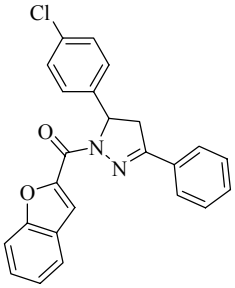
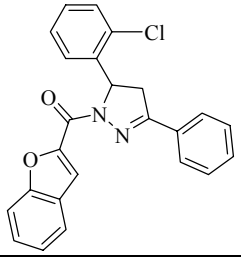
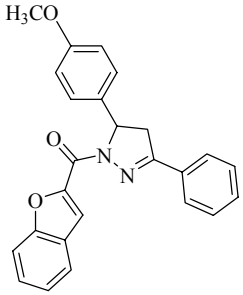
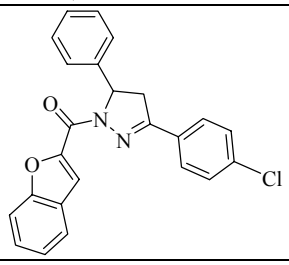
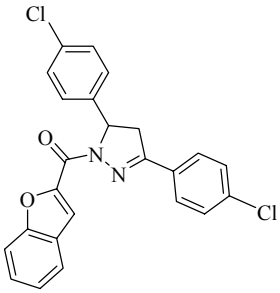
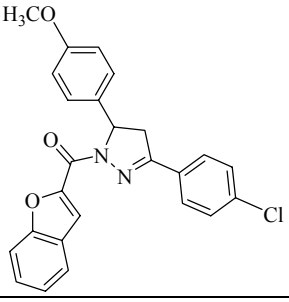
11		SBF-04 (0.5mg/ml)	2 µg/ml	R123	503,27	186,01	867,73	26.83	749
12			20 µg/ml	R123	506,21	169,73	934,62	29.90	842
13		SBF-05 (0.5mg/ml)	2 µg/ml	R123	495,45	187,53	432,99	13.39	358
14			20 µg/ml	R123	497,24	192,26	648,69	20.06	609
15		SBF-06 (0.5 g/ml)	2 µg/ml	R123	494,27	189,19	225,53	6.97	156
16			20 µg/ml	R123	500,82	203,13	269,80	8.34	187
17		SBF-07 (0.5mg/ml)	2 µg/ml	R123	468,48	179,83	469,62	14.51	417
18			20 µg/ml	R123	494,92	148,31	520,81	16.10	406

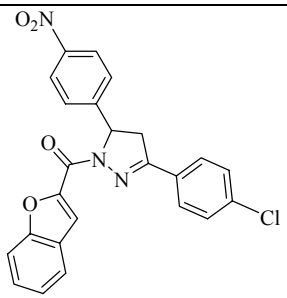
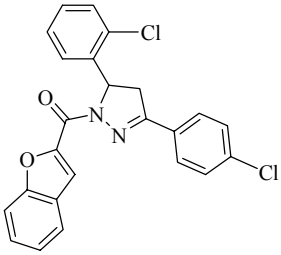
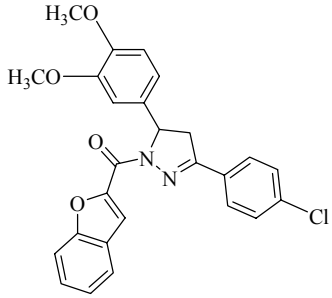
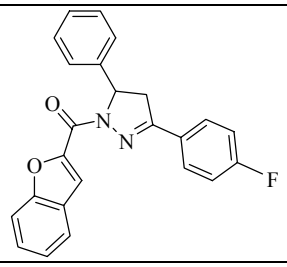
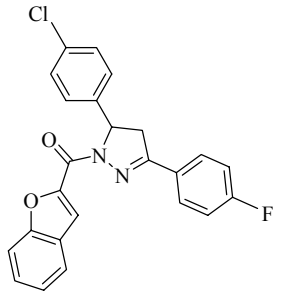
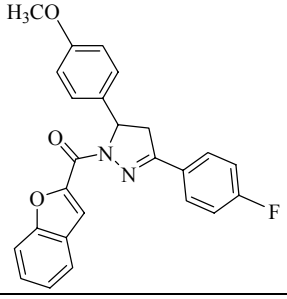
19		SBF-08 (0.5mg/ml)	2 µg/ml	R123	480,18	179,57	770,70	23.83	743
20			20 µg/ml	R123	487,55	169,41	865,82	26.77	784
21		SBF-11 (0.5mg/ml)	2 µg/ml	R123	471,87	183,87	192,83	5.96	194
22			20 µg/ml	R123	475,43	200,91	218,84	6.77	268
23		DMSO	4%	R123	468,46	176,40	22,18	0.69	19
24		MDR	-	R123	465,74	177,60	23,92	-	20

FAR* : Fluorescence Activity Ratio

TABLE-7.3 - IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-2

COMPOUND CODE	STRUCTURE	IC_{50}
SBF-01		7.58
SBF-02		16.602
SBF-03		4.866
SBF-04		6.888
SBF-05		9.936

SBF-06		5.134
SBF-07		6.219
SBF-08		4.569
SBF-11		13.632
SBF-12		8.496
SBF-13		5.743

SBF-14		34.125
SBF-15		44.79
SBF-16		6.29
SBF-21		11.765
SBF-22		>50
SBF-23		22.997

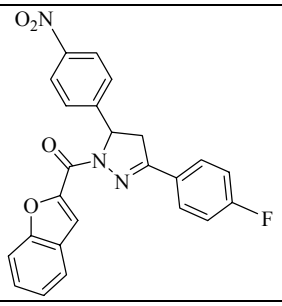
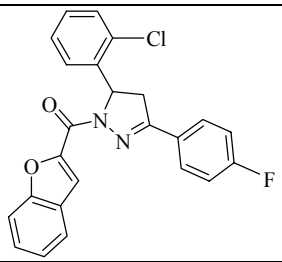
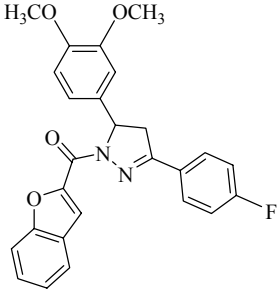
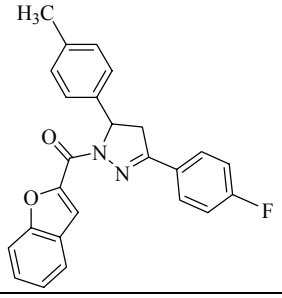
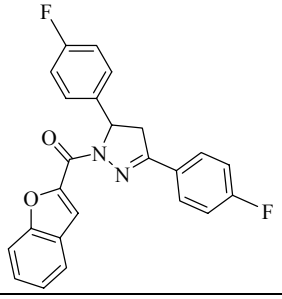
SBF-24		>50
SBF-25		45.193
SBF-26		>50
SBF-27		>50
SBF-28		>50

TABLE-7.4 – IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-3 (SECTION-A)

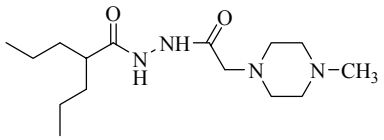
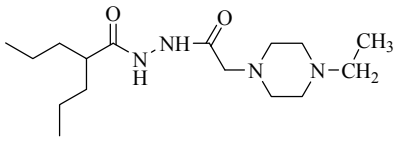
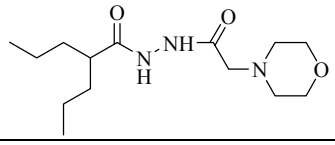
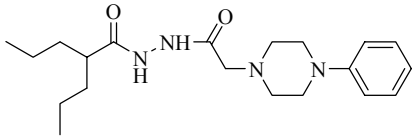
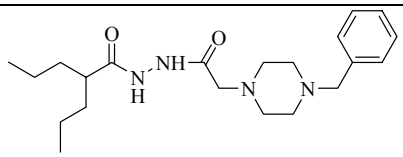
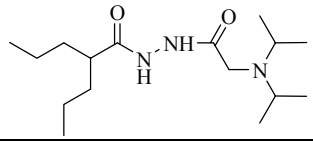
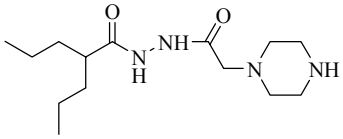
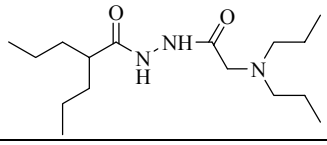
COMPOUND CODE	STRUCTURE	IC_{50}
SPSC-01		>50
SPSC-02		>50
SPSC-03		>50
SPSC-04		>50
SPSC-05		>50
SPSC-06		>50
SPSC-07		>50
SPSC-08		>50

TABLE-7.5 – IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-3 (SECTION-B)

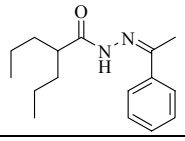
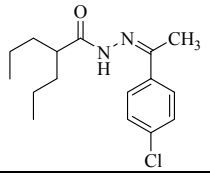
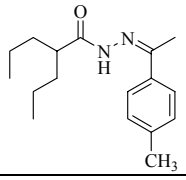
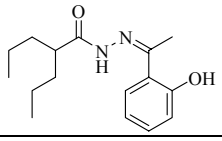
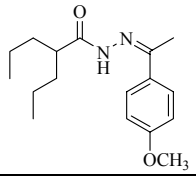
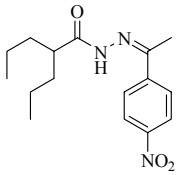
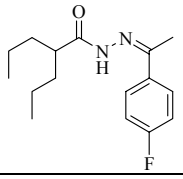
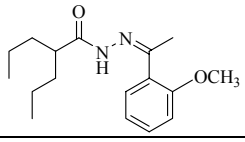
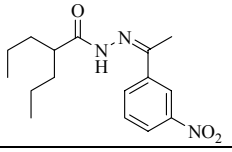
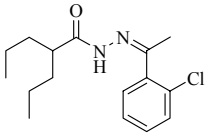
COMPOUND CODE	STRUCTURE	IC_{50}
SPA-01		>50
SPA-02		>50
SPA-03		1.569
SPA-04		2.077
SPA-05		3.792
SPA-06		30.981
SPA-07		>50
SPA-08		21.222
SPA-09		1.83
SPA-10		>50

TABLE-7.6 – IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-3 (SECTION-C)

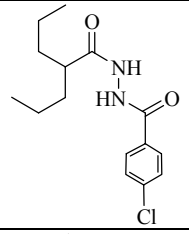
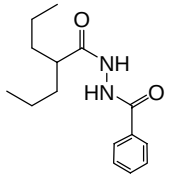
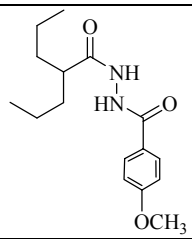
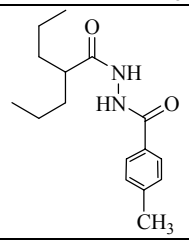
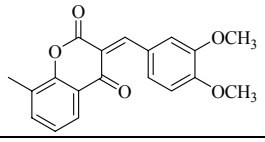
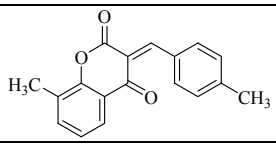
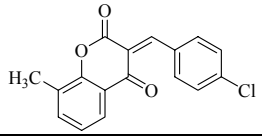
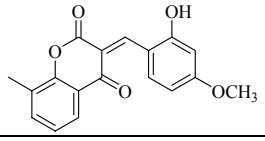
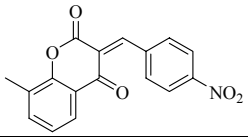
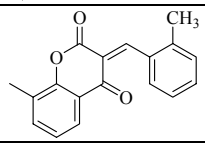
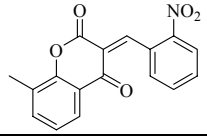
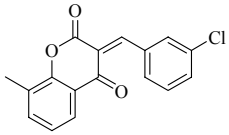
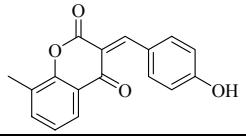
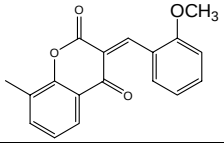
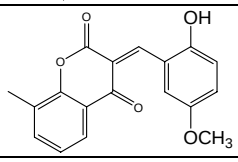
COMPOUND CODE	STRUCTURE	IC_{50}
SPAM-01		>50
SPAM-02		>50
SPAM-03		>50
SPAM-04		12.123

TABLE-7.7 – IC_{50} VALUE OF NEWLY SYNTHESIZED COMPOUNDS CHAPTER-4 (SECTION-B)

COMPOUND CODE	STRUCTURE	IC_{50}
SPC-01		>50
SPC-02		>50
SPC-03		12.881
SPC-04		17.754
SPC-05		>50
SPC-06		8.307
SPC-07		35.577
SPC-08		30.679
SPC-09		>50
SPC-10		22.535
SPC-12		5.353

7.6 RESULTS AND 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.[64,65] Herein fluorescence activities shows that SBF-02, SBF-06 and SBF-11 from the synthesized compounds shown to be moderately active. This means that the above stated compounds were moderately effective in reversal of MDR efflux pump activity and rests of the compounds are poorly active.

While antiproliferative activities in terms of IC_{50} shows that molecules like SBF-03, SBF-08, SPA-03, SPA-04, SPA-05, SPA-09 among the synthesized compounds seems to be moderately active and molecules like SBF-01, SBF-02, SBF-04, SBF-05, SBF-06, SBF-07, SBF-11, SBF-12, SBF-13, SBF-14, SBF-15, SBF-16, SBF-21, SBF-23, SBF-25, SPA-06, SPA-08, SPAM-04, SPC-03, SPC-04, SPC-06, SPC-07, SPC-08, SPC-10 and SPC-12 seems to be poorly active.

7.7 CONCLUSION

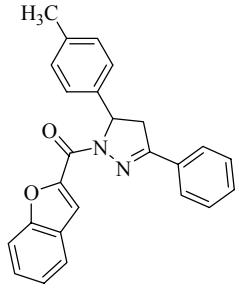
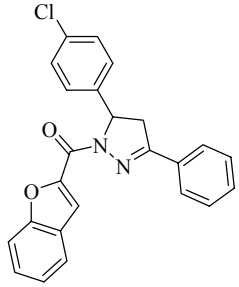
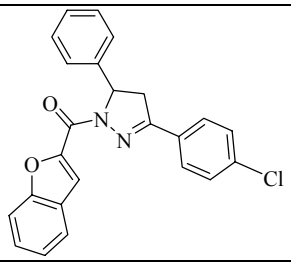
Among the synthesize compounds, three compounds which are benzofuran analogous have shown good MDR reversal activity against cancer cell lines described in the protocol.

While atleast six compounds have shown good antiproliferative activity where in SBF-03 and SBF-08 are benzofuran analogues, while the others SPA-03, SPA-04, SPA-05 and SPA-09 are novel valproic acid analogues first time reported for such activity.

Please refer Table **7.8** and Table **7.9**.

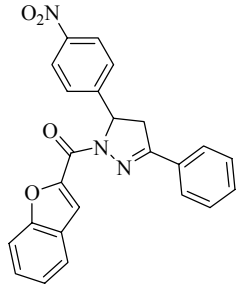
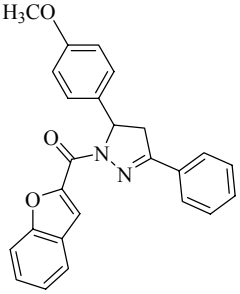
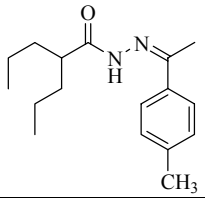
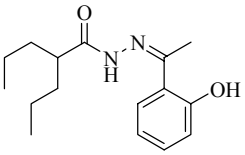
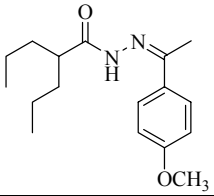
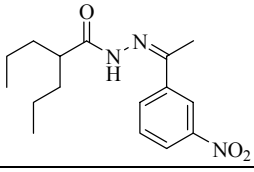
Thus, **Following molecules are identified for MDR reversal activity.**

TABLE-7.8

Sr. No.	Series	Code	Structure
1	SBF	SBF-02	
2	SBF	SBF-06	
3	SPA	SBF-11	

Following molecules are showing good antiproliferative activity.

TABLE-7.9

Sr. No.	Series	Code	Structure
1	SBF	SBF-03	
2	SBF	SBF-08	
3	SPA	SPA-03	
4	SPA	SPA-04	
5	SPA	SPA-05	
6	SPA	SPA-09	

Pyrazole bearing Benzofuran derivatives and hydrazides from valproic acid are good ‘scaffold’ for antiproliferative activity.

On the basis of the above interesting results, new synthetic programmes can be develop to more active molecules.

7.8 REFERENCES

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SUMMARY

The work represented in the thesis entitled ***"Synthesis and Characterization of Pharmacologically Active compounds"*** is divided into seven chapters which can be summarized as under.

Chapter-1 covers an introduction to Medicinal Chemistry depicting drug discovery Pathways and brief history of evolution of heterocycles as drug molecules.

Chapter-2 deals with the introduction of oxygen heterocycle – Benzofuran-2-Carboxylic acid ester and various chalcones. It deals with synthesis of 24 new molecules derived from Benzofuran and chalcones to arrive at variously functionalized pyrazoles. The chapter covers Reaction mechanism, IR, ^{1}H NMR, Mass spectral and other Physical data to support the structure elucidation.

Chapter-3 Section – A is an effort to modify known antiepileptic drug Valproic acid to convert into various analogs, in order to explore other biological activities. Total 12 compounds are synthesized.

In Section – B, reactions between Valproic acid hydrazide and substituted acetophenone afforded to give 10 novel derivatives. The microwave assisted methods was used.

In Section – C, The condensation products are obtained having important amide linkage between Valproic acid and aroyl nucleus. Another 10 compounds are obtained in this section.

All compounds are characterized by spectral and elemental analysis.

Chapter-4 is subdivided into two sections.

In Section – A is preparation of several pyrazole bearing arylidine derivatives appended at N-methyl 4-hydroxy 2-quinolone.

In Section – B is again novel arylidine derivatives obtained from 8-methyl-4-hydroxy coumarin and various aldehydes.

The synthesis was carried out using microwave assisted as well as conventional method. Total 26 compounds were synthesized and characterized by IR, ^{1}H NMR,

Mass spectra and elemental analysis.

Chapter-5 Section – A deals with hybridized analogs of known drug Aripiprazole. The core structure motif was utilized to obtain 12 molecules which were adequately characterized.

In Section – B, twelve new Mannich base were prepared using secondary amines and indole-2-carboxylic acid. The structural elucidations of all compounds were carried out.

Chapter-6 is an effort to develop analytical method for purity check of newly synthesized compounds, mentioned in the five chapters earlier. In all sixteen HPLC assay determination are given along with the experimental condition and protocols.

Chapter-7 is revolved to the biological activity. The MDR reverting activity against resistant cancer cell lines was carried out and the anti proliferative study was also done on several newly synthesized molecules.

In all 55 compounds were screened. three new compounds were shown to the posses moderate MDR reversal activity, while six new compounds are showing good anti proliferative activity.

In entire thesis, total 106 compounds were prepared and more than half of them are screened. The remaining compounds are also under study.

CONFERENCES/SEMINARS/WORKSHOPS ATTENDED

- ISCB Conference ***"International conference on chemical biology for discovery: perspectives and Challenges"*** at CDRI, Lucknow, 15-18 Jan., 2010.
- ***"International Seminar on Recent Developments in Structure and Ligandbased Drug Design"*** jointly organized by Schrodinger LLC, USA; National Facility for Drug Discovery through New Chemicals Entities Development & Instrumentation support to Small Manufacturing Pharma Enterprises and DST FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot, dated December, 23rd, 2009.
- ***"National seminar on Alternative Synthetic Strategies for Drugs & Drug Intermediates"*** at Institute of Pharmacy, Nirma University, Ahmedabad on 13th November, 2009.
- ***"Two Days National Workshop on Patents & Intellectual Property RightsRelated Updates"*** Sponsored by TIFAC & GUJCOST and Organized by DST-FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry,Saurashtra University, Rajkot, dated September, 19-20, 2009.
- DST-FIST, UGC (SAP) supported and GUJCOST Sponsored ***"National Conference on Selected Topics in Spectroscopy and Stereochemistry"*** organized by the Department of Chemistry, Saurashtra University, Rajkot, dated March, 18-20, 2009.
- ***"A National Workshop On Updates In Process and Medicinal Chemistry"*** jointly organized by National Facility for Drug Discovery through NewChemicals Entities Development & Instrumentation support to SmallManufacturing Pharma Enterprises and DST FIST, UGC-SAP & DST-DPRP Funded Department of Chemistry, Saurashtra University, Rajkot dated March, 3-4, 2009.
- DST-FIST, UGC (SAP) supported and GUJCOST Sponsored ***"National Workshop on Management and Use of Chemistry Database and Patent Literature"*** organized by GUJCOST & Dept. of Chemistry of Saurashtra University, Rajkot, (Gujarat), dated February, 27-29, 2008.

Paper/Poster presented at the International Conference:

- ***"synthesis and anticoagulant activity of dimeric 4-hydroxycoumarins having indole and chromone as central linkers"***

  Shrey Parekh and Shailesh Thakrar, ***Anamik Shah*.***

  Poster Presented at 14th ISCB International conference on chemical biology for discovery: perspectives and Challenges, CDRI, Lucknow, 15-18 Jan., 2010.