STUDIES ON SOME BIOACTIVE HETEROCYCLIC MOIETIES

A
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IN
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BY

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

The work included in the thesis is my own work under the supervision of Prof. Anamik Shah and leads to some contribution in the field of synthetic Chemistry and is supported by recent references.

Date: 18th September 2006

Place: Rajkot **Kuldip D. Upadhyay**

CERTIFICATE

This is to certify that present work submitted for the Ph.D. Degree of Saurashtra University by Mr. Kuldip D. Upadhyay has been the result of work carried out under my supervision and is a good contribution in field of Chemistry of "Triazolotriazinoindoles, 1,5-Benzothiazepines, Pyrano quinolones, Pyrroles, Styryl coumarins" and related heterocycles with a special emphasis on biological activities.

Date: 18th September 2006

Place: Rajkot

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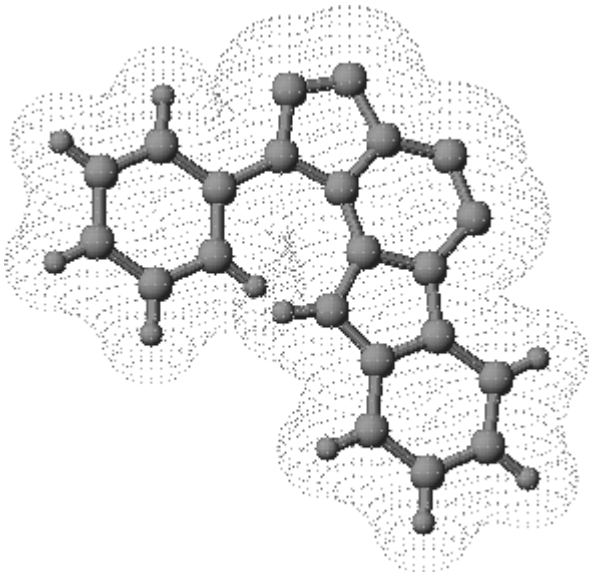
CONTENTS

CHAPTER-1

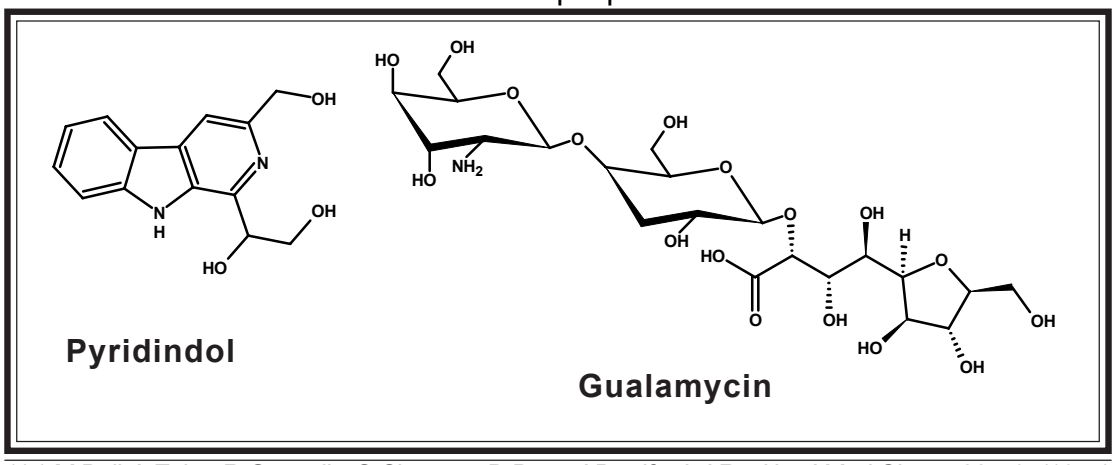


Synthesis and Characterization of Some Novel 1-(Un)substituted phenyl-10H-[1,2,4]-triazolo [3',4':3,4][1,2,4]triazino[5,6-b]indoles

Introduction

Azoles and azines are well known for their diversed biological activities. It is also known that fusion of the heterocyclic nuclei enhances the pharmacological activities more than its parent nucleus. The importance of the indole nucleus is well established in pharmaceutical chemistry as it's corresponding derivatives are used as antipyretic, anticonvulsant, antianalgesic and antidepresant agents[1].

A few naturally occurring members of this class of compounds, pyridindolol [2,3] and the two antibiotics CV-1[4] and gualamycin[5-7], have been isolated from different species of Streptomyces. Valuable biological activities are known to be associated with the 1,2,4-triazolo[4',3':2,3]-1,2,4-triazino[5,6-b]indole skeleton. 3-hydrazino-1,2,4-triazino[5,6-b]indoles show antihypertensive[8,9], antiviral[8], blood-platelet aggregation inhibitory[9,10], analgesic[11], and antibacterial activities[12]. Hydrazone derivatives of 3-hydrazino-1,2,4-triazino[5,6-b]indoles have been found to possess antiumor activity against P388 lymphocytic leukemia in mice[13] and antibacterial activity[14,15]. In addition, 1,2,4-triazolo[3',4':3,4]- and -[4',3':2,3]-1,2,4-triazino[5,6-b]indoles exhibit antiviral[16-19] and antibacterial properties[20].



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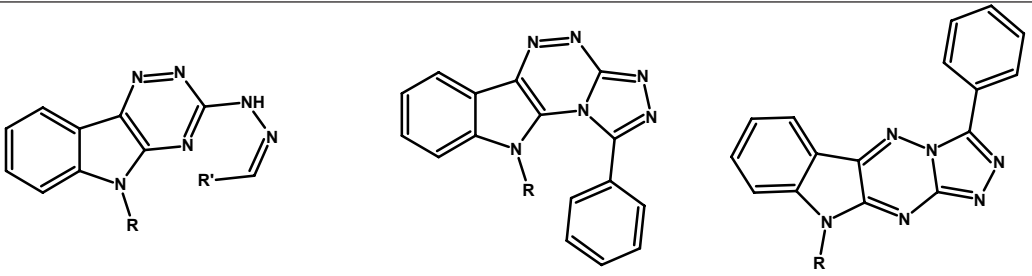
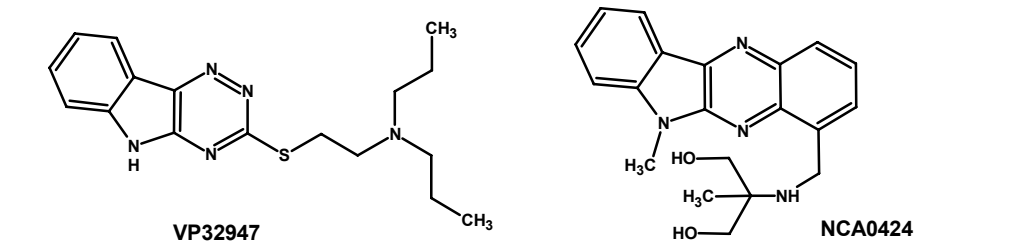


Polycyclic fused heteroaromatics of functionalized isatin derivatives are of interest because of their potential biological activity in analogy to the antiviral drug VP32947[21] and the DNA-intercalating drug/DNA topoisomerase II inhibitor NCA0424[22].



Polycyclic fused heteroaromatics with established biological activies

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The triazino[5,6-b]indole derivatives have aroused considerable interest as a result of their broad spectrum of antibacterial, antifungal and anti-parasitic activities[23]. The majority of the 5H-as-triazino[5,6-b]indoles are active *in vitro* against a variety of viruses including several strains of rhinovirus[24]. Since they are purine analogues, it is expected that they should exert their pharmacological effects by interfering with DNA metabolism.

Pharmacology

Triazino[5,6-b]indole derivatives are Purine analogues and exhibit diversed pharmacological activities. They have been described to have activity against *A.niger* and when provided as seed dressing, they protected sorghum seeds from infection with this organism[25]. The modes of action of these drugs are not known. Some triazinoindoles have antiviral properties. SK&F 30097 was active against picornavirus *in vitro* and RNA synthesis was inhibited[26], while SK&F 21681 had activity against herpes simplex virus, in which DNA synthesis was suppressed [27]. Triazinoindoles were also shown to have antihypertensive properties [28] and inhibit thromboxane synthetase in rats. Some triazinoindoles are also mutagenic[29]. The literature survey reviewed on pharmacological profile of Triazino[5,6-b]indole- and-Triazolotriazino[5,6-b] derivatives highlighed the following classification of the major therapeutic activity as per mention below.

❖ Antiviral activity ❖ Antifungal Activity ❖ Anticancer Activity

❖ Antimalarial activity

❖ Antihypertensive and Platelet aggregation Inhibitors / Thromboxine synthetase Inhibitors

❖ Antibacterial activity

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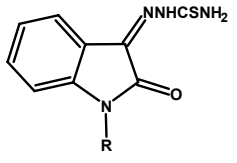
❖ Antiviral activity

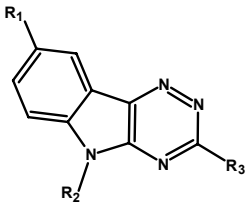
The antiviral activity of as-triazines[30] and clinical efficacy of methisazone[31] were well documented. The action of isatin-3-thiosemicarbazone is known to have antiviral activity against certain poxviruses[32,33]. The antiviral spectrum of 1-methylisatin 3-thiosemicarbazone, which reportedly extends to adenoviruses, may be rather broader but is still limited to several groups of DNA viruses[33,34]. It was therefore an unexpected finding that these compounds are also active against certain rhinoviruses. J.M.Z.Gladych[35] and co-workers have studied Isatin thiosemicarbazone analogues among which two compounds were found to have activity against all rhinovirus strains used so far. Human rhinovirus (HRV) is a primary cause of mild upper respiratory infection in humans[36]. Although rhinovirus infection, as the "common cold" is known to spread easily throughout human populations, only higher primates are susceptible [37,38]; thus, the virus possesses a narrow host range. This narrow host range is reflected in tissue culture in that the HRV replicates only in cells of primate origin.

In vitro testing of the 5H-triazino[5,6-b]indoles shown a broad spectrum antiviral activity against both DNA and RNA viruses. A notable feature of the series is the wide spread action against several strains of rhinovirus which is most consistently shown by compounds containing hydroxyalkylamino substituents at C-3.



Isatin-3-thiosemicarbozones

R = CH_3 Methisazone or (1-methylisatin-3-thiosemicarbazone)



5H-as-triazino[5,6-b]indoles

R_1 = H,Cl, NO_2, Me, Pr, NO, OMe R_2 = H, Me, $C_6H_5(CH_2)_2$, $C_6H_5CH_2$ R_3 = $OH(CH_2)_2NH$, $OH(CH_2)_3NH$, NH_2, Me_2N, Morphonyl, $Me_2CH(CH_2)_2NH$, $Me(CH_2)_{11}NH$, $CH_3(CH_2)_{17}NH$, $OHCH_2CH(OH)CH_2NH$, $OH(CH_2)_4NH$, $MeO(CH_2)_3NH$, $OH(CH_2)_2NH(CH_2)_2NH$, $Me_2N(CH_2)_3NH$, $OH(CH_2)_6NH$ etc.

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The search for effective antirhinovirus agents has yielded a wealth of compounds belonging to widely varying chemical classes: thiosemicarbazones[35], isoquinolines [39,40], triazinoindoles[41], guanidines[42], benzoates[43], furanyls[44], benzimidazoles[45], thiourea[46], diketones[47,48,49], flavans[50,51], flavones[52], chalcones[53], nitrobenzenes[54,55], and isoxazoles[56,57]. The most recently developed antirhinovirus compounds[50, 56, 53, 57] reach their MICs within the range of 0.001 to 0.01 μg/ml.

A wide variety of purine nucleoside (mainly tubercidin and adenosine) analogs, which had previously been shown to inhibit the replication of a broad spectrum of RNA viruses, were evaluated for their antirhinovirus activity in human diploid (WI-38) fibroblasts[58]. Among the 5- or 6-substituted tubercidin analogs, several derivatives, i.e., tubercidin, sangivamycin, toyocamycin, 5-(1-hydroxyethyl)tubercidin, and 5-(2-buten-1-yl)tubercidin (the later being a mixture of E and Z isomers at a 2:1 ratio)(Figure-1), inhibited the cytopathogenicity of rhinovirus types 1A, 1B and 9 at concentrations well below 1μg/ml, the most potent being tubercidin itself with an MIC_{50} of 0.03 μg/ml. Of the sugar-modified tubercidin analogs, only 2'-deoxytoyocamycin exhibited an MIC_{50} of <1 μg/ml (against rhinovirus types 1B and 9). Of the carbocyclic adenosine analogs, carbocyclic 7-deazaadenosine proved the most potent, with an MIC_{50} of 2 μg/ml (against rhinovirus types 1B and 9). Acyclic adenosine analogs were totally ineffective as antirhinovirus agents, and from the miscellaneous reference compounds formycin A, pyrazofurin, and 3-deazaguanine showed the lowest MIC_{50}s (within the range of 5 to 25 μg/ml).

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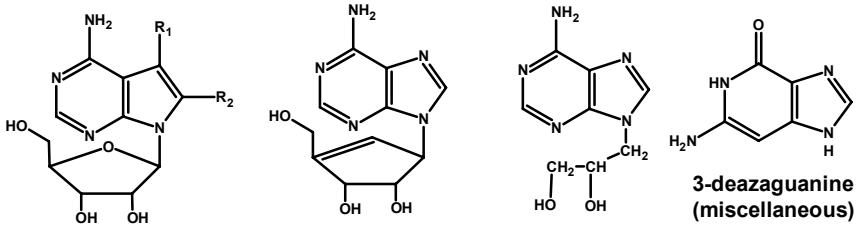
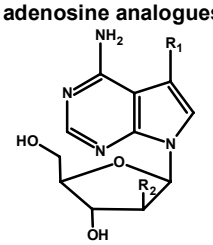
Despite their higher potency as antirhinovirus compounds, tubercidin, 5-(1-hydroxyethyl)tubercidin, 5-(2-buten-1-yl)tubercidin, sangivamycin, and toyocamycin did not display much selectivity in their antirhinovirus action. Their selectivity indexes fell in the range of 3 to 30 (index A) and 1.3 to 4 (index B). Of the sugar-modified tubercidin analogs, only the *ara* analog of sangivamycin showed some selectivity (index A = 8.6; index B = 3.1). Of the carbocyclic and acyclic adenosine analogs, none proved to be a selective inhibitor of rhinovirus; and of the miscellaneous group of compounds, 3-deazaguanine showed the highest selectivity (index A = 50; index B = 33).



R_1	R_2	Compound Name
H	H	Tubercidin (7-deazaadenosine)
$CH(OH)CH_3$	H	5-(1-hydroxyethyl)tubercidin
$CHOCH_2CH_3$	H	5-(1-methoxyethyl)tubercidin
$CH_2CH=CHCH_3$	H	5-(2-buten-1-yl)tubercidin
$CONH_2$	H	Sangivamycin
CN	H	Toyocamycin
H	Br	6-Bromotubercidin



Sugar modified tubercidin analogues R_1 = H, CN,$CONH_2$ and R_2=H (2'deoxy) or OH(*ara*)

Fig-1. Structural formulas of selected purine nucleoside analogues.

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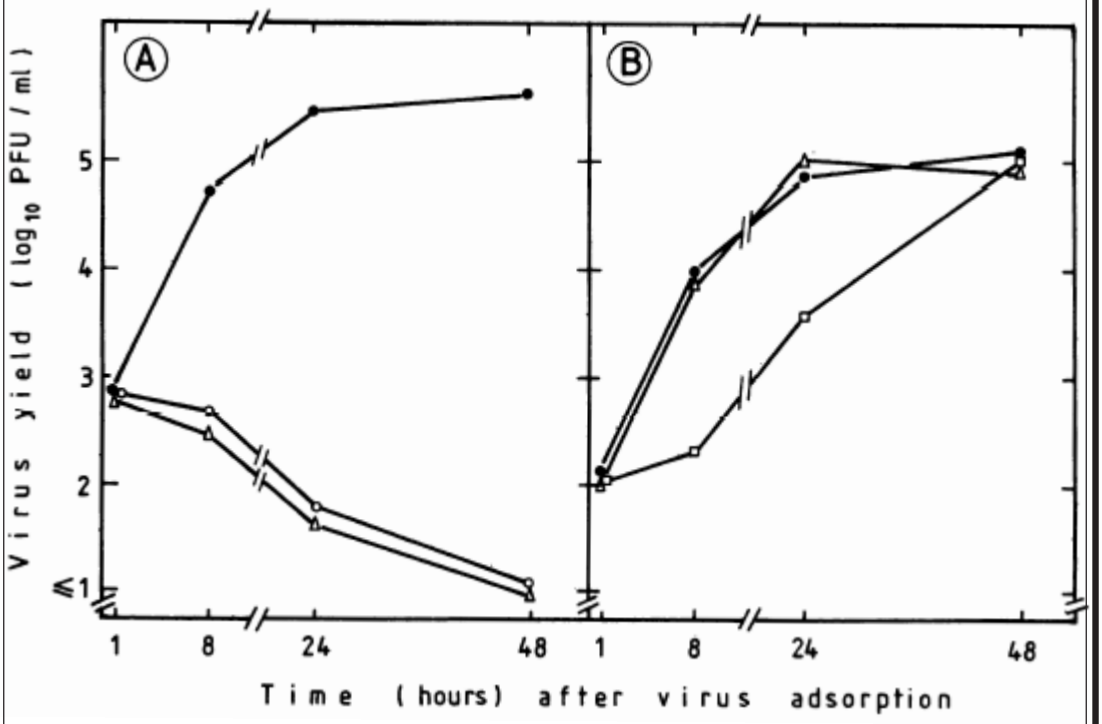

FIG. 2. Effects of 5-(2-buten-1-yl)tubercidin (A) and 3-deazaguanine (B) on the replication of rhinovirus type 1A in WI-38 cells.Concentration of the compounds: 100 ì g/ml (□), 10ì g/ml (△) or control (●)

With the compounds that proved most potent or selective or both in inhibiting rhinovirus-induced cytopathogenicity, i.e., 5-(2-buten-1-yl)tubercidin, 5-(1-hydroxyethyl)tubercidin, sangivamycin, and 3-deazaguanine, further experiments were undertaken to determine their inhibitory effects on the production of rhinovirus type 1A progeny (yield). 5-(2-Buten-1-yl)tubercidin effected a 3 to 4 $\log_{10}$ reduction in virus yield at a concentration as low as 1ì g/ml (Fig. 2). 3-Deazaguanine achieved a 1.5 to 2 $\log_{10}$ reduction in virus yield, but only if added at a concentration of 100 ì g/ml and in contrast with the virus yield reductions obtained with 5-(2-buten-1-yl)tubercidin (Fig. 2A) and the two other compounds [5-(1-hydroxyethyl)tubercidin and sangivamycin] the virus yield reduction achieved by 3-deazaguanine (Fig. 2B) was only transient. The reduction of rhinovirus type 1A yield by 5-(1-hydroxyethyl)tubercidin, 5-(2-buten-1-yl)tubercidin, sangivamycin, and 3-deazaguanine may be interpreted to mean that their inhibitory effects on rhinovirus cytopathogenicity probably result from an inhibition of virus replication.

Isatin-3-thiosemicarbazone exhibits antiviral activity, mainly against poxviruse[59,60]. A group of 3-substituted triazinoindoles, which are structurally related to isatin thiosemicarbazone, inhibit the growth of several rhinoviruses [35,17]. Ehud Katz et al[27]suggested SK&F 21681 (3,10-dimethyl-10-H-s-triazolo[4',3':2,3]-astriazino-[5,6-b]indole) is an inhibitor of the growth of herpes simplex viruses types 1 and 2 at a concentration of 60ì g/ml without causing any morphological alterations in BSC1 cells. The DNA synthesis rate of BSC1 affected by 16% between 0.5 to 7 hrs and this inhibition increased upto 33% between 2 to 22 h.p.i.

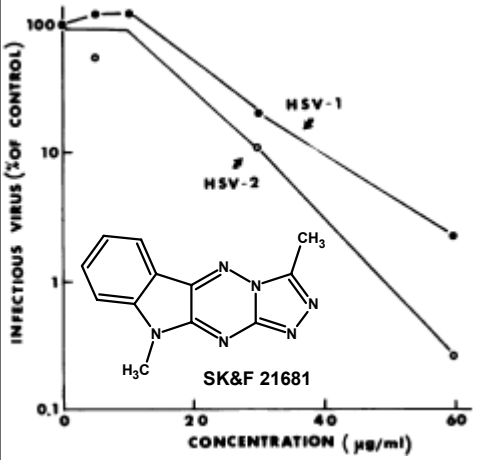

FIG. 3. Growth of HSV-1 and HSV-2 in the presence of different concentrations of SK&F 21681. BSC1 cells were infected with the viruses at a multiplicity of infection of 1 PFU per cell. After adsorption, maintenance medium containing different concentrations of SK&F 21681 was added. The cultures were harvested at 22 h p.i., and virus titers were determined.

TABLE 1. Effects of removal and addition of SK&F 21681 upon HSV-infected cells at different intervals p.i.[a]

Virus	Removal (h)	Addition (h)	Virus titer PFU/ml	Virus titer %
HSV-1	2		7.7×10^7	79.3
	4		1.0×10^8	106.9
	7.5		3.0×10^7	31.0
		2	1.9×10^6	1.9
		4	5.7×10^6	5.9
		7.5	7.7×10^6	7.9
	Not added		9.7×10^7	100.0
	Not removed		2.7×10^6	2.8
HSV-2	2		9.7×10^6	67.4
	4		8.0×10^6	55.8
	7.5		1.1×10^6	7.7
		2	6.7×10^4	0.5
		4	2.2×10^5	1.5
		7.5	2.0×10^6	14.0
	Not added		1.4×10^7	100.0
	Not removed		8.0×10^4	0.6

[a] To BSC1 cells infected with HSV-1 and HSV-2 at a multiplicity of infection of 1 PFU per cell, medium containing SK&F 21681 (60ug/ml) was added or removed at different time intervals p.i. The cultures were harvested at 22 h p.i., and virus titers were determined.

The growth of HSV-1 and HSV-2 in BSC1 cells was followed in the presence of different concentrations of SK&F 21681. It was shown that the highest rate of inhibition occurred at the concentration of 60ì g/ml (the upper limit of solubility of this compound in aqueous solution); decreasing the concentration resulted in a parallel loss in the efficiency of the inhibition (Fig. 3). Concentrations of SK&F 21681 lower than 10 ì g/ml were not active. The results also suggest that the susceptibility of the growth of HSV-2 to this compound is higher than that of HSV-1 (Fig. 3). When formation of plaques of HSV in the presence of SK&F 21681 (60 p.g/ml) was studied, a remarkable inhibition was observed.

The examination with respect of inhibition caused by SK&F 21618 was reversible upon removal of the compound suggested(Table-1) that when the compound was removed at 2 or 4 h p.i., it was still possible to recover most of the virus infectivity, but when the removal of the compound was delayed to 7.5 h p.i., the rate of rescue of viral infectivity was much lower.

The study of effect on formation of viral particles indicated that in untreated cultures infected with HSV-1 or HSV-2, a clear peak which was resistant to DNase was observed. The position of this peak was similar to that of purified HSV particles. In SK&F 21681-treated cultures, virus particles were almost entirely missing (Fig. 4).

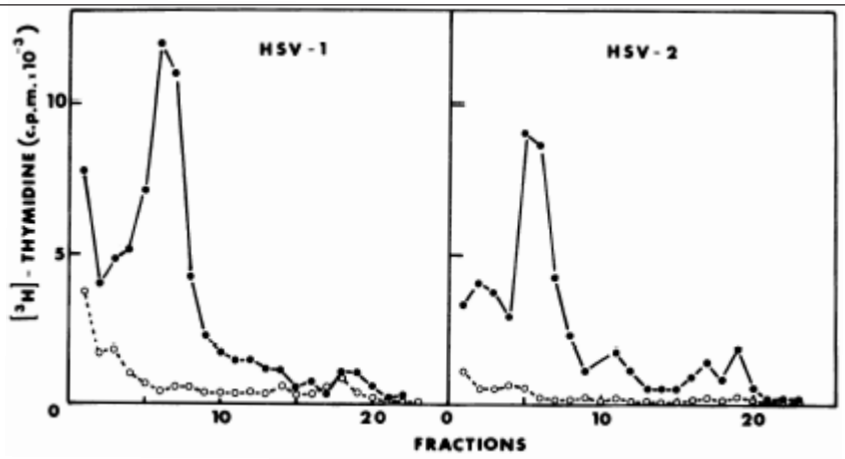

FIG. 4. Sedimentation of HSV particles in sucrose gradients.[3H]thymidine-labeled infected cells were analyzed for virus particles as described in the text. Symbols ●,control ; ○;K&F21681(60ìg/ml)

Since HSV virus particles are not formed in the presence of SK&F 21681 (Fig. 4), it is possible that an inhibition in viral DNA synthesis does occur under these conditions. The experimental results indicated (Fig.5) that although the inhibitory effect of SK&F 21681 on the synthesis of cellular DNA is very minor, the synthesis of viral DNA of both HSV-1 and HSV-2 is highly suppressed by this compound.

The polypeptide profile of HSV-1was significantly different from that of HSV-2, but the profiles of both viruses were very similar in the absence and presence of SK&F 21681 (60ìg/ml). Mutants of herpes simplex viruses types 1 and 2 which are able to grow in the presence of SK&F 21681 were isolated.

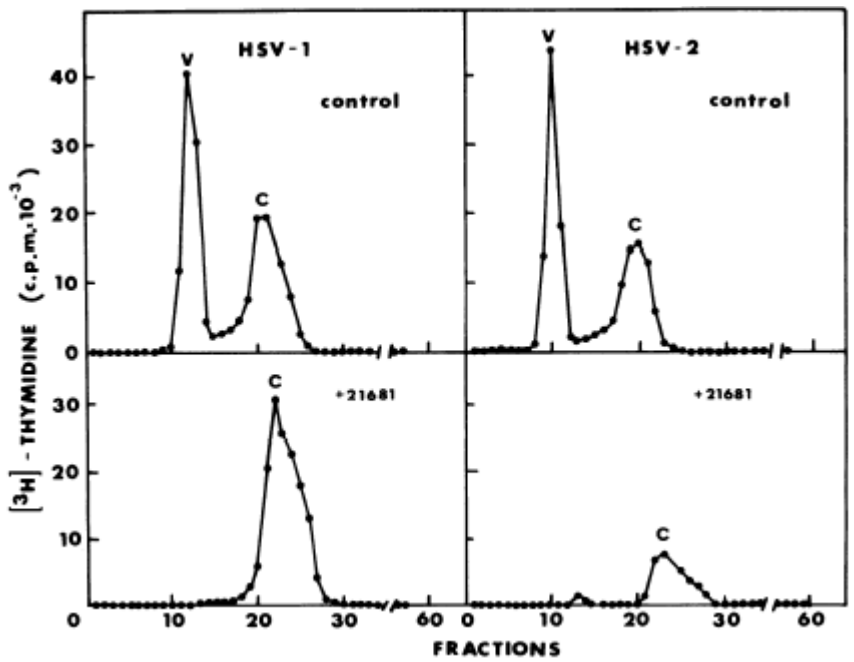

Fig-5.Cellular (C) and viral (V) DNA in CsCl gradients. Analysis of the DNA made in HSV-1- and HSV-2-infected BSC1 cells in the absence and presence of SK&F 21681 (60 ì g/ml)

Scott Baginski and co-researchers[61] have reported the discovery of a small molecule inhibitor of pestivirus replication. The compound, designated VP32947, inhibits the replication of bovine viral diarrhea virus (BVDV) in cell culture at a 50% inhibitory concentration of approximately 20 nM. VP32947 inhibits both cytopathic and noncytopathic pestiviruses, including isolates of BVDV-1, BVDV-2, border disease virus and classical swine fever virus. However, the compound shows no activity against viruses from unrelated virus groups.

Bovine viral diarrhea virus (BVDV), the prototypic representative of the pestivirus genus, family *Flaviviridae*, is ubiquitous and causes a range of clinical manifestations, including abortion, teratogenesis, respiratory problems, chronic wasting disease, immune system dysfunction, and predisposition to secondary viral and bacterial infections[62]. Certain BVDV strains can cause acute fatal disease with mortality rates of 17–32%[63,64,65]. BVDV is also able to establish persistent infections in fetuses[66,67]. When born, these persistently infected animals remain viremic throughout life and serve as continuous sources for virus spread in herds. Persistently infected animals may also succumb to fatal mucosal disease on superinfectionwith closely related BVDV strains. Vaccines are used in some countries in an attempt to control pestivirus disease with varying degrees of success[62,68].

Time of drug addition studies indicated that VP32947 acts after virus adsorption and penetration and before virus assembly and release. Analysis of viral macromolecular synthesis showed VP32947 had no effect on viral protein synthesis or polyprotein processing. The investigation of the effect of VP32947 on viral RNA synthesis indicated when virus-infected cells, treated with actinomycin D(to inhibit cellular RNA synthesis) with or without VP32947 in the cell culture medium. There was a clear inhibition of incorporation of ^{3}H-uridine into viral RNA found in the drug treated culture(Fig. 6). Although the drug-free culture showed the time-dependent incorporation of ^{3}H-uridine into viral RNA. When this experiment was performed in the absence of actidomycin D, no change of ^{3}H-uridine uptake in the presence of drug was observed for cellular RNA synthesis, indicating that VP32947 had no obvious effect on uridine uptake by the cells or on cellular RNA synthesis. These results suggest that VP32947 exerted its antiviral effect at the level of viral RNA synthesis.

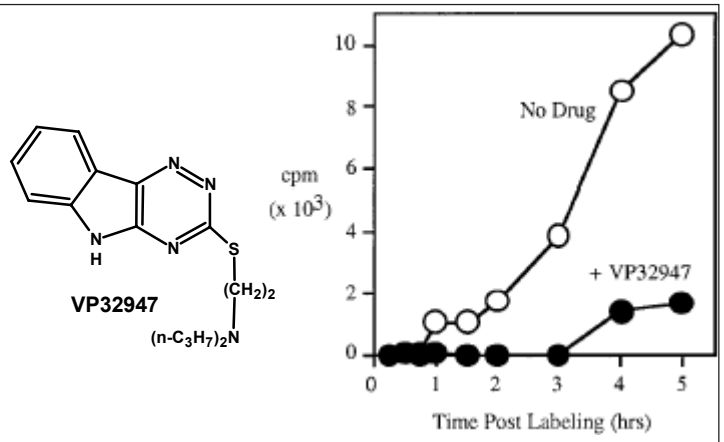

Fig. 6. Effect of VP32947 on viral RNA synthesis in WT virus-infected cells.Incorporation of 3H-uridine into viral RNA in the absence○or presence●of 167 nM VP32947 was determined as described in Materials and Methods.

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Hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV) are both members of the *Flaviviridae* family and share many molecular and virological similarities [69,70]. They both have single-strand RNA genomes that replicate via negative strand intermediates and produce a single polyprotein that is cleaved into individual proteins by a combination of host and viral proteases. Although HCV is a hepacivirus and BVDV is a pestivirus, there is a low degree of sequence homology between their respective nonstructural proteins and both RNAdependent RNA polymerases are structurally very similar [71].

Current therapies are limited to interferon treatment, either alone or in combination with ribavirin[72,73], but patient response for certain genotypes is still unsatisfactory. This unmet medical need has created an urgent demand for the development of new drugs to treat chronic hepatitis C. However, the lack of an *in vitro* infection system has hampered HCV drug development. For this reason, BVDV has sometimes been used as a surrogate infectivity model *in vitro*.

In search of HCV-specific, non nucleoside-based antivirals Nigel Horscroft et al[74] have developed a bi-cistronic, subgenomic replicon for BVDV that replicates efficiently in Huh-7 cells. This have made enable to compare RNA replication of HCV and BVDV in similar cellular backgrounds and to identify antiviral compounds specific for HCV in the absence of significant cytotoxicity. Although BVDV and HCV are closely related, there was low sequence similarity or identity between the corresponding proteins. The high degree of structural similarity between the polymerase motifs of BVDV and HCV NS5B [71,75] suggests that compounds that inhibit viral replication by targeting the catalytic site of the polymerase (e.g., nucleosideor nucleotide-based inhibitors) will likely be inhibitory in both systems. However, compounds that are inhibitory but target less-conserved regions of the polymerase enzyme may not be cross-active against both viruses.

To validate such an approach, the Huh-7–HCV and Huh-7–BVDV replicons were tested in parallel against a number of inhibitor compounds with known specificities (Fig. 7). 2'-â-C-methyl ribonucleosides are known to inhibit HCV polymerase by incorporation into viral RNA and causing chain termination[76]

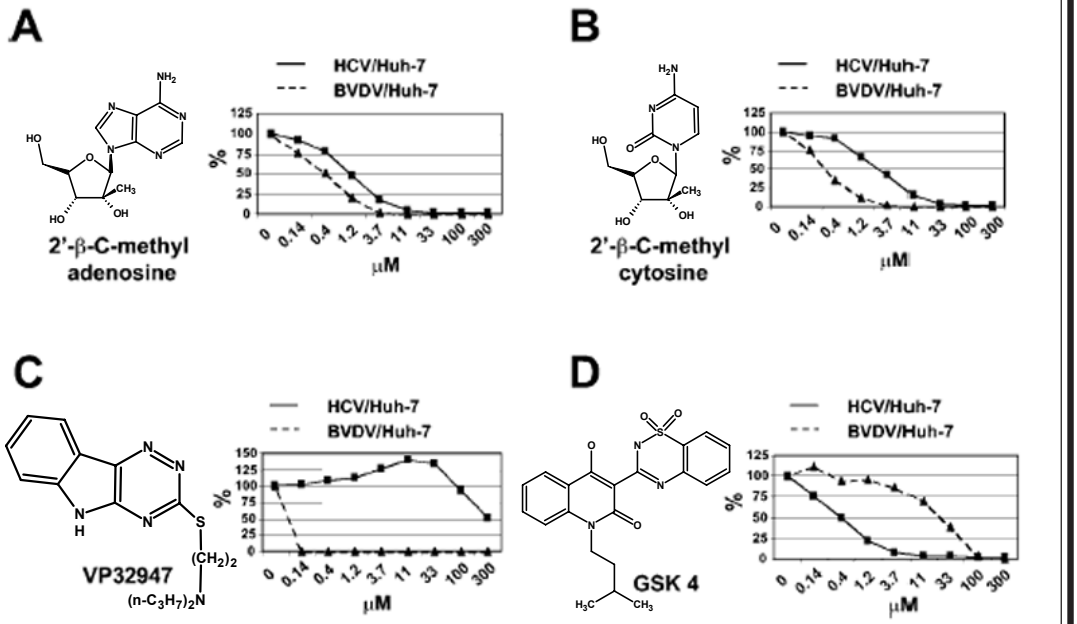

FIG-7. Demonstration of viral specificity with known inhibitor compounds. BVDV/Huh7 or HCV/Huh7 cells expressing luciferase were plated in white 96-well plates and allowed to attach overnight (~18 h) before treatment with known small-molecule inhibitors for a further 48 h. (A and B) 2-â-C-methyl adenosine (A) and 2'-â-C-methyl cytosine (B) showed comparable EC50 values in the parallel replicon assays. (C) VP32947, a triazinoindole derivative, showed potent activity against BVDV/Huh-7, with an EC50 of < 0.14 M, but was not active against HCV/Huh-7.(D) GSK-4, a benzothiadiazine derivative, showed specific activity against HCV/Huh7 but much lower activity against BVDV/Huh7.

As substrate-based inhibitors targeting the catalytic center of the polymerase enzyme, this class of compounds would be predicted to be active against BVDV as well. As expected, 2'-â-C-methyl adenosine (Fig. 7A) and 2'-â-C-methyl cytosine (Fig. 7B) showed comparable EC50 values in the parallel replicon assays. For 2'-â-C-methyl adenosine, the EC50 values against Huh-7–BVDV and Huh-7–HCV were 0.4 & 1.2ì M, respectively, while for 2'-â-C-methyl cytosine those values were 0.3 & 3.0 ì M, respectively. In contrast, when two nonnucleoside polymerase inhibitors were tested, a greater difference in virus specificity was observed. VP32947 is a triazinoindole derivative that is a potent inhibitor of BVDV polymerase which acts by targeting a region near the amino terminus and away from the active site[77]; GSK compound 4 (GSK-4) is a benzothiadiazine derivative that shows specific activity against the HCV polymerase[78]. As shown in Fig. 7C, VP32947 showed potent activity against Huh-7–BVDV, with an EC50 of < 0.14 ì M, but was not active against Huh-7–HCV.

The EC50 of VP32947 against BVDV–Huh-7 was subsequently determined to be 25 nM, giving a selectivity index (SI) of 12,000. Conversely, for GSK-4

the EC50 values against Huh-7–BVDV and Huh-7–HCV were 25 & 0.4 ìM, respectively, which resulted in an SI of 63 (Fig. 7D). The relatively low SI for GSK-4 may be due to the intrinsic cytotoxicity associated with the compound, which affected both replicons. Taken together, these results illustrated the utility of the parallel replicon assays in quickly separating antiviral activity from cytotoxicity and the identification of compounds with high specificity towards HCV.

❖ **Antifungal activity**

Triazinoindoles have been described to have activity against *A.niger.* and when provided as seed dressing, they protected sorghum seeds from infection with this organism[25]. The description of the antifungal activities of triazinoindole compounds was new and no mode of action is suggested from the literature. Oonagh Kinsman and co-workers[77] have described GR99060 and GR99062 as representatives of a series of 1,2,4-triazino[5,6-b]indole compounds. This series possessed broad-spectrum antifungal activity *in vitro*. The MIC ranges of the two compounds were as follows: 0.25 to 4 ìg/ml for *Candida albicans*, 0.25 to 16 ìg/ml for *Candida sp.*, 1 to 8 ìg/ml for *Asperigllus spp.*, and 0.25 to 16 ìg/ml for *Cryptococcus neoformans.* GR99062 was metabolized to corresponding N-oxide analogue GR107867 *in vitro* by a mouse liver microsomal preparation, while GR99060 was stable. GR99060 was efficacious in a murine model of systemic candidiasis by oral or parenteral administration, although no clear dose-response was achieved, suggesting that other factors adversely affected the compound's *in vivo* activity.

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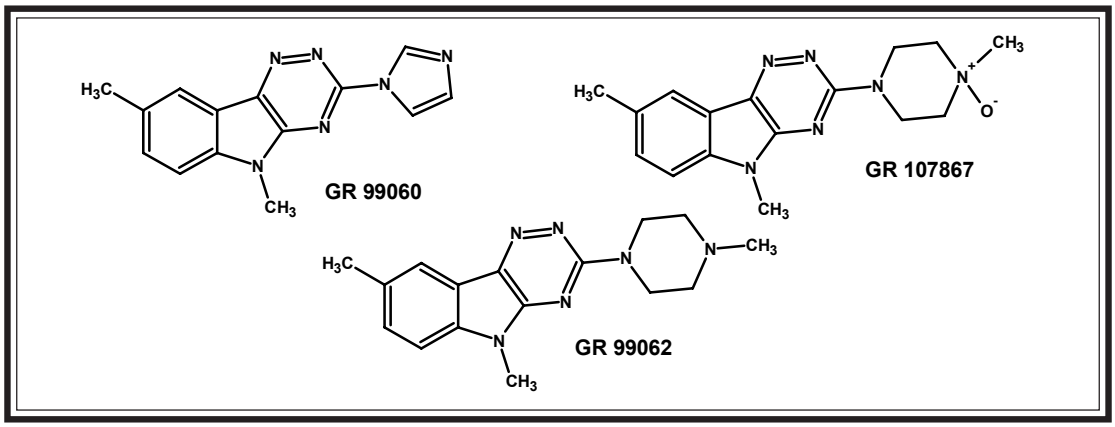


GR99060 showed a therapeutic effect in mice with systemic *C. albicans* infections when it was administered by both the oral and subcutaneous routes. Since the levels of the drug in blood after subcutaneous dosing were low, the compound may be released slowly over a longer period of time. In the efficacy test, the dose-response was variable by both routes. Toxicity in infected animals may account for the reduced activity at high doses, since a lower survival rate was noted in mice that received 100 mg/kg than in those that received lower doses. Variations in bioavailability and possible further metabolism may also account for the lack of good dose-responses. Similarly, in the subacute infection model, the oral route of administration was more effective than the subcutaneous route in reducing kidney counts when a dose of 100 mg/kg was used.

3. Anticancer activity(c-MET Inhibitors)

Protein kinases(PKs) are the enzyme that catalyses the phosphorylation of hydroxy group on tyrosine, serine and threonine residues of protein. The consequences of this seemingly simple activity are staggering cell growth, differentiation and proliferation i.e. virtually all aspects of cell life in one way or another depend on PK activity. The PKs can be conviniently broken into two classes, the protein tyrosine kinase(PTKs) and the serine threonine kinase(STKs).

One of the prime aspect of PTK activity is their involvement in growth factor receptors. Growth factor receptors are cell-surface proteins, when bound by a growth factor ligand, converted into an active form which interacts with proteins on the inner surface of a cell membrane, leads to phosphorylation

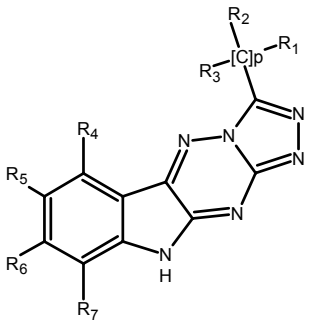
on tyrosine residue of the receptor and other proteins and to the formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that in turn effect numerous cellular responses such as cell division(proliferation), cell differentiation, cell growth, expression of metabolic effects to the extracellular microenvironment etc[78].

Growth factor receptors with PTK activity are known as receptor tyrosine kinase(RTKs). They comprise a large family of transmembrane receptors with diversed biological activity. There were 19 distinct families of RTKs have been identified[79] including EGFR(epithelial growth factor receptor), Insulin receptor(IR), PDGFR(platelet derived growth factor receptor, FGR(fibroblast growth factor etc.

Still another member of the tyrosine kinase growth factor receptor family is MET often referred as c-MET or hepatocyte growth factor receptor or scatter factor receptor. c-MET is thought to play a role in primary tumor growth and metastasis. c-MET is a receptor tyrosine kinase that is encoded by the Met protooncogene[80], tranduces the biological effect of hepatocyte growth factor(HGF) and their expression confined predominantly to cell of epithelial and mesenchymal origin. c-MET and HGF are required for normal mammalian development and have been shown to be important in cell migration, cell proliferation, survival, morphogenic differentiation and organization of 3-dimentional tubular structures(eg. renal tubular cells, gland formation etc.). c-MET is an attractive target from a clinical perspective because (1) c-MET has been implicated to in the growth and metastases of most types of cancer, (2) growth at the secondary site appears to be the rate limiting step in metastases and (3) by the time of diagnosis, it likely that the disease has already spead.

Tomas Vijkovsky et al[81] have patented compounds of formula I and II for treating c-MET related disorders or in another preferred embodiment, the cancer selected from the group consisting breast cancer, lung cancer, prostate cancer, pancreatic cancer, glioma, liver cancer, gastric cancer, throat

cancer, melanoma, renal cancer, leucamia, myeloma and sarcoma. The investion deals with a family of novel tetracyclic compounds which exhibits c-MET modulationg activity and have ameliorating effect against disorders related to abnormal c-MET activity.



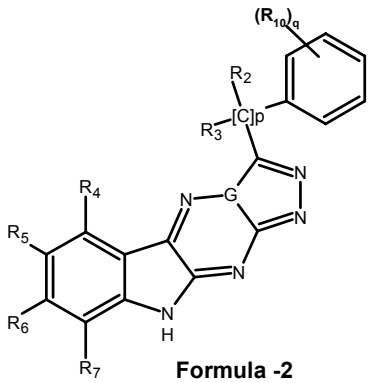
Formula -1

R_1=substituted aryl or heteroaryl group

R_2 & R_3=H, halogen, OH, OR, $N(R)_2$, -CN, -COR, COOR, $CON(R)_2$, CF_3, lower alkyl, cycloalkyl, heterocycle, alkenyl and alkynyl or R_2 & R_3 togather form cycloalkyl or heterocyle

R_4, R_5, R_6 and R_7= H, halogen, OR, $N(R)_2$, -CN, -COR, COOR, $CON(R)_2$, NO_2, $S(O)_nR$ (where n=0,1,2), CF_3,lower alkyl, cycloalkyl, heterocycle, alkenyl, alkynyl or aryl

p=0,1,2,3,4 or 5



Formula -2

R_{10}=OH,OR, COR, COOR, $CON(R)_2$, $N(R)_2$, CN, NO_2, SO_2R, $SO_2N(R)_2$, CF_3, lower alkyl, cycloalkyl, heterocycle, alkenyl or aryl

q=1,2,3,4 or 5 G = C or N

R_2 & R_3=H, halogen, OH, OR, $N(R)_2$, -CN, -COR, COOR, $CON(R)_2$, CF_3, lower alkyl, cycloalkyl, heterocycle, alkenyl and alkynyl or R_2 & R_3 togather form cycloalkyl or heterocyle

R_4, R_5, R_6 and R_7= H, halogen, OR, $N(R)_2$, -CN, -COR, COOR, $CON(R)_2$, NO_2, $S(O)_nR$ (where n=0,1,2), CF_3,lower alkyl, cycloalkyl, heterocycle, alkenyl, alkynyl or aryl

p=0,1,2,3,4 or 5

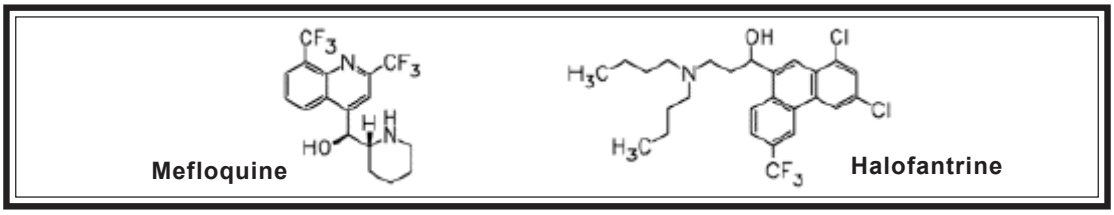
❖ Antimalarial activity

Recent hypothesis seem to suggest that quinoline-type antimalarial drugs coordinate to the malarial parasite's endogenous antimalarial agent, Ferriprotoporphyrin IX(FP), thus disrupting the conversion of haematin to haemazoin(malaria pigment)[82]. A free FP as a toxic substance to the malaria parasites, serves as a receptor for the accumulation of antimalarial drugs in the food vacuoles[83]. The parasites which are lacking in heam oxygenase are unable to detoxify the free FP by metabolism, but the malaria parasites have evolved an autocatalytic detoxification process in which FP is oxidised to heamatin[84]. In this process the malarial parasites convert the haematin to a haemazoin, a repeating array of coordinated dimers held

together in a crystalline matrix by hydrogen bonding interactions[85,86]. The accumulation of FP allows for a significant concentration of the free haematin and haematin-antiviral drug complex to remain in the food vacuole and in this way, the ability of the parasite and host red blood cells to maintain cationic gradients is impaired, leading to the death of parasites[87-90].

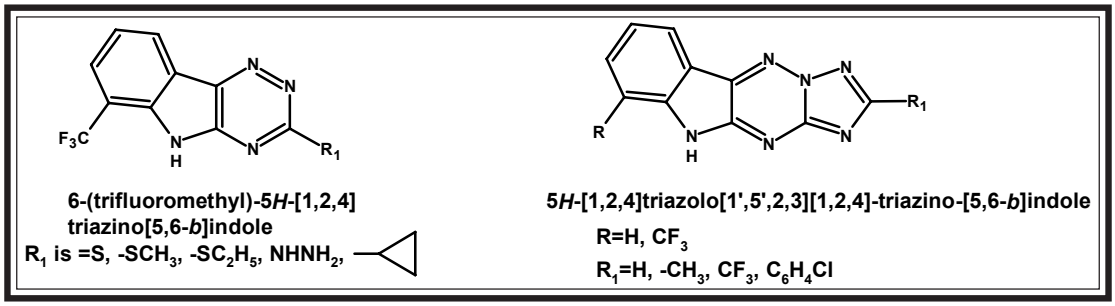
The activities of these antimalarial drugs are a function of both the ability of the drug to accumulate to pharmacologically relevant concentrations at the site of drug action, and the ability of the drugs to the to interfere with the haemozoin formation[91]. It is also possible that haematin-antimalarial drug complex cause paracytic death by multiple mechanism[92], with evidence suggesting that haem, rather than the haem drug complex, is responsible for actual lysis of the vacuolar membrane[88]. The free FP and its complex with chloroquine-type drugs inhibits protease, which are essential for the degradation of haemoglobin, and thus affect the growth of the parasite[93]. The binding of the quinoline-type antimalarial drugs may stabilize the ì -oxo dimer to the monomers, shifting the dimerization equilibrium to the right and reducing the amount of haematin monomers available for incorporation into the growing haemazoin[94].

Since the discovery of the non-phototoxic, but highly effective quinolinemethanol antimalarial agent, mafloquine[95] and halofantrine, the trifluoromethyl group has aroused considerable interest as a pharmacophore. Both compounds being effective against multidrug resistant *Plasmodium falciparum*, including strains, which are highly resistant to chloroquine[96,97] and associated with reduced concentration of the drug from the acid food vacuoles of the parasite due to increased efflux of the drug from cell[99]. The emergence of multidrug-resistant strain of *Plasmodia*, has created a near desperate situation, where the need for new antimalarials to circumvent the parasite's resistance mechanism has become vital[98].



Mefloquine **Halofantrine**

Joseph Kgokong et al[100] have synthesized the unsubstituted and the 6-trifluoromethyl-1,2,4-triazino[5,6b]indole analogues and evaluated for antimalarial activity *in vitro* against the chloroquine sensitive and chloroquine-resistant strains of P. falciparum. The results have revealed that the CF_3 group tends to lead to an increased *in vitro* antimalarial activity of the 1,2,4-triazino[5,6b]indole albeit to a smaller degree in some compounds. Analogues without the trifluoromethyl group, which were evaluated simultaneously, were all devoid of activity even at concentrations as high as 400 ì M. The increased activity resulting from the presence of this group could be ascribed to the increased lipophilicity of the compound, as this group is known to be more hydrophobic than even the fluorine atom.[101] In this series, the size of the substituent on the thiol group have no significant effect on *in vitro* antimalarial activity. Replacement of a thiol group by a hydrazino group in the trifluoromethyl substituted derivatives leads to compounds with half the potency of those with alkyl groups on the thiol group. On the other hand, the activity of the 6-trifluoromethyl-5H-1,2,4-triazolo[1',5',2,3]-1,2,4-triazino[5,6b]indole series was almost identical to those of the 6-trifluoromethyl-1,2,4-triazino[5,6b]indole-3-alkylthiols, with the exception of compound containing the 6-CF_3 and the 3-CH_3 groups. The later exhibited a four fold improvement in activity against the chloroquine-sensitive strains. However, introduction of a second CF_3 particularly at position 3 tends to lead to compounds with diminished antimalarial activity. Further consideration of the structure–activity relationship of compounds in the series without the 6-CF_3 indicates that compound with 3 –CH_3 substitution was the most active against the chloroquine-sensitive strains followed by unsubstituted skeleton. The former exhibited activity against the chloroquine-sensitive strains in the same molar range as chloroquine and mefloquine under identical experimental conditions, while later was the most active against the chloroquine-resistant strains of P. falciparum. Both –CF_3 and –C_6H_4Cl groups at position 3 without CF_3 at position 6 lead to relatively inactive compounds. The fact that the P. falciparum lactate dehydrogenase



(PfLDH)activity can be distinguishable from the host LDH[102] by using the 3-acetyl pyridine adenine dinucleotide analogue of nicotinamide adenine dinucleotide (APAD) has afforded an opportunity for the development of an enzymatic method for the evaluation of antimalarial compounds.[103] The compounds selected for screening against the chloroquine-resistant strain of P. falciparum have shown identical but much higher *in vitro* activity than against the chloroquine-sensitive strain, with the exception of compound having no substitution, which has a IC50 value of 48.0 ì M, which was very close to that of chloroquine (IC_{50} of 277.5 ì M) against this strain. The 3-CF_3 substitution did not lead to any improvement on the *in vitro* antimalarial activity than it did when attached at position 6. The 3,6-bis(trifluoromethyl)-5H- 1,2,4-triazolo-[10,50,2,3]-1,2,4-triazino-[5,6b]indole has a much lower activity against both the chloroquine- sensitive and chloroquine-resistant strains of P.falciparum than the other 6-CF_3 substituted derivatives. As a result of a direct relationship between the level of drug accumulation in the parasite food vacuole and antimalarial drug potency, but no simple relationship between accumulation and either pKa or lipophilicity,[84,104,105,106] it could be inferred that the bulkiness of the molecule conferred by the second CF_3 group will affect the relative membrane permeability of the molecule, leading to reduced drug accumulation within the parasite food vacuole.

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❖ Antihypertensive and Platelet aggregation Inhibitors/Thromboxine synthetase Inhibitors

The action of antithrombotic drugs that act on platelet aggregation has been largely due to inhibition of platelet cyclooxygenase, the foremost example being acetylsalicylic acid (ASA)[107]. A more effective approach may be the selective inhibition of thromboxane (TXA_2) synthetase[108]. TXA_2, which rapidly hydrolyzes under physiological conditions to TXB_2 is a potent vasoconstrictor and platelet aggregating agent[109]. An additional advantage would be that some precursor of TXA_2, like prostaglandin H_2(PGH_2), may increase the production of the vasodilator prostacyclin (PGI_2) in the vessel wall[110]. The control of the PGI_2/TXA_2 system may have a great biological significance in the treatment or prophylaxis of several cardiovascular diseases[111].

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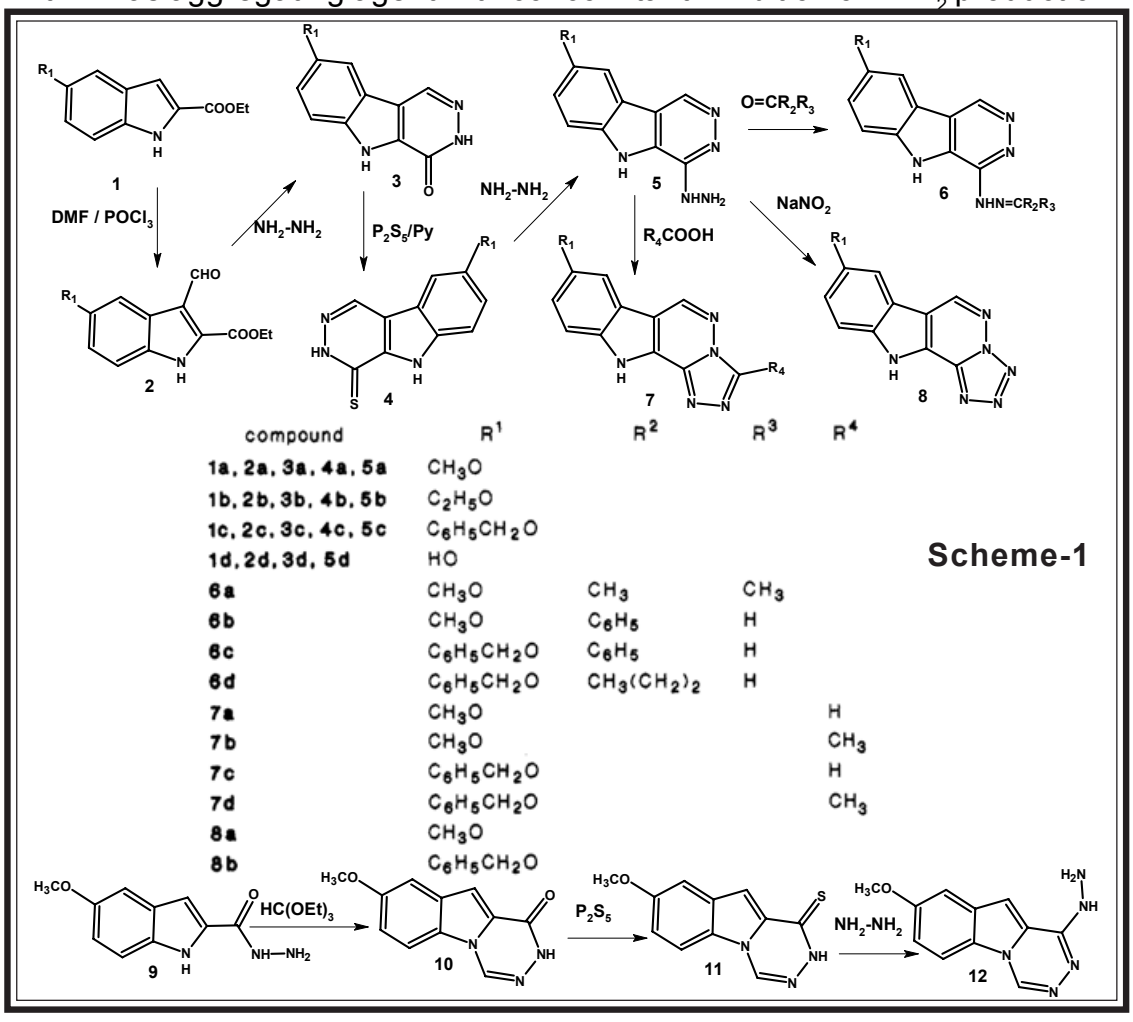
The antiaggregation properties of several indolic derivatives were well documented[112] and there are also many examples of selective TXA_2 synthetase inhibitors[113].

Based on the these consideration Monge A. et al[114] have studied the regulation of the PGI_2/TXA_2 system with antihypertensive effects by synthesizing a series of 4-hydrazino-5H-pyridazino[4,5-b]indole and 4-hydrazinopyridazino[4,5-a]indole derivatives (Scheme-1), structurally related to hydralazine[115] (1-hydrazinophthalazine) and dihydralazine(1,4-dihydrazinophthalazine), peripheral vasodilators used in the treatment of hypertension. The demonstration of selective inhibitory actions of the compounds was determined in concordance with the Gorman mode[116], namely, the inhibition of the second wave of platelet aggregation induced by adenosine 5'-diphosphate (ADP), inhibition of aggregation induced by arachidonic acid (AA), and prostaglandin H, (PGH_2) and prostaglandin H_2(PGH_2) production with AA as aggregating agent with concomitant inhibition of TXA_2 production.



compound	R^1	R^2	R^3	R^4
1a, 2a, 3a, 4a, 5a	CH_3O			
1b, 2b, 3b, 4b, 5b	C_2H_5O			
1c, 2c, 3c, 4c, 5c	$C_6H_5CH_2O$			
1d, 2d, 3d, 5d	HO			
6a	CH_3O	CH_3	CH_3	
6b	CH_3O	C_6H_5	H	
6c	$C_6H_5CH_2O$	C_6H_5	H	
6d	$C_6H_5CH_2O$	$CH_3(CH_2)_2$	H	
7a	CH_3O			H
7b	CH_3O			CH_3
7c	$C_6H_5CH_2O$			H
7d	$C_6H_5CH_2O$			CH_3
8a	CH_3O			
8b	$C_6H_5CH_2O$			

Scheme-1

Compounds 3,5, and 10-12 were studied as potential inhibitors of the blood platelet aggregation induced by ADP and/or AA, PGH2, and adrenaline. Compounds 3c and 5a.HCl were found to be the most potent inhibitors in all of these experiments. These compounds were inhibitors of the *in vitro* platelet aggregation induced by ADP, AA, and PGH,, and compound 5a.HCl also inhibited the aggregation induced by adrenaline.

Compounds 3c and 5a-HCl were subsequently studied with ASA as reference as potential inhibitors of thromboxane synthetase in the *in vitro* blood platelet aggregation induced by AA and in the ex vivo blood platelet aggregation induced by ADP and AA. In both types of experiments, 3c and 5a.HCl showed an inhibitory effect on the synthesis of TXB_2 and a stimulation of the synthesis of prostaglandin E_2 (PGE_2). Both effects are more significant with 5a-HCl. To a 100 ì M concentration, this compound as compared with ASA showed no significant inhibitory effect on the PGI_2 release from rat aortic tissue.

The antihypertensive effect of the compounds was measured in spontaneously hypertensive rats (SHR), after intraperitoneal, oral, and intravenous administration to SHR for compounds 3-8, 12 and hydralazine. The introduction of the hydrazino group in the position 4 of the 5H-pyridazino[4,5-b]indole system considerably increased the antihypertensive activity and toxicity. However, compounds 6a and 6b were less active as compared with compounds 5. Considering the results together following decreasing order of activity may be stated for compounds 5:

hydralazine >/= 5a = 5d >/= 5 (R' = H)>/=5b >> 5c.

The results for 5a, 5b**,** 5d, and hydralazine for a short time after iv administration revealed that decreasing order of activity seems to be

5d > hydralazine = 5a > 5b.

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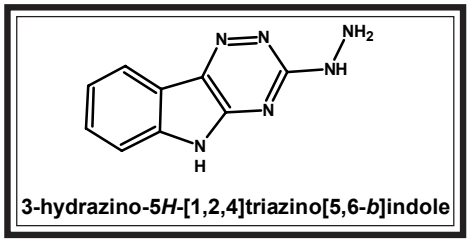
The effects on cardiac rhythm (CR) caused by the titled compounds suggested the following increasing order of stimulation.

hydralazine = 5a = 5b << 5d and at a higher dose 5b = 5a << 5d.

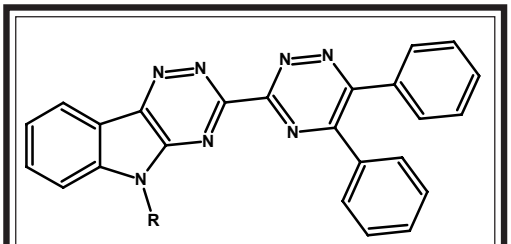
After this, the same group have extended their study with a series of 5H-triazino[5,6-b]indole. Only one compound 3-hydrazino-5*H*-[1,2,4]triazino [5,6-*b*] indole hydrochloride was found to be most potent as antihypertensive agent with slighly affecting the cardiac rhythm but did not present any antiaggregative effect.



3-hydrazino-5*H*-[1,2,4]triazino[5,6-*b*]indole

❖ Antibacterial activity

Morsy J.M. and Abd el-Monem W.R.[118] have synthesized 3-(5,6-Diphenyl-1,2,4-triazin-3-yl)-5-substituted-1,2,4-triazino[5,6-b]indole derivatives showed pronounced effect on the Cellobiase produced by Thermomyces lanuginosus and Chaetomium thermophilum.



3-(5,6-diphenyl-1,2,4-triazine-3-yl)-5-substituted-1,2,4-triazino[5,6-b]indoles

Ethyl 1,2,3-triazino[5,4-b]indole-4-carboxylate N(3)-oxide (13) and 2-chloroethyl 1,2,3-triazino-[5,4-b]indole-4-carboxylate N(3)-oxide (14) have shown potent platelet antiaggregating and hypotensive activity, and their precursors 2-ethoxy-carbonylmethyl-1-methylindole-3-carboxylic acid (15) and 2-(2-chloroethoxy carbonylmethyl)-1-methylindole-3-carboxylic acid (16) were tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA97 and TA102) using the standard plate incorporation technique. 15 & 16 were not mutagenic whereas 13 was mutagenic to all the strains and 14 was mutagenic to TA97, TA98 and TA100[119].

117a. A.Monge, J.Palop, C.Ramirez, M.Font, E.Fernandez-Alvarez, *Eur.J.Med.Chem.*,**26**, 179(1991)
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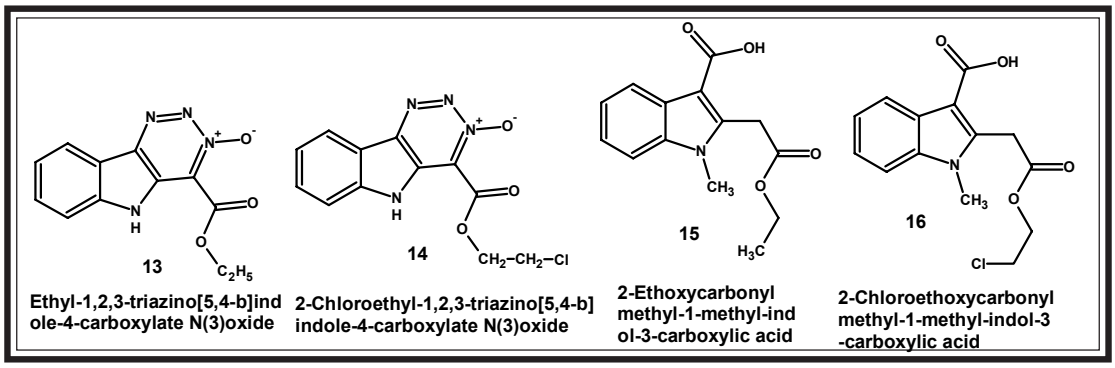
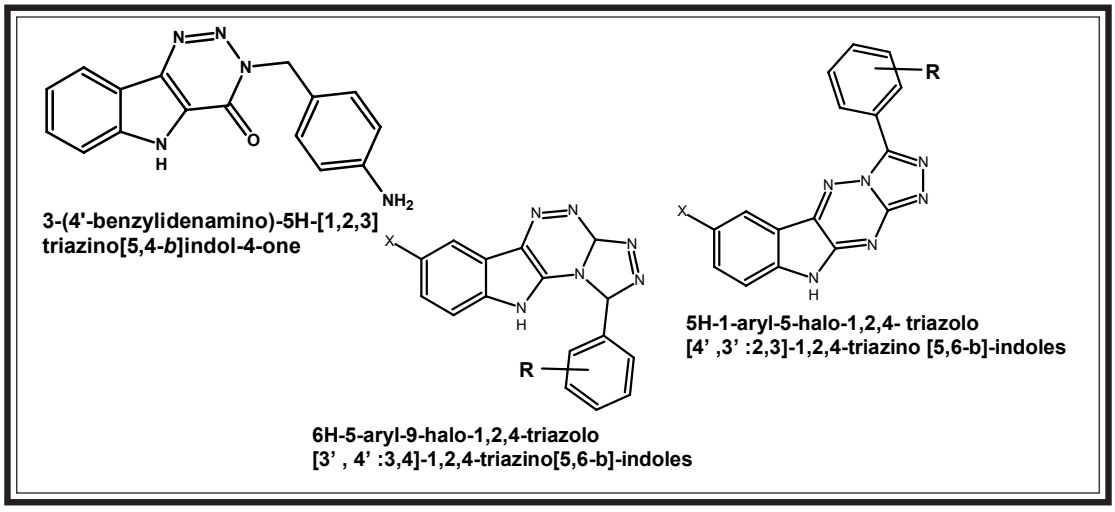


In continuation with this, Garcia et al[120] have studied quantitative structure-mutagenic activity relationship of 3-(4'-benzylidenamino)-5H-1,2,30triazin[5,4-b]indole-4-one derivatives. The titled compounds was assayed with the Ames test using the *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102. The adaptive least-squares method (ALS method) was used to carry out a quantitative structure-activity relationship (QSAR) analysis. Three equations, based on 10 congeners, were found for strains TA97, TA98 and TA100. The results suggest that lipophilicity of the substituent decreases the mutagenicity of the series.

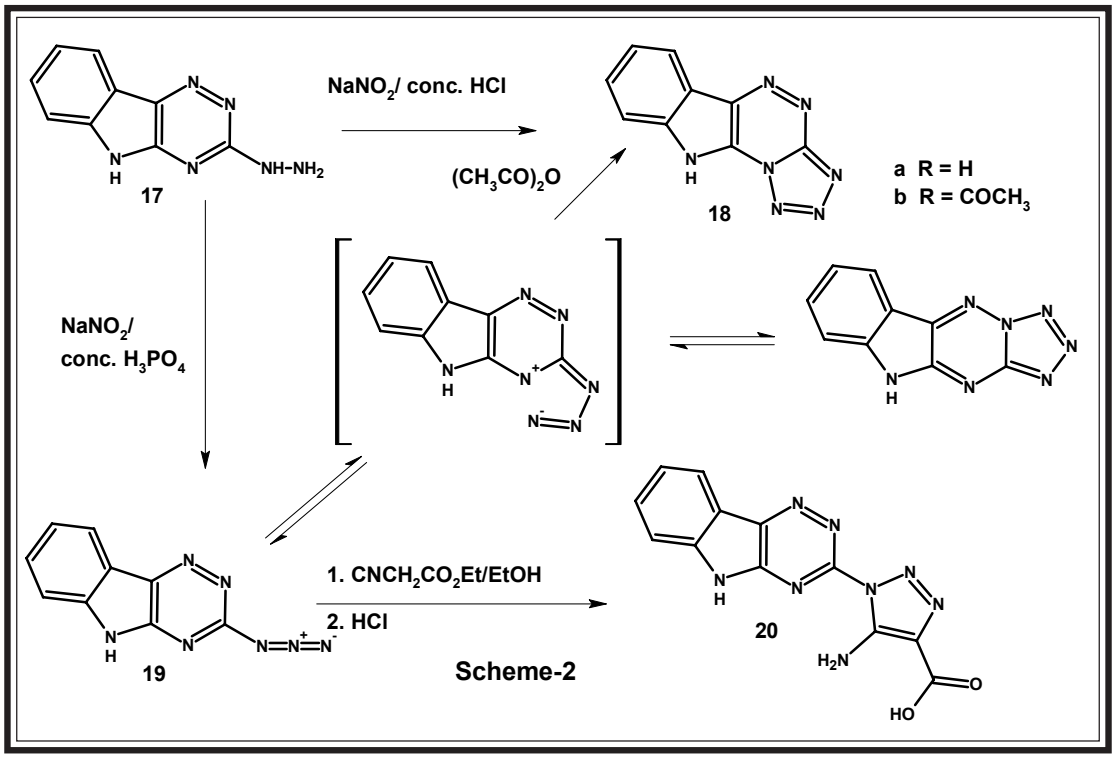


Holla B.S. et al[121] synthesized a series of 6H-5-aryl-9-halo-1,2,4-triazolo [3' , 4' :3,4]-1,2,4-triazino[5,6-b]-indoles and 5H-1-aryl-5-halo-1,2,4- triazolo [4' ,3' :2,3]-1,2,4-triazino [5,6-b]-indoles. The titled compounds assayed against *B. subtilis*, *E.coli*, *P. aeruginosa* and *S.aureus*.

120.E.Garcia, A.Lopez-de-Cerain , Martinez-Merino, A.Monge, *Mutat. Res*, **268**(1),1(1992)
121. B.Holla, N.Shashidhara, K.Udupa, B.Poojary, *Ind.J.Heterocycl.Chem.*, **14**(4), 347(2005)

Synthetic approaches and Chemistry

Joshi et al[122] reported that the reaction of 3-hydrazino[1,2,4]triazino[5,6-b]indole (17) with nitrous acid (sodium nitrite/PPA) gave 10H-tetrazolo[5',1':3,4][1,2,4]triazino[5,6-b]indole(18), where as reaction of (17) with nitrous acid gave azide(19)[123-124], the structure of the azide was confirmed by its analytical and spectral data. The azide (19) was cyclized to tetrazolo compound (18b) on treatment with acetic anhydride, while cyclization with ethyl cyanoacetate in presence of sodium ethoxide afforded 5-amino-4-carboxy[1,2,3]-triazolyl-[1,2,4]-triazino-[5,6-b]indole (20)[127]. On the other hand treatment of (17) with sodium nitrite/concentrated hydrochloric acid gave the corresponding tetrazolo compound 18a (Scheme-2).



F.F.Abdel-Latif et al[128] have studied reactivity of hydrazino compound (21) toward different condensing cyclization reagents[129](Scheme-3) , found that when (21) treated with acetic anhydride affords the fused triazolo compound (22) namely 10-acetyl-1-methyl-10H-[1,2,4]triazolo[3',4':3,4[-1,2,4]triazino[5,6-b]indole. Refluxing (21) with carbon disulphide produced 1,2-dihydro-10H-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-b]indol-1-thione (23a). In the same manner, refluxing (21) with ethyl chloro formate produced

1,2-dihydro-10H-[1,2,4] triazolo[3',4':3,4][1,2,4]triazino[5,6-b]indol-1-one (23b). On the other hand refluxing (21) with 1-napthaldehyde in absolute ethanol produced 1-naphthylidine-(5H-[1,2,4]-triazino[5,6-b]indol-3-yl)hydrazone (24a), where as upon heating in glacial acetic acid gave corresponding triazolo compound, namely 10-acetyl-1-(1-naphthyl)-10H-[1,2,4]triazolo[3',4':3,4] triazino[1,2,4] [5,6-b]indole (25a). Treatment of (21) with benzaldehyde or p-nitrobenzaldehyde in ethanol and/or in glacial acetic acid similarly afforded both benzylidene((5H-[1,2,4]-triazino[5,6-b]indol-3-yl)hydrazone(24b) and 10-acetyl-1-(p-nitrophenyl)-10H-[1,2,4]triazolo[3',4':3,4]triazino[1,2,4] [5,6-b]indole (25b) respectively. Cyclization of (21) with ethyl acetoacetate yielded the pyrazolino compound-3-(5'-hydroxy-3'-methyl-1H-pyrazol-1-yl)-5H-[1,2,4]triazino[5,6-b]indole (26a). Similarly, refluxing (21) with acetyl acetone produced 3(3',5'-dimethyl-1H-pyrazolo-1-yl)-5H-[1,2,4]triazino[5,6-b]indole (26b). Condensation of (21) with malanonitrile resulted 3-(3',5'-diamino-1H-pyrazol-1-yl)-5H-[1,2,4]triazino[5,6-b]indole (27a), while treatment of (21) with ethyl cyanoacetate gave 3-(3'-amino-5'-hydroxy-1H-pyrazol-1-yl)-5H-[1,2,4]triazino[5,6-b]indole (27b).

The reaction of (21) with á-dicarbonyl compounds was assumed to involve one or both carbonyl groups in the condensation. Thus, heating of (21) and diacetyl in ethanol gave only the corresponding mono hydrazone namely 2-(5H-[1,2,4]triazino[5,6-b]indol-3-yl)-hydrazono-3-oxobutane (28). However in the presence of excess of (21) the corresponding 2,3-bis(5H-[1,2,4]triazino[5,6-b]indol-3-yl)hydrazonobutane (29) was formed.. The same result was also obtained if the monohydrazone (28) was heated with excess of hydrazine(21). All the resulting compounds were well characterized by IR and NMR spectral data and elemental analysis.

122. K.Joshi, P.Chand, *J.Heterocycl. Chem.*, **17**, 1783(1980)
123.E.Alcalde, J.Mendoza, J.Elguero, *J.Heterocycl.Chem.*, **11**,921(1974)
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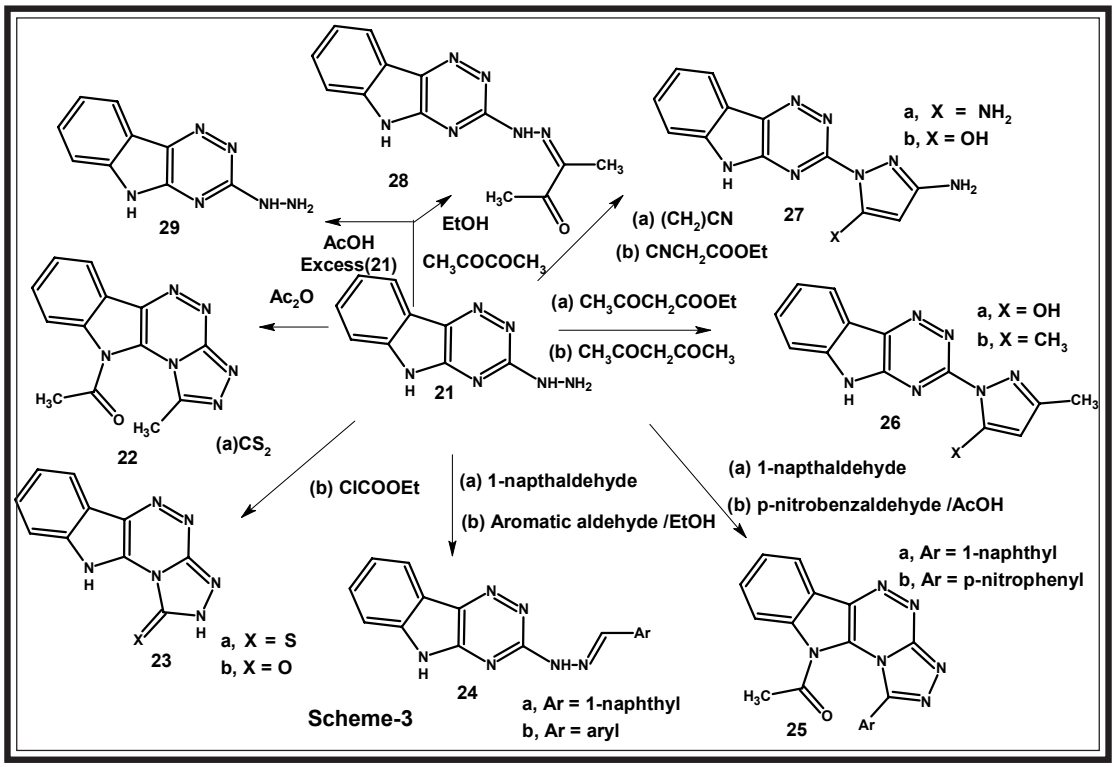
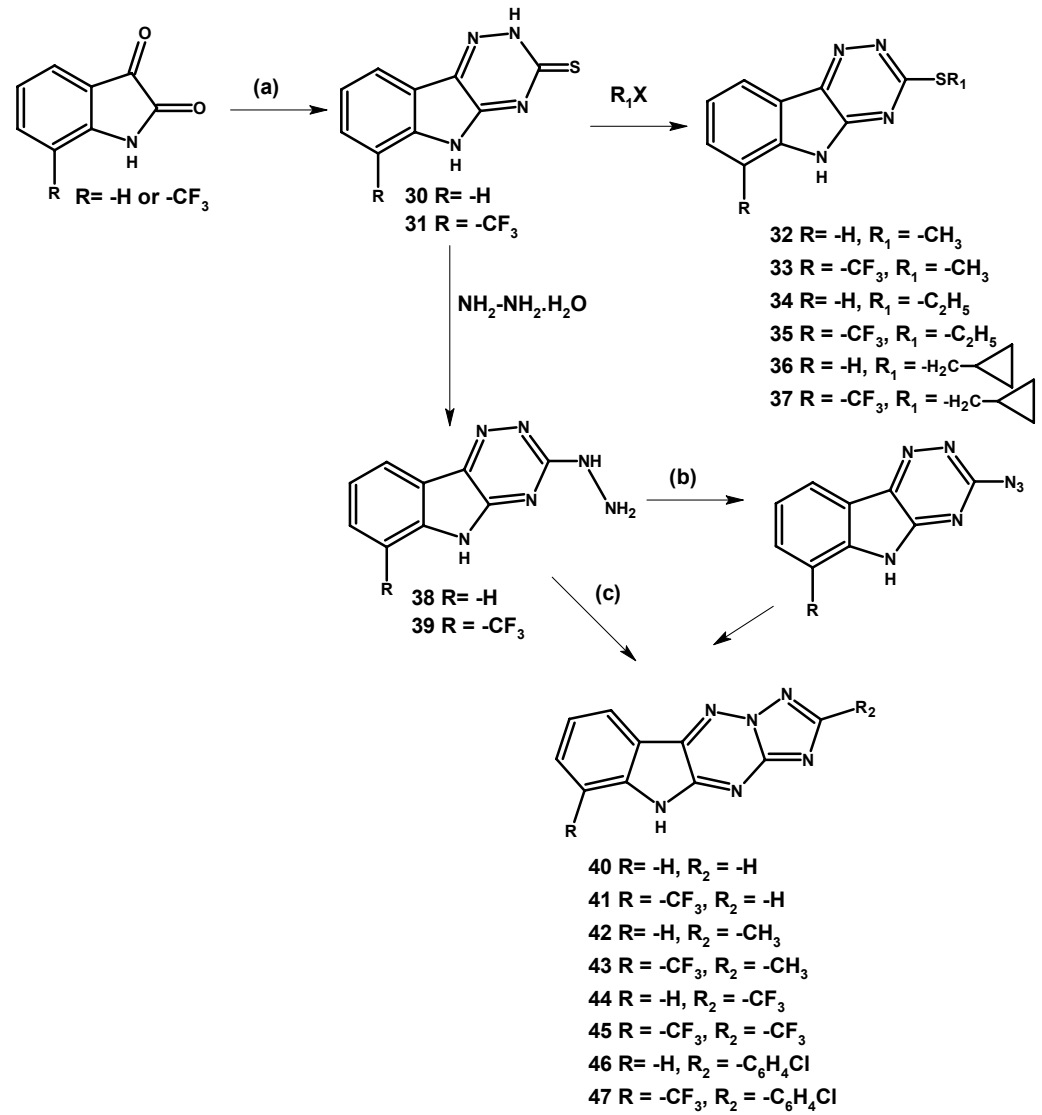
126.R.Granados, M.Rull, J.Vilarrasa, *J.Heterocycl.Chem.*, **13**,281(1976)
127.J.Hoover, A.Day, *J.Am.Chem.Soc.*, **78**, 5832
128.F.Abdel-Latif, R.Shaker, S.Mahgoub, M.Badr, *J.Heterocycl.Chem.*,**26**,769(1980)



Joseph Kgokong et al[100] have synthesized a series of unsubstituted and 6-trifluoromethyl-1,2,4- triazino[5,6b]indole and 5H-1,2,4-triazolo[1',5',2,3]-1,2,4-triazino[5,6b]indole derivatives and their chemical structures confirmed by ^{1}H NMR and ^{13}C NMR, elemental, IR and mass spectrophotometric analyses. The synthetic stretegy discribed as Isatin or the 6-trifluoromethyl isatin was condensed with thiocarbazide in K_2CO_3 solution to form 1,2,4-triazino-[5,6b]indole-3-thione (30) or 6-trifluoromethyl-1,2,4-triazino-[5,6b]indole-3-thione (31) as show in scheme below. Each of one of these products was alkylated with each of methyl chloride, ethylchloride and chlorocyclopropylmethane to form 3-methylthio- (32), 3-methylthio-6-trifluoromethyl- (33), 3-ethylthio- (34), 3-ethylthio-6-trifluoromethyl-(35) and 3-cyclopropylmethylthio-(36) and 3-cyclopropylmethylthio-6-trifluoromethyl-(37) 1,2,4-triazino-[5,6b]indole derivatives following a known procedure[23] Refluxing a mixture of 30 or 31 with hydrazine hydrate in HCl resulted in the formation of 3-hydrazo-1,2,4-triazino-[5,6-b]indole (38) or 3-hydrazo-6-trifluoromethyl-1,2,4-triazino-[5,6b]indole (39), which on reaction with sodium nitrite resulted in the formation of 3-azido derivatives. In the presence of HCl, formic, acetic,

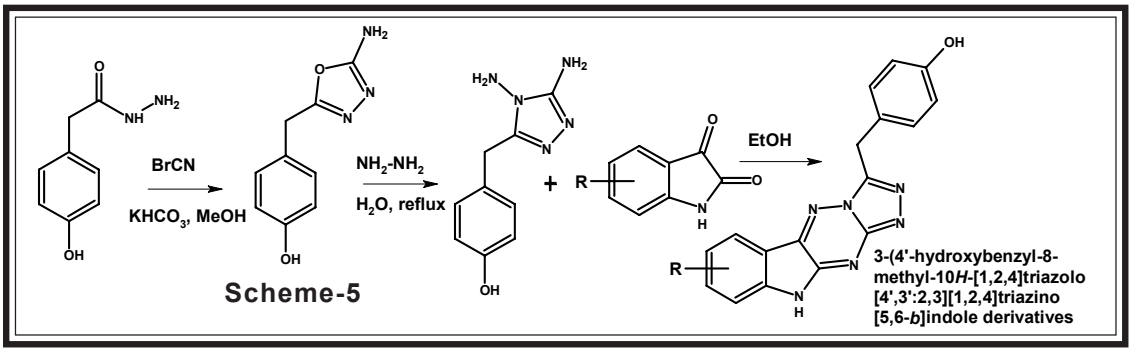
trifluoroacetic or chlorobenzoic acid, the hydrazo and azido derivatives cyclize to form fused tetrazole compounds, 5H-1,2,4-triazolo-[1',5',2,3]-1,2,4-triazino-[5,6-b]indole derivatives (40 and 41), 3-methyl- (42 and 43), 3-trifluoromethyl-(44) and 3,6-bis(trifluoromethyl)-(45) and 3-(2-chlorophenyl)- (46 and 47) 5H-1,2,4-triazolo-[1',5',,2,3]-1,2,4-triazino-[5,6-b]indole derivatives (Scheme-4).



Scheme 4. Reagents: (a) $NH_2CSNHNH_2$, K_2CO_3; (b) $NaNO_2$/HCl; (c) HCOOH; CF_3COOH; ClC_6H_4COOH in 4% NaOH.

Tomas Vijkovsky et al[81] have described general procedure for synthesis of 3-(4-hydroxybenzyl-10*H*-[1,2,4]triazolo[4',3':2,3][1,2,4]triazino[5,6-*b*]indole derivatives(Scheme-5) .

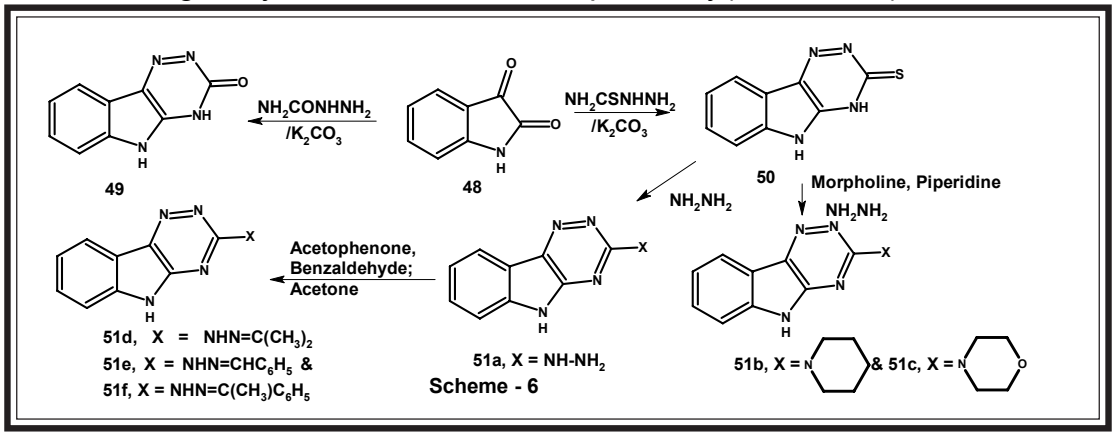
129.M.Badr, S.Mahgoub, A.Fahmey, F.Abdel-Latif,*Acta.Chim.Hung.*,**124**,413(1987)



Scheme-5

The synthetic approach suggested that p-hydroxyphenyl acetic acid methyl ester was condensed with neat hydrazine-hydrate to afford corresponding hydrazide. The hydrazide was treated with cyanogen bromide in the presence of potassium hydrogen carbonate to yield appropriate 1,3,4-oxadiazole, which on treatment with hydrazine hydrate would converted into 1,2-diamino-1,3,4-triazole derrivative. Condensation of later with substituted isatins afforded the 3-(4-hydroxybenzyl-10*H*-[1,2,4]triazolo[4',3':2,3][1,2,4]triazino[5,6-*b*]indole derivatives.

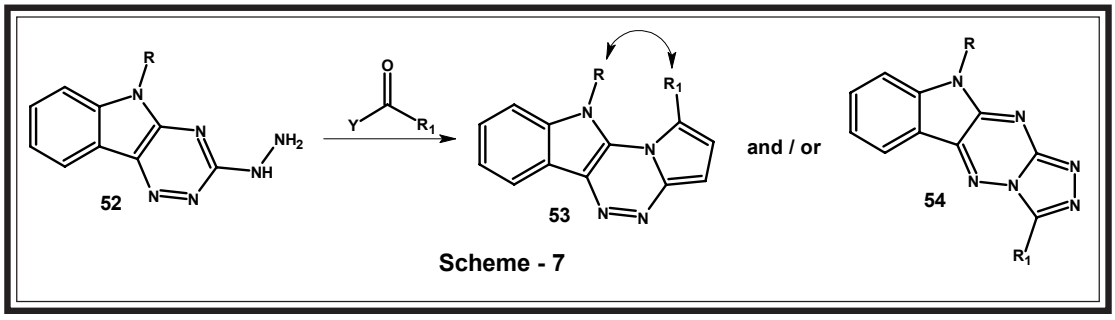
Monge et al[117a] have reported a series of 5H-1,2,4-triazino[5,6-b]indole derivatives of type 50 and 51 as per shown in scheme below. The 1,2,4-triazino[5,6-b]indoles (49 & 50) were obtained by cyclization of isatin(48) with semicarbazide or thiosemicarbazide in the presence of potassium carbonate. When a mixture of 50 and hydrazine hydrate was boiled, 90% of 51a was obtained. The compound was characterized as the free base and its monohydrochloride by elemental analysis and spectroscopic data (IR &^{1}H-NMR). Several other hydrazones, not reported earlier (51d, 51e, and 51f), were prepared by standard methods and also characterized. Upon boiling, solutions of 50 with piperidine and morpholine, compound 51b and 51c were obtained in good yields 80 and 83% respectively(Scheme-6).



Scheme - 6

Sterically Controlled Regiospecific Cyclization

Synthesis of 1,2,4-triazolo-1,2,4-trizino[5,6-b]indoles by cyclocondensation of various 3-hydrazono and 3-thiosemicarbazido derivatives of 1,2,4-triazino[5,6-b]indoles generated contradictions[130,131] regarding assignment of their correct structures. Products of heterocyclization of 5-substituted-3-hydrazino-1,2,4-triazino[5,6-b]indoles such as 52($R \neq H$) with one carbon cyclizing agents were assigned to either angularly annulated 1,2,4-triazolo[3',4':3,4]-1,2,4-triazino[5,6-b]indole 53 structure[16,17,132] or it's linearly annulated regioisomeric 1,2,4-triazolo[4',3':2,3]-1,2,4-triazino[5,6-b]indole 54[133-137](Scheme-7); in no case has the formation of a mixture of both regioisomers(53 and 54) been reported. Assignment of either of these two structures was based on only on electronic factors that enhance the nucleophilicities of N_2 and N_4 in the 1,2,4-triazine ring. Unexpectedly, steric factors that might dominate electronic influences in determinating the regiospecific(or regioselective) outcome of the reaction havebeen overlooked. **Inspection of molecular models indicated that cyclization of the N_5 unsubstituted hydrazine 52(R=H) with one carbon cyclizing reagents would only be electronically controlled and might, accordingly, lead to 53(R=H) or 54(R=H) depending on factors affecting the comparative nucleophilicities of N_2 or N_4 of the 1,2,4-triazine ring.**



Scheme - 7

Molecular models and computer optimized geometries (Figs.8-11)predicted that introduction of even the least bulky methyl group at N_5 (52,R = CH_3)would impose a steric control upon regiochemical outcome of cyclization. Proximity of C_1 and N_{10} substituents in the angular regioisomers (53, R=CH_3, R_1=CH_3, Ph)(Figs.8 & 9) would generate enough steric interaction to force the direction

of cyclization towards the formation of linear regioisomers(54, R = CH_3, R_1 = CH_3, Ph)(Figs. 10 & 11)which are free of unfavorable steric interactions.

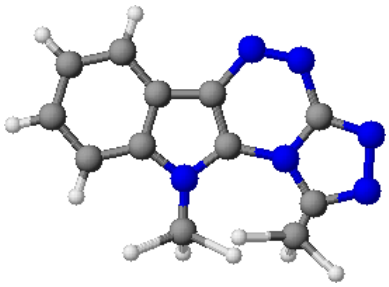


Fig.8 (R = R_1 = CH_3)

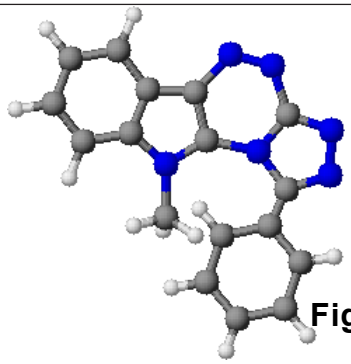


Fig.9 (R = CH_3, R_1 = Ph)

Computer optimized Geometry

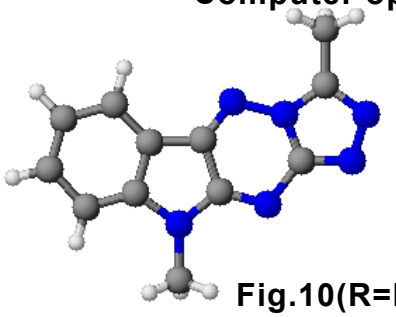


Fig.10(R=R_1=CH_3)

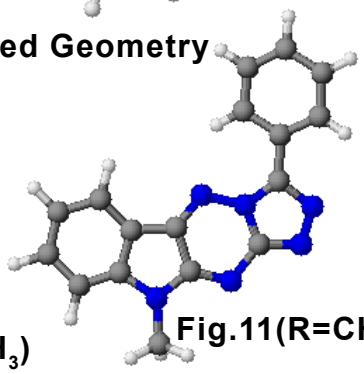
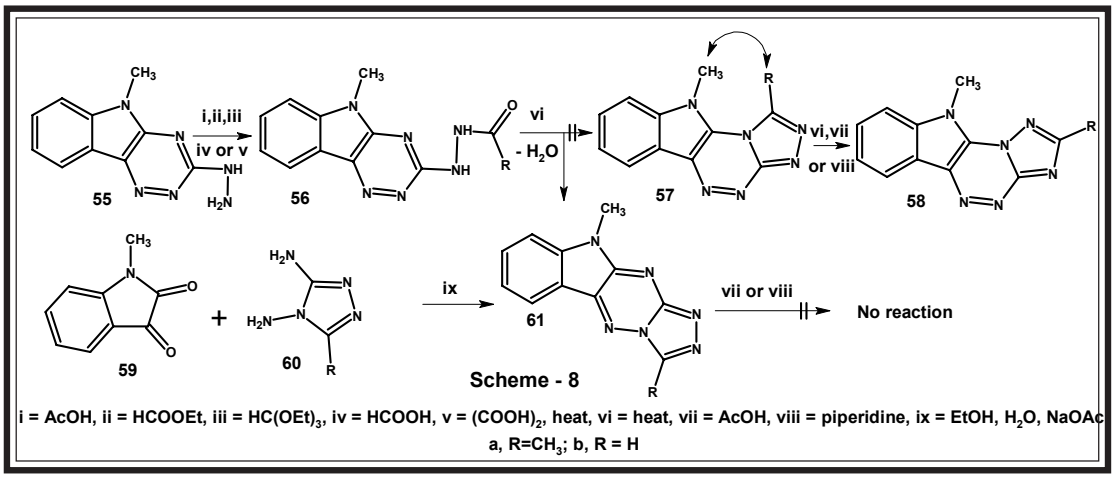


Fig.11(R=CH_3, R_1=Ph)

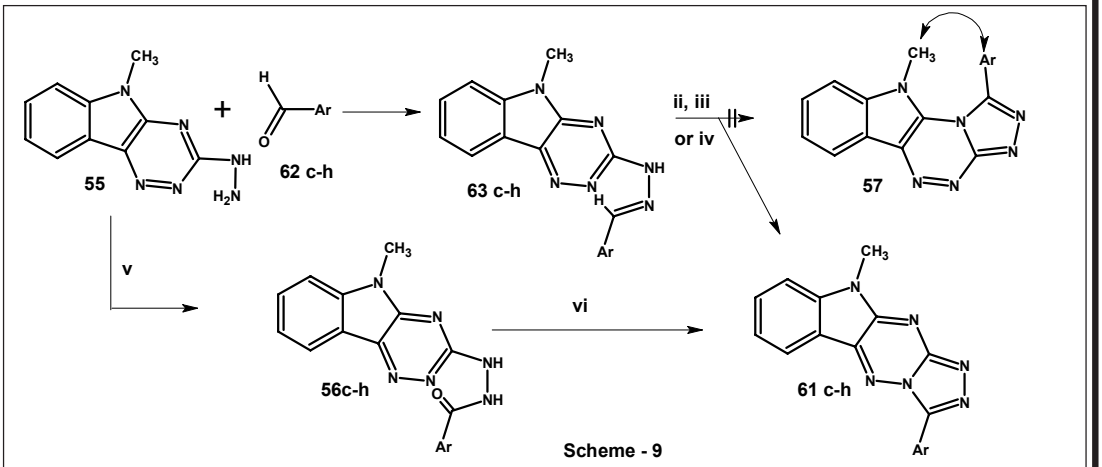
In order to validate or refute the aforementioned observations, Mohammed[135] some relevant heterocyclization reactions of 3-hydrazino-5-methyl-1,2,4-triazino[5,6-b]indole(55) have been studied[138]. In description, heating the later hydrazine with acetic acid(i) gave the single product which showed IR and ^{1}H NMR spectral data consistent with either of the angular or the linear tetracyclic structures 57a or 61a(Scheme-8), which must have been formed through the dehydrative cyclization of the unisolable hydrazide 56a. The UV spectrum of the product was identical with the spectrum of authentic samle 61a unequivocally prepared by cyclocondansation of 1-methylisatin (59) with 3,4-diamino-5-methyl-1,2,4-triazole(60a)[135]. Corraboration of the assigned linear structure 61a has been pinpointed by its failure to undergo Dimroth-like rearrangement upon heating with acetic acid or piperidine. This result agreed with a 1,2,4-triazolo[4,3-b]-1,2,4-triazine type of fusion (eg.61a), which was known to be unable to undergo Dimroth rearrange-ment and not with the 1,2,4-[3,4-c]-1,2,4-triazine type fusion (eg.57a)which undergoes facile rearrangement to yield thermodynamically

more stable 1,2,4-triazino[5,1-c]-1,2,4-triazine type compound 58a[139,140]. It was evident that the enhanced nucleophilicity of N_2 of triazine ring together with steric interruption by N_5 substituted methyl group in 55 operated synergistically toward the regiospecific formation of sterically prefered linearly annulated regioisomer 61a(Fig.3).



Cyclization of 55 with a methine moiety has been made under neutral conditions by heating with ethyl formate(ii) or triethylorthoformate(iii) as well as under acidic conditions by the heating with formic acid(iv) or oxalic acid(v). The intermediate obtained with ethyl formate showed NH and CONH IR absorption and a formyl hydrazino and a hydrazido ^{1}H NMR proton signals . This data along with elemental analysis indicated that the intermediate is the formayl hydrazino derivative 56b(scheme 8). Fusion of 56b (vi) caused its dehydrocyclization to a single product which was assigned the linearly annulated tetracyclic structure 61b rather than angularly annulated 57b on the following bases: (a) direct UV spectral comparison with an unequivocally prepared sample obtained by cyclocondansation between 1-methylisatin(59) and 3,4-diamino-5-methyl-1,2,4-triazole(60b)[135]. (b)resistance of the obtained product to undergo acid, base or thermaly induced Dimroth rearrangement to 58b. (c) proton of triazole ring showed ^{1}H NMR absorption in down field region at chemical shift value of 9.53 äppm which is closer to those (ä = 9.15-9.30) for the more deshielded proton of linearly annulated 1,2,4-triazolo[4,3-b]-1,2,4-triazines(61b) than proton of angularly cyclized 1,2,4-triazolo-[3,4-c]-1,2,4-triazines(57b)[139,141](Scheme-8).

Condensation of 55 with aromatic aldehydes(62c-h) gave the corresponding 3-arylidenehydrazino-1,2-4-triazinoindoles(63c-h)(scheme-9). Oxidative cyclization of 63c-h was accomplished by heating with 10% ethanolic iron(III)chloride, thionyl chloride or 30% aqueous sodium hypochloride solution in dioxane. All the three reagents produced same cyclic product from a perticular hydrazone, which was again assigned to be linear structure(61) and not the angular regioisomeric structure (57) on the basis of the afforementioned steric and electronic parameters and corroborated by their failure to undergo acid,base or thermally induced Dimroth rearrangement. Compound 61c was also prepared through the benzoylation of 55 with benzoyl chloride followed by the acid catalysed dehydrocyclization of the benzoylhydrazide intermediate (56c)(scheme 9).



Scheme - 9

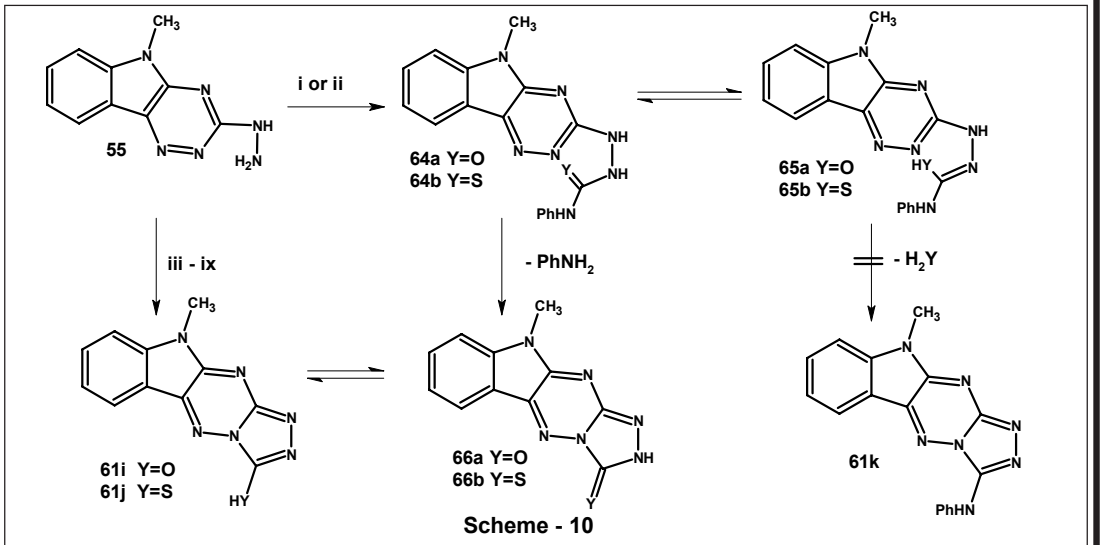
i = EtOH, heat, ii = 10%$FeCl_3$/EtOH, iii = $SOCl_2$, iv = 30% NaOCl/H_2O, dioxane, v = ArCOCl, pyr. RT, vi = AcOH, heat

c, Ar = C_6H_5; d, Ar = 4-$MeOC_6H_4$; e, Ar = 4-ClC_6H_4; f, Ar = 4-BrC_6H_4; g, Ar = 4-$Me_2NC_6H_4$; h, Ar = 4-$O_2NC_6H_4$

Reaction of 55 with phenyl isocyanate at 100°C afforded the phenylsemicarbazide 64a(Scheme 10). Heating 64a above its melting point gave only one product to which the 10-methyl-3-oxo-1,2,4-triazolo[4',3':2,3]-1,2,4-triazino[5,6-b]indole(66a)structure was assigned. Formation of 66a from 64a took place throughout the elimination of an aniline molecule. Under this thermal cyclization condition, aniline appears to be a better leaving entity than water since the 3-phenylamino derivative 61k has not been formed. Molecular models showed that, whereas the C_3 carbonyl and N_{10} methyl groups in the linear structure 66a were far apart, the C_1 carbonyl

and N_{10} methyl groups in the corresponding angular structure were in close proximity to each other and should suffer mild steric crowding. ^1H NMR spectra of 66a showed an exchangeable one-proton broad signal at ä = 5.30 ppm indicating that, amide-imidic acid equilibrium[142] exist in this compound. Compound 66a was also obtained directly by heating 4 with phenyl isocyanate, urea or ethyl chloroformate(Scheme 10)

Reaction of 55 with phenyl isothiocyanate gave the phenylthiosemicarbazide 64b which was thermally cyclized to the 10-methyl-3-thioxo-1,2,4-triazolo [4',3':2,3]-1,2,4-triazino[5,6-b]indole(66b). Compound 66b was also obtained by reacting 55 with phenyl isocyanate, thiourea, phenylthiourea or caron disulfide.



Scheme - 10

i = PhNCO/100°C, ii = PHNCS/100°C, iii = PhNCO, 185°C; iv = PhNCS, 185°C, v = $(H_2N)_2CO$, vi = $(H_2N)_2CS$, vii = $PhNHC(S)NH_2$, viii = ClCOOEt, ix = CS_2

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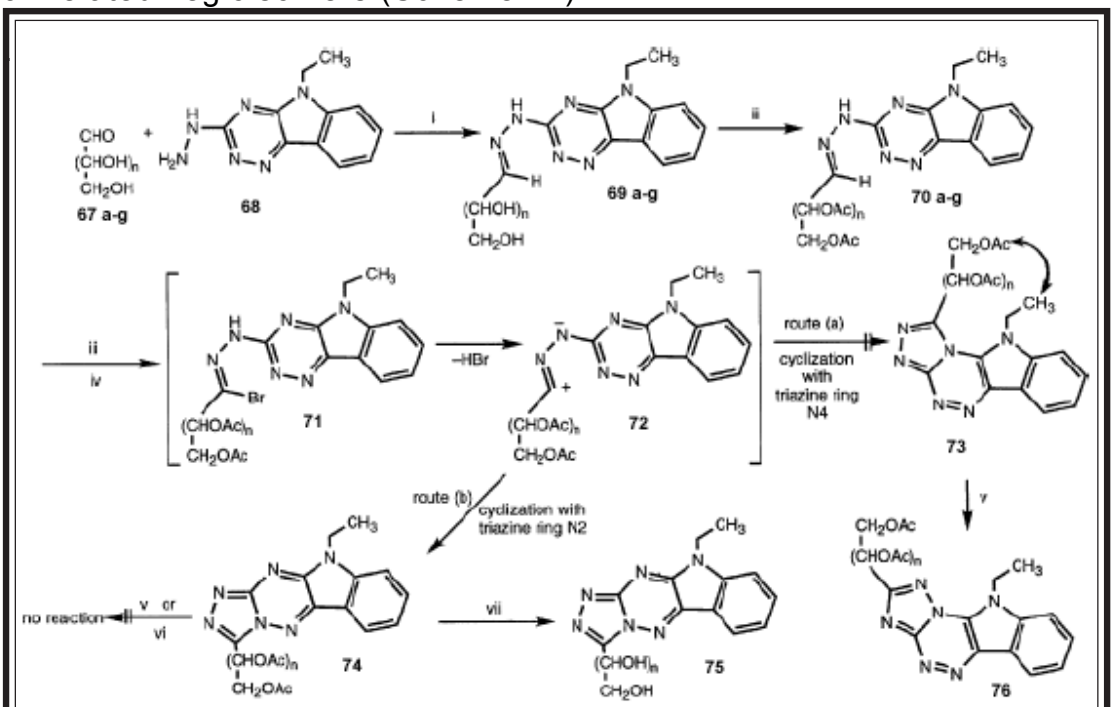
In continuation with this study, Shaban and Morgaan[143] have described that condensation of aldoses with 5-ethyl-3-hydrazino-1,2,4-triazino[5,6-b]indole gave the corresponding aldose-5-ethyl-1,2,4-triazino[5,6-b]indol-3-ylhydrazones which were acetylated to their poly-O-acetyl derivatives. The later underwent sterically controlled regiospecific oxidative cyclization with bromine in acetic acid and sodium acetate to sterically favourable linearly annelated 3-polyacetoxyalkyl-10-ethyl-1,2,4-triazole[40,30:2,3]-1,2,4-triazino[5,6-b]indoles rather than to their sterically unfavourable angularly annelated regioisomers.(Scheme-11)



Scheme11 Reagents and conditions: i, H_2O/EtOH, 100°C, 15 min, RT, 24 h; ii, Ac_2O/pyridine, RT, 24 h; iii, Br_2/AcOH; iv, NaOAc, RT, 2 h ; v, AcOH, reflux, 2 h; vi, fusion; vii, NH_4OH/MeOH, RT, 24 h $n = 3$: **a** = *D*-arabino-, **b** = *L*-arabino-, **c** = *D*-ribo-, **d** = *D*-xylo-; $n = 4$: **e** = *D*-galacto-, **f** = *D*-gluco-, **g** = *D*-manno-

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Oxidative cyclization of the hydrazone acetates 69a-g with bromine in acetic acid in the presence of anhydrous sodium acetate or one-pot oxidative cyclization afforded a single crystalline product. These spectroscopic data together with the elemental analyses of the cyclization products were in agreement both with the angularly annelated 1-polyacetoxyalkyl-10-ethyl-1,2,4-triazolo[3',4':3,4]-1,2,4-triazino[5,6-b]indole (73) and the linearly annelated 3-polyacetoxyalkyl-10-ethyl-1,2,4-triazole[4',3':2,3]-1,2,4-triazino[5,6-b]indole (74a-g) structure types (Scheme 11). Formation of 73 or 74 presumably took place via hydrazonoyl bromide (71) and nitrilimine (72) intermediates[144] as a result of nucleophilic attack of the triazine ring N_4 (route a) or N_2 (route b) on the nitrilimine carbon.

Previous results on heterocyclization of 5-hydrazino-1,2,4-triazino[5,6-b]indoles with one-carbon cyclizing reagents indicated that the choice of routes (a[5-7,17-19] or b[17,131,135-137,23,147-149]) depends on electronic factors that enhance the nucleophilicity of N4 or N2 of the triazine ring [130,16,138-140,150-155]. Surprisingly, steric factors that may dominate electronic factors in determining the regiospecificity (or regioselectivity) of this cyclization have been disregarded. In contrast, molecular models and computer optimized geometries predicted that cyclization of N_5-substituted 3-hydrazino-1,2,4-triazino[5,6-b]indoles (e.g. 68) with one-carbon cyclizing reagents would be sterically controlled to preferably produce the linearly annelated 3,10-disubstituted regioisomer (e.g. 74) rather than the angularly annelated 1,10-disubstitutesd regioisomer (e.g. 73). The angular structure (e.g. 73a) suffers crowding of the C_1 and N_{10} substituents (Fig. 12).

Entry 3 shows the adverse steric interaction between the ethyl group and the alditolyl chain even when the methyl portion of the ethyl group is directed away from the alditolyl chain. Entry 4 shows, on the other hand, that the linear structure (e.g. 74a) is free of such adverse steric interactions. This argument is taken to favor the assignment of the linearly annelated structure (74) to the cyclization products which also concords with electronic factors favoring cyclization at the more nucleophilic N_2 of the 1,2,4-triazine ring rather than at the less nucleophilic N_4 [138-140,149-154]. Evidently, both electronic

steric factors operated synergistically towards the regiospecific production of the linear isomer (74). Experimental evidences in favour of the linear structure 74 were discussed as per described in previous part of this study. Fortunately however, crystals of 10-ethyl-3-(4-methoxyphenyl)-1,2-4-triazolo[40,30:2,3]-1,2,4-triazino[5,6-b]indole (77), prepared by a similar oxidative cyclization of the corresponding hydrazone[156] were found amenable for X-ray analysis and shown to possess the linear and not the angular skeleton[157]. This X-ray analysis result may reasonably be extended to confirm the assigned linear structure 74.

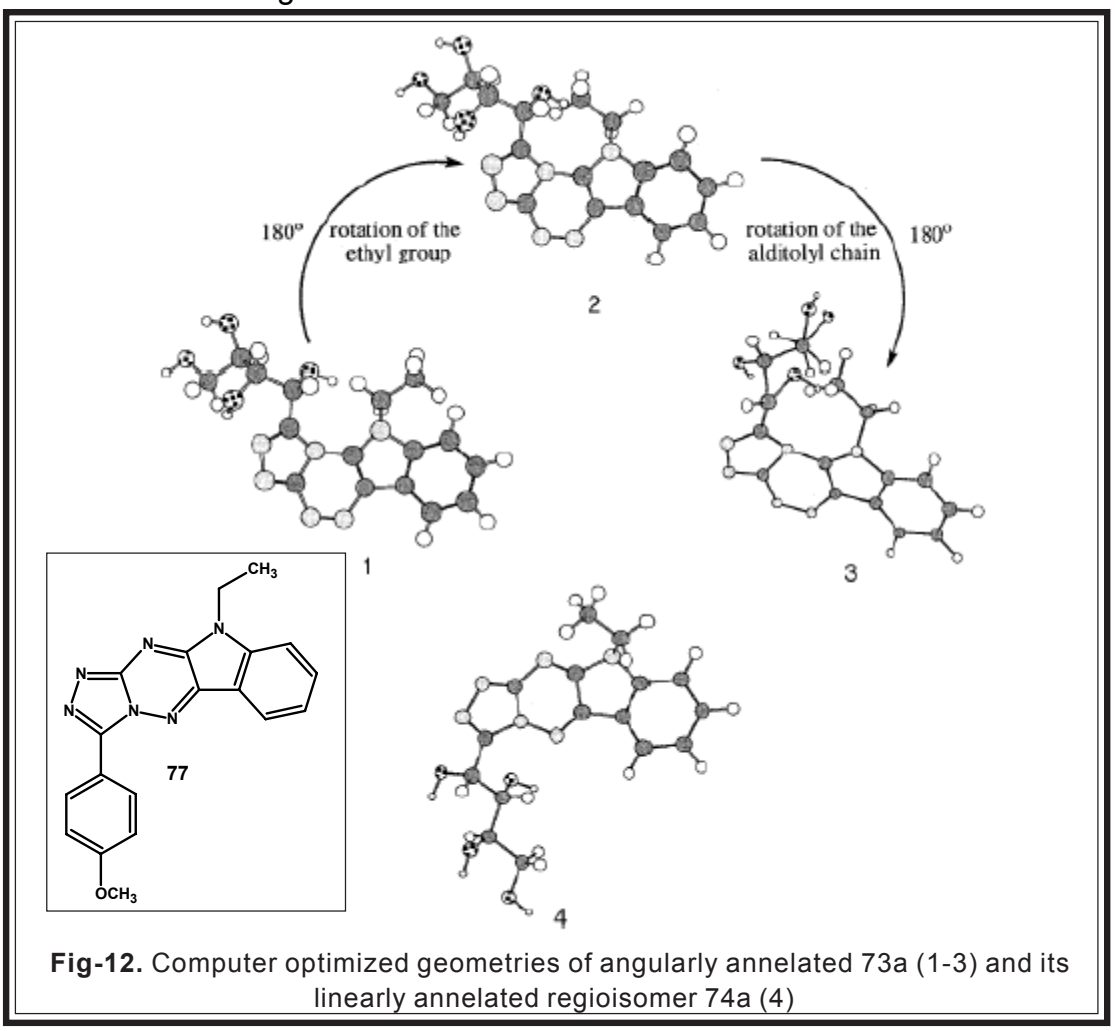

Fig-12. Computer optimized geometries of angularly annelated 73a (1-3) and its linearly annelated regioisomer 74a (4)

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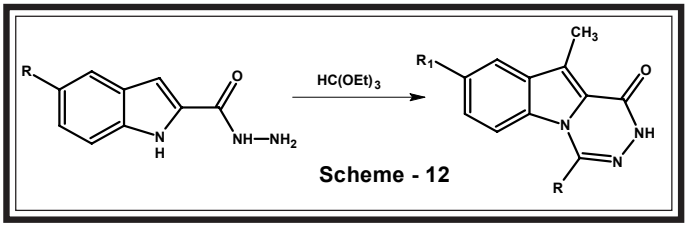
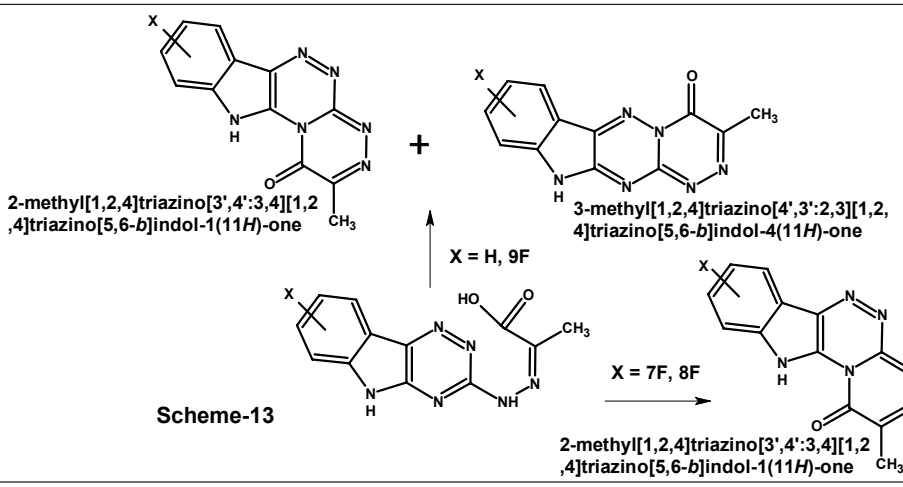
158.S.Rajur, A.Merwade, L.Basanagoudar, P. Kulkarni, *J.Pharm.Sci.*,**78**(9),780(1989)
159.A.Dandia,N.Ahmed, V.Sehgal, M.Saha, *Asian Journal of Chemistry*, **9**(3), 501(1997)

Rajur S.B. et al[158] have synthesized ten new 4,8-disubstituted 10-methyl-1,2-dihydro-1-oxo-1,2,4-triazino(4,5-a)indoles by refluxing various 5-substituted indole-2-carbohydrazides with triethylorthoformate or triethylorthoacetate in dimethylformamide. These derivatives were evaluated for their antimicrobial activity. Some of the title compounds possess fairly potent antimicrobial activity (Scheme-12).



Scheme - 12

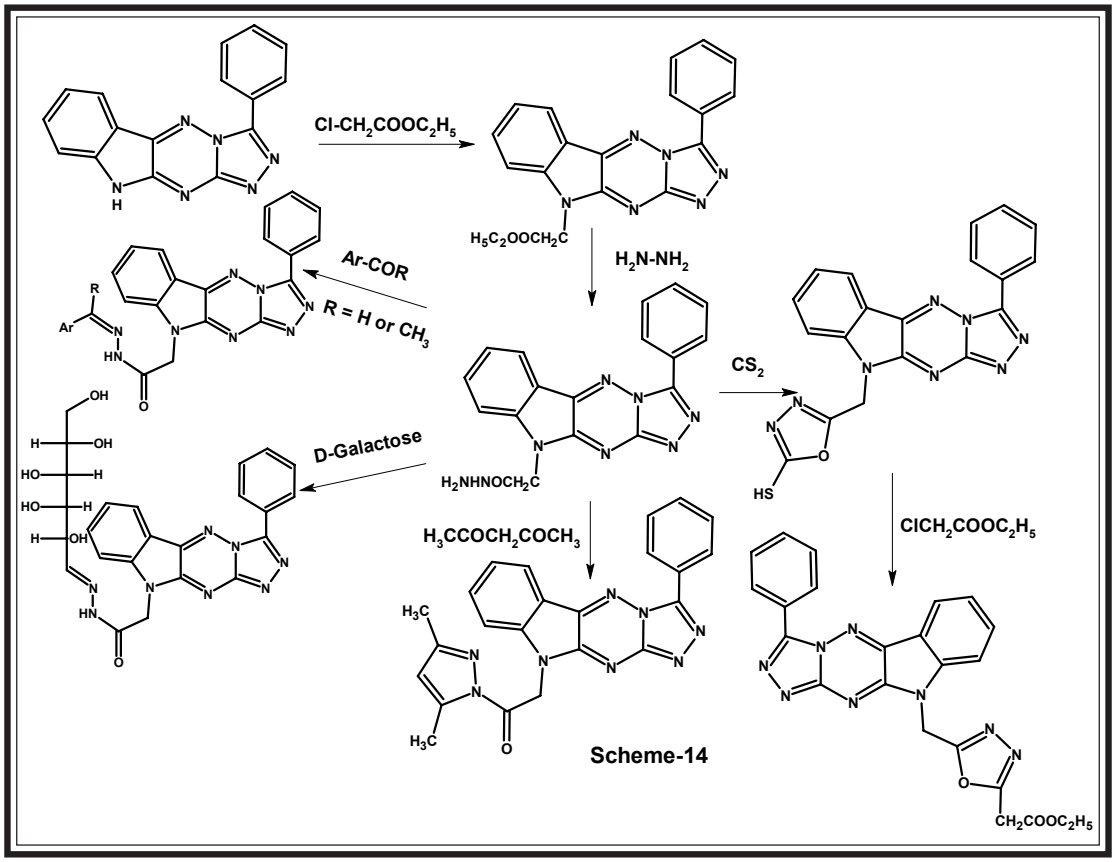
A.Dandia et al[159] have synthesized tetracyclic ring systems viz. 2-methyl-1-oxo-11H-1,2,4-triazino [32 ,42 :3,4] [1,2,4] triazino [5,6-b] indole and 3-methyl-4-oxo11H-1,2,4-triazino [42 ,32 :2,3] [1,2,4] triazino [5,6-b] indole by the cyclization of 2-oxo-propionic acid [5H-1,2,4-triazino] [5,6-b] indole-3-yl hydroazone. Cyclization of 7-fluoro- as well as 8-fluoro -2-oxo-propionic acid [5H-1,2,4-triazino] [5,6-b] indole-3-yl hydroazone afforded the angular product exclusively, while cyclization of defloro and 9-CF_3 substitution gave a mixture of angular and linear products in the ratio 3 : 1 (Scheme-13).



Scheme-13

N.Rashed et al[149] have suggested that reaction of 3-phenyl-10H-1,2,4-triazolo[4',3':2,3][1,2,4]triazino[5,6-b]indole with ethyl chloroacetate gave 10-carbethoxymethyl-3-phenyl-1,2,4-triazolo[4',3':2,3][1,2,4]triazino[5,6-b]indole. Condensation of the later with hydrazine hydrate gave (3-phenyl-1,2,4-triazolo[4',3':2,3][1,2,4]triazino[5,6-b]indol-10-yl)a cetylhydrazine.

Reactions with a number of aromatic aldehydes, acetophenone & D-galactose gave the corresponding hydrazones, while condensation with acetylacetone gave the pyrazole . Cyclization with CS_2 gave (3-phenyl-1,2,4-triazolo[4',3':2,3][1,2,4] triazino[5,6-b]indol- 10-yl)(2-thiol-1,3,4-oxadiazol-5-yl)methane, while reaction with ethyl chloroacetate gave the carbethoxy alkylated derivative (3-phenyl-1,2,4-triazolo[4',3':2,3][1,2,4]triazino[5,6-b]indol-10-yl)[2-(thiocarbethoxymethyl)1,3,4-oxadiazol-5-yl]methane (Scheme-14).



Scheme-14

Current Work

The importance of the indole nucleus is well established in pharmaceutical chemistry. Azoles and azines are well known for their diversed biological activities. It is also known that fusion of the heterocyclic nuclei enhances the pharmacological activities more than its parent nucleus.

On the other hand, as described in the chemistry part, cyclization of the N_5 unsubstituted 1,2,4triazino[5,6-b]indol-3-hydrazine derivatives with one carbon cyclizing reagents would only be electronically controlled and might, accordingly, lead to angularly annulated 1,2,4-triazolo[3',4':3,4]-1,2,4-triazino[5,6-b]indoles or it's linearly cyclized 1,2,4-triazolo[4',3':2,3]-1,2,4-triazino[5,6-b]indole derivatives depending on factors affecting the comparative nucleophilicities of N_2 or N_4 of the 1,2,4-triazine ring. In order to study the mode of cyclization of 1,2,4triazino[5,6-b]indol-3-hydrazine derivatives based on only on electronic factors, by affecting the comparative nucleophilicities of N_2 or N_4 of the 1,2,4-triazine ring, we have synthesized a series of angularly annulated 1,2,4-triazolo[3',4':3,4]-1,2,4-triazino[5,6-b]indole derivatives afforded by electronically controlled cyclization of N_5 unsubstituted 1,2,4triazino[5,6-b]indol-3-hydrazine derivatives by using thionyl chloride as cyclizing agent. All the synthesized compounds were well characterized by spectroscopic data and elemental analysis revealed to agree with the cyclization of the N_5 unsubstituted 1,2,4triazino[5,6-b]indol-3-hydrazine derivatives with one carbon cyclizing reagents would only be electronically controlled and because of annulation with thionyl chloride forced toN_5 comparatively become more nucleophilic, resulting into angularly cyclized 1,2,4-triazolo[3',4':3,4]-1,2,4-triazino[5,6-b]indoles.In addition to this the angular cyclization of the synthesized compounds were also confirmed by it's characteristic to undergo facile acid or base catalysed or thermally induced Dimroth rearrangement to convert into thermally stable rearranged 1,2,4-triazino[5,1-c]-1,2,4-triazine type product.

The reaction scheme involves four steps for the synthesis of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole in which first step deals with the condensation between isatin and thiosemicarbazide in the presence of potassium carbonate & water as solvent to yield 2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione, which underwent hydrazinolysis in second step, to afford as-triazino[5,6-b]indol-3-yl-hydrazine. In the third step different substituted aromatic aldehydes were condensed with 3-hydrazino-5*H*-[1,2,4]triazino[5,6-*b*]indole to give corresponding hydrazones.

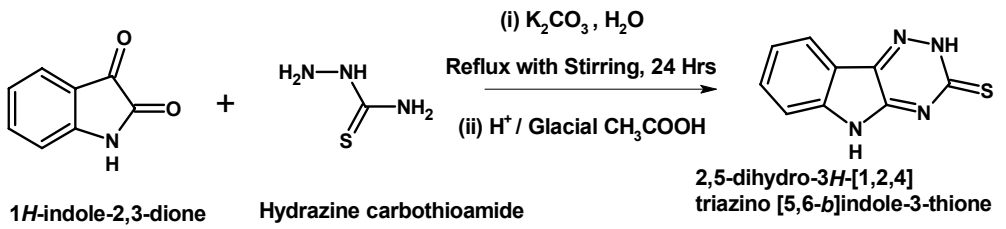
Cyclization of the above hydrazones using thionyl chloride affored angular cyclization to give 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4] triazino[5,6-*b*]indole, which were finally characterized by FT-IR, NMR and Mass spectral data.

The appearance of a base peak at m/z 346 in the mass spectrum of compound is consistant with its molecular formula $C_{18}H_{14}N_6O_2$. The appearance of an intense molecular ion peak also indicates the aromatic nature of this angularly fused Triazolotriazinoindole.

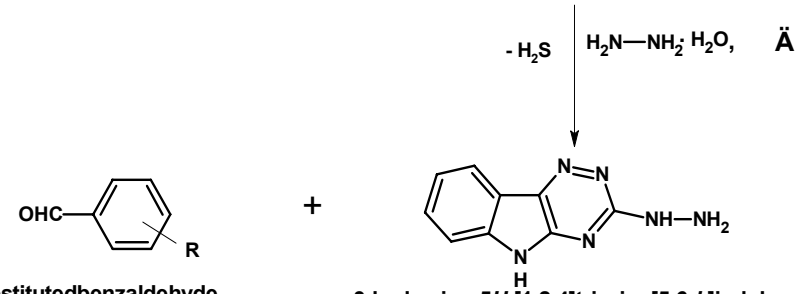
The molecular ion, in this case, underwent fragmentation with lose of C_7H_3N (m/z 102) which could be attributed to the benzonitrile diradical obtained by the initial fragmentation of the triazole ring and nitrogen molecule to give an ion at m/z 173. An ion at m/z 128 is obtained by the loss of nitrogen molecule and HCN. However, products obtained by cycliztion using bromine in acetic acid are found different and characterized as linearly cyclized compounds.

The newly synthesized compounds were screened for antitubercular, antimicrobial, anti HIV, antiinflammatory, anticancer and antihypertensive activities

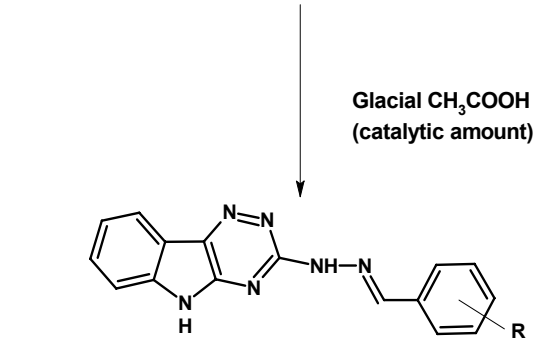
REACTION SCHEME



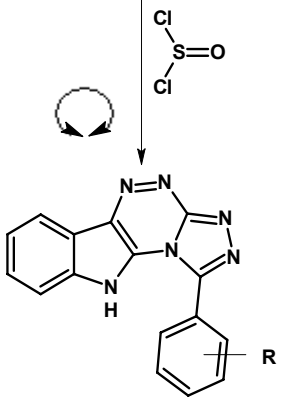
1*H*-indole-2,3-dione + Hydrazine carbothioamide → 2,5-dihydro-3*H*-[1,2,4] triazino [5,6-*b*]indole-3-thione



(Un)substitutedbenzaldehyde + 3-hydrazino-5*H*-[1,2,4]triazino[5,6-*b*]indole



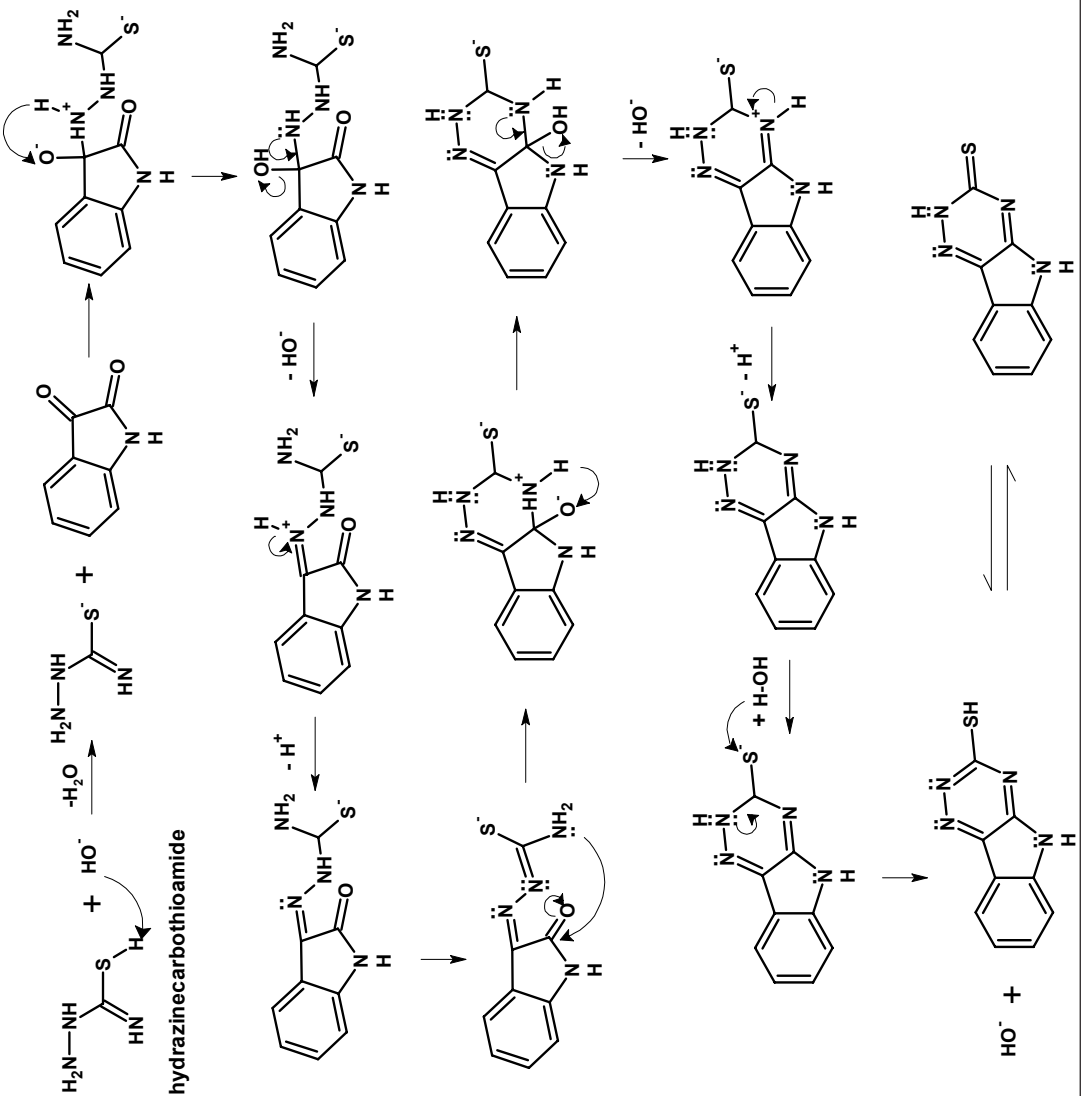
5*H*-[1,2,4]triazino[5,6-*b*]indol-3- arylhydrazones

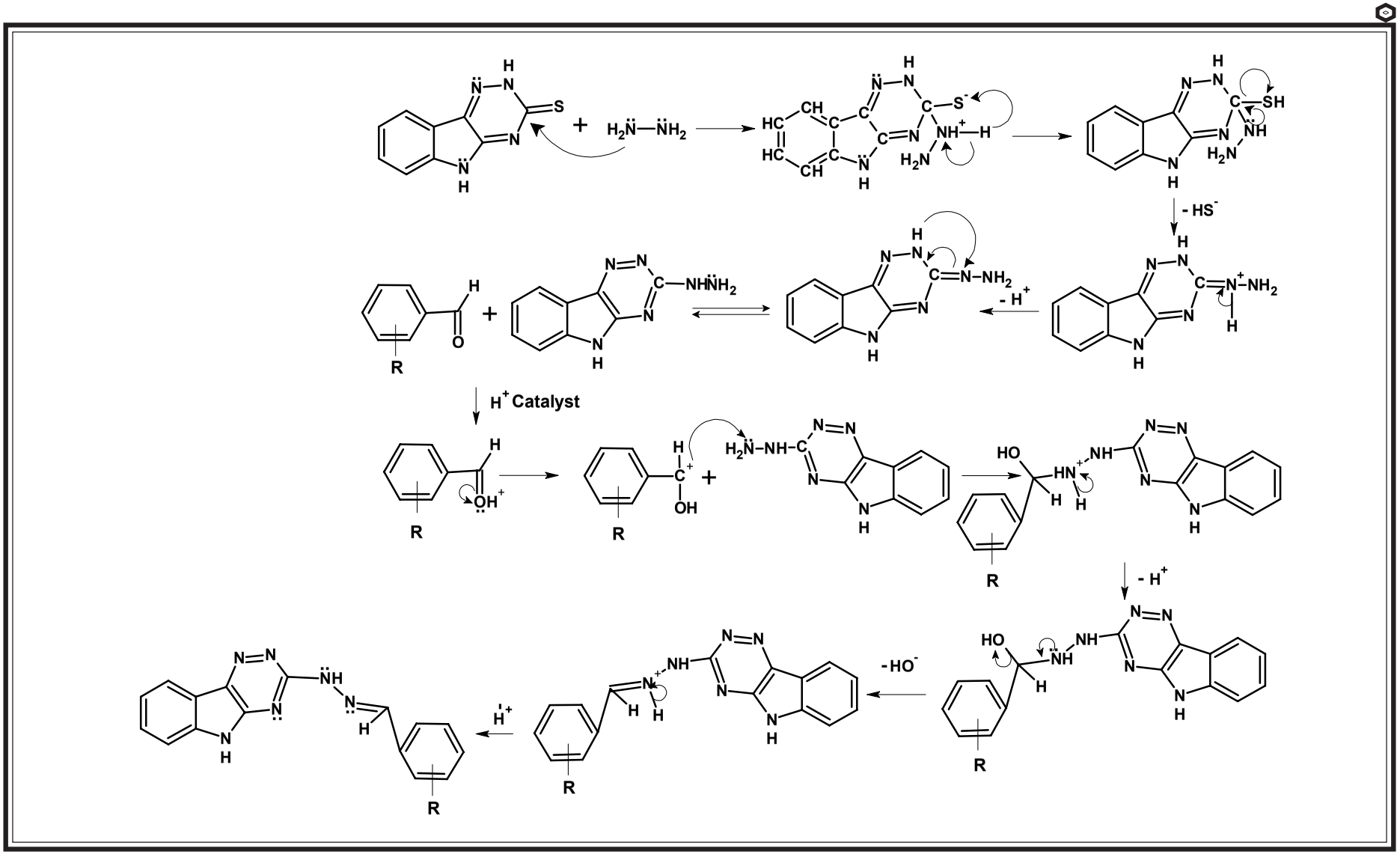


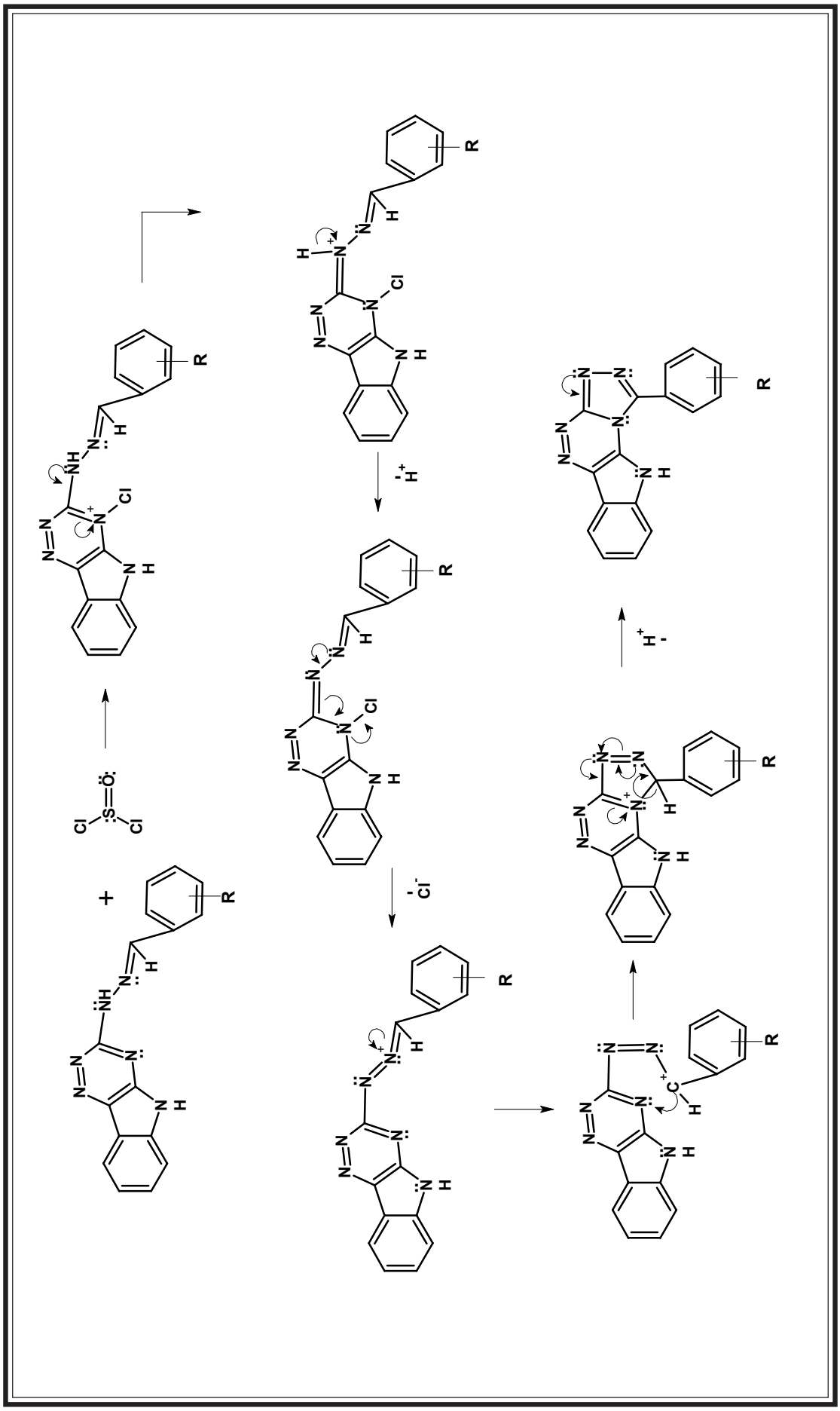
1-(un)sub.phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles

R = Different electron donating or electron withdrawing groups

REACTION MECHANISM




+ H2N—NH2
- HS-
H+ Catalyst
- H+
-HO-
H+



Experimental Protocols

All the starting materials, Isatin, thiosemicarbazide, hydrazine and substituted aromatic aldehydes were obtained from the commercial sources. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature using standard Zeal's thermometer and are uncorrected. The commencement of the reaction and purity of the synthesized compounds were ensured using thin layer chromatography (TLC) silica gel-G, used as stationary phase, the TLC plates were purchased from Merck India Ltd. Ethyl acetate: Hexane was used as the mobile phase. However, other solvent systems like acetone :benzene and chloroform:methanol were also employed but the best results observed with ethyl acetate:hexane system.

3-Hydrazino-as-triazino[5,6-b]indoles were used for the synthesis of linearly fused triazinoindoles, however treatment with nitrous acid was reported to yield angularly fused triazolotriazinoindoles. The present investigation deals with cyclisation of as-triazino[5,6-b]indolyl hydrazones in the presence of thionyl chloride to afford angularly fused triazolotriazinoindoles.

Experimental

Synthesis of 2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione:

The appropriate isatin (0.1mole), thiosemicarbazidde (0.11mole), potassium carbonate(0.15mole) and distilled water (500ml) were stirred and refluxed for 7-10hrs. On cooling, the mixture was filtered and acidified with acetic acid. The solid was filtered off, washed with water and dried. Recrystallized from dimethylformamide. Yield 78%, m.p. >300°C(Reported m.p. >300°C[24]). Purity was checked by TLC using ethylacetate:hexane(4:6) as mobile phase.

Synthesis of 3-hydrazino-5*H*-[1,2,4]triazino[5,6-*b*]indole :

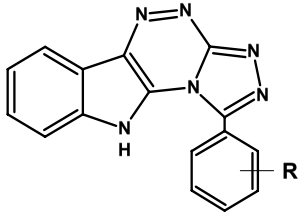
2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione (0.01mole) was refluxed with 99% Hydrazine hydrate (15ml) for 4 hrs. On cooling, crystals seperated which were filtered washed with ethanol to afford 1.4gms (71%) Yield. Recrystallized from dimethylformamide. Yield 60% m.p. 275-278°C(Reported m.p. >276-277°C[122]). Purity was checked by TLC using ethyl acetate:hexane (4:6) as mobile phase.

Synthesis of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4] triazino [5,6-*b*]indole(General Method):

A mixture of substituted benzaldehyde (0.01mole) and 3-hydrazino-5*H*-[1,2,4]triazino[5,6-*b*]indole (0.01mole) with catalytic amount of glacial acetic acid were refluxed in ethanol, on cooling it gives crystals of corresponding aryl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl-hydrazone in good yield(70-80%), which were purified by recrystallization from dimethylsulfoxide. Purity of each compound was checked by TLC using ethylacetate:hexane (5:5)as mobile phase. A mixture of appropriate hydrazone (0.3gms) and thionyl chloride (10ml) was refluxed on water bath for four hrs. The excess of thionyl chloride was removed by distillation under reduced pressure. The residue was washed with hot petroleum ether and cooled. The product so obtained was recrystallized from DMSO to yield powdery crystals of title compounds. The purity of each compound was checked by TLC using Ethyl acetate:Hexane (5:5) as mobile phase.

The elemental analysis of all the compounds performed by **PERKIN ELMER 470R** are in total agreement with the theoritical values.The physical constants and elemental analysis of all compounds were shown in **Table-1.1**

Table 1.1: Physical constants of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
1	KUIITT-1	3,4-Di OCH_3	$C_{19}H_{15}N_5O_2$	345.3	289-292	62.42 62.45	4.07 4.06	24.27 24.29
2	KUIITT-2	4-OCH_3	$C_{18}H_{13}N_5O$	315.3	282-285	64.55 64.53	3.82 3.81	26.57 26.58
3	KUIITT-3	4-SCH_3	$C_{18}H_{13}N_5S$	331.4	241-244	61.43 61.45	3.64 3.63	25.28 25.3
4	KUIITT-4	3-Br	$C_{16}H_9BrN_6$	365.2	>310	52.62 52.65	2.48 2.48	23.01 23.03
5	KUIITT-5	4-F	$C_{16}H_9FN_6$	304.3	164-165	63.16 63.18	2.98 2.97	27.62 27.63
6	KUIITT-6	2-Cl	$C_{16}H_9ClN_6$	320.7	247-249	59.92 59.9	2.83 2.82	26.20 26.21

Note:- The underline values denote the calculated percentage of composition

Table 1.1: Physical constants of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
7	KUITT-7	4-Cl	$C_{16}H_9ClN_6$	320.7	>310	59.92 59.9	2.83 2.82	26.20 26.21
8	KUITT-8	3-NO_2	$C_{16}H_9N_7O_2$	331.3	>310	58.01 58.03	2.74 2.75	29.60 29.61
9	KUITT-9	4-NO_2	$C_{16}H_9N_7O_2$	331.3	253-255	58.01 58.03	2.74 2.75	29.60 29.61
10	KUITT-10	H	$C_{16}H_{10}N_6$	286.3	221-224	67.12 67.1	3.52 3.51	29.35 29.36
11	KUITT-11	2-NO_2	$C_{16}H_9N_7O_2$	331.3	279-282	58.01 58.03	2.74 2.75	29.60 29.61
12	KUITT-12	3-OC_6H_5	$C_{22}H_{14}N_6O$	378.4	283-285	69.83 69.8	3.73 3.74	22.21 22.2
13	KUITT-13	2,5-DiOCH_3	$C_{19}H_{15}N_5O_2$	345.3	249-250	62.42 62.41	4.07 4.05	24.27 24.25
14	KUITT-14	3-OCH_3	$C_{18}H_{13}N_5O$	315.3	205-207	64.55 64.52	3.82 3.83	26.57 26.54

Note:- The underline values denote the calculated percentage of composition

Table 1.1: Physical constants of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
15	KUITT-15	2-OCH_3	$C_{18}H_{13}N_5O$	315.3	225-228	64.55 64.52	3.82 3.83	26.57 26.54
16	KUITT-16	3-Cl	$C_{16}H_9ClN_6$	320.7	234-237	59.92 59.9	2.83 2.82	26.20 26.21
17	KUITT-17	2-OH	$C_{16}H_{10}N_6O$	302.3	301-305	63.57 63.54	3.33 3.32	27.80 27.83
18	KUITT-18	4-OH	$C_{16}H_{10}N_6O$	302.3	>310	63.57 63.54	3.33 3.32	27.80 27.83
19	KUITT-19	3-OH	$C_{16}H_{10}N_6O$	302.3	254-257	63.57 63.54	3.33 3.32	27.80 27.83
20	KUITT-20	3-OCH_3, 4-OH	$C_{17}H_{12}N_6O_2$	332.3	258-262	61.44 61.47	3.64 3.63	25.29 25.32
21	KUITT-21	4-CH_3	$C_{17}H_{12}N_6$	300.3	287-291	67.99 67.95	4.03 4.02	27.98 27.96
22	KUITT-22	9-Anthryl	$C_{24}H_{14}N_6$	386.4	>300	74.60 74.63	3.65 3.66	21.75 21.72

Note:- The underline values denote the calculated percentage of composition

SPECTRAL CHARACTERIZATION

The constitution of newly synthesized compounds were supported by FT - IR, ^{1}H NMR and EI-Mass spectral study.

IR Spectral Study

Instrument: SHIMADZU FT-IR 8400 Spectrophotometer

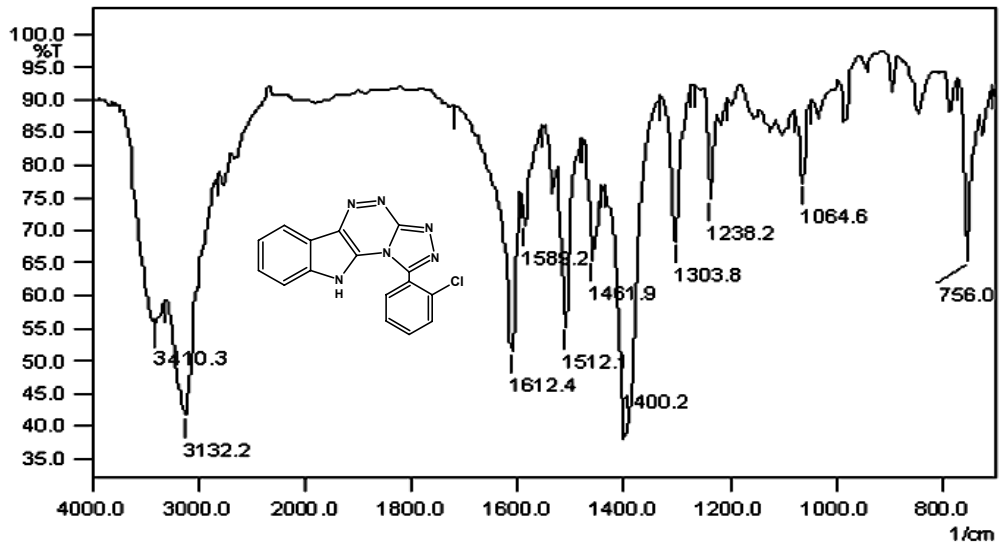
Sample technique: KBr pellet

Frequency range: 400-4000 cm^{-1}

The 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles (KUITT Series) indicates the presence of very specific functional groups like 2^{o}amino group of indole skeleton, azo group of triazine residue, C=N vibration of triazole ring, aromatic ring skeleton etc., which absorb IR radiations of specific frequency and show sharp, medium or weak intense signals and hence supports in identifiction and confirmation of the molecule.

The IR spectrums of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles shows common frequences as 3430-3350cm^{-1} for 2^{o}amino group, 3150-3100cm^{-1} for aromatic C-H str.,1620-1605cm^{-1} for C=Nstr., N=N(azo)str. merges with aromatic ring skeleton observed between 1590-1510cm^{-1} where as aromatic region was observed extended upto 1450cm^{-1}. Tertiary N-Cstr. was observed range between 1400-1300cm^{-1},while medium intense bands between 1370-1220cm^{-1} for secondary N-C str. mono,di & tri Phenyl substitutions observed between 970-730cm^{-1}. While characteristic vibration corresponds to substitution at phenyl residue was clearly observed in each case. The IR spectral data of all compounds of KUITT series were shown in **Table-1.2**

IR Spectrum of 1-(2-chloro)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (KUITT-6)



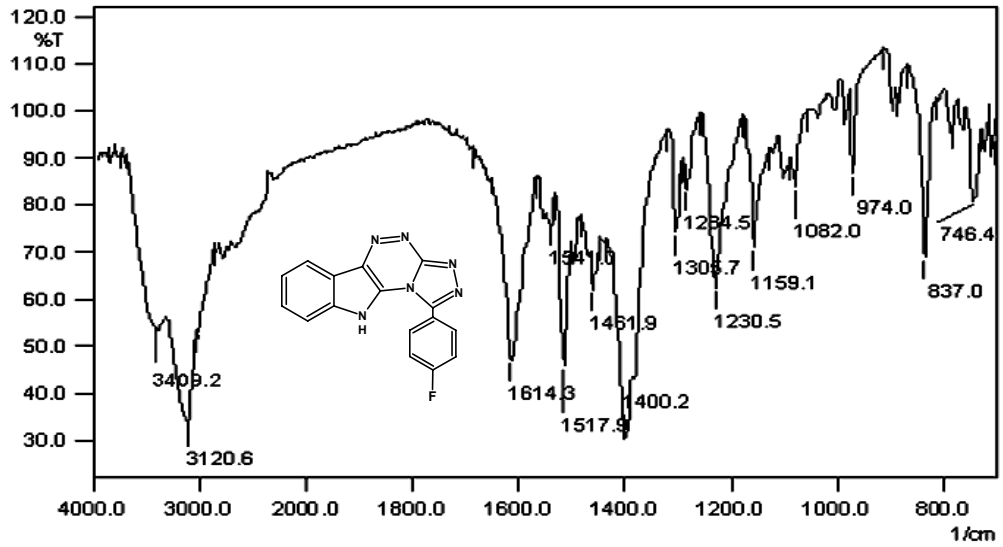
KUITT-6

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3410
-CH Aromatic	-C-H str.	3132
-C=N	C=N str.	1612
Azo / Aromatic Skeleton	-N=N-str. / -C=C- str.	1588,1512,1461
	1:2 di substitution	735
3°N-C	-N-C str.	1303
2°N-C	-N-C str.	1238
Halogen	-C-Cl str.	756

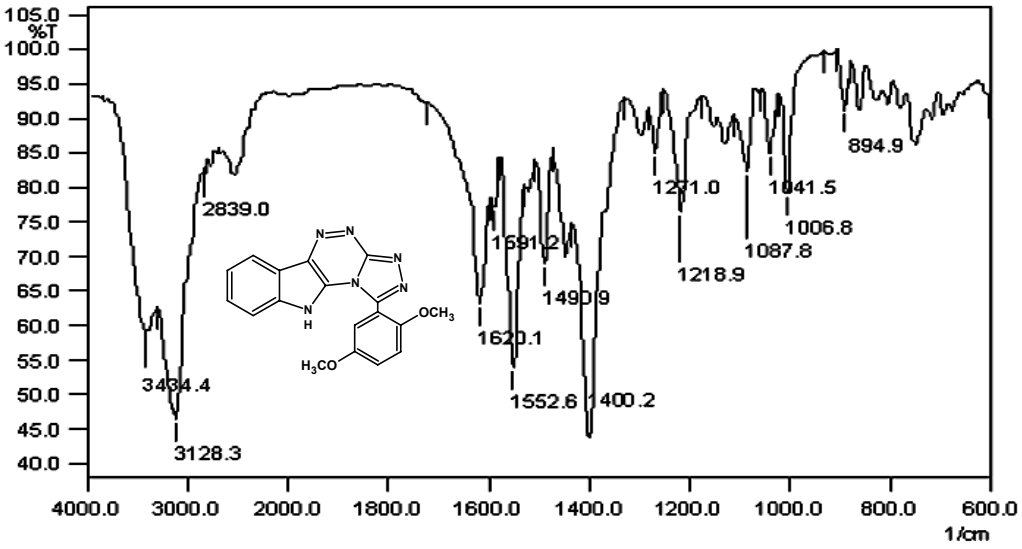
KUITT-5

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3410
-CH Aromatic	-C-H str.	3132
-C=N	C=N str.	1612
Azo / Aromatic Skeleton	-N=N-str. / -C=C- str.	1588,1512,1461
	1:2 di substitution	735
3°N-C	-N-C str.	1303
2°N-C	-N-C str.	1238
Halogen	-C-Cl str.	756

IR Spectrum of 1-(4-fluoro)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (KUITT-5)



IR Spectrum of 1-(2,5-dimethoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole



KUITT-13

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3434
-CH Aromatic	-C-H str.	3128
-C=N	C=N str.	1620
Azo / Aromatic Skeleton	-N=N-str. / -C=C- str.	1552,1490,1450
	1:2:5 tri substitution	894
3^{o}N-C	-N-C str.	1400
2^{o}N-C	-N-C str.	1271
$-OCH_3$	-C-Hstr, -C-O-C str.	2839, 1218

KUITT-15

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3423
-CH Aromatic	-C-H str.	3124
-C=N	C=N str.	1616
Azo / Aromatic Skeleton	-N=N-str. / -C=C- str.	1587,1550,1483
	1:2 di substitution	735
3^{o}N-C	-N-C str.	1400
2^{o}N-C	-N-C str.	1253
$-OCH_3$	-C-H str.,-C-O-C-str.	2974,1217

IR Spectrum of 1-(2-methoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoleKUITT-15)

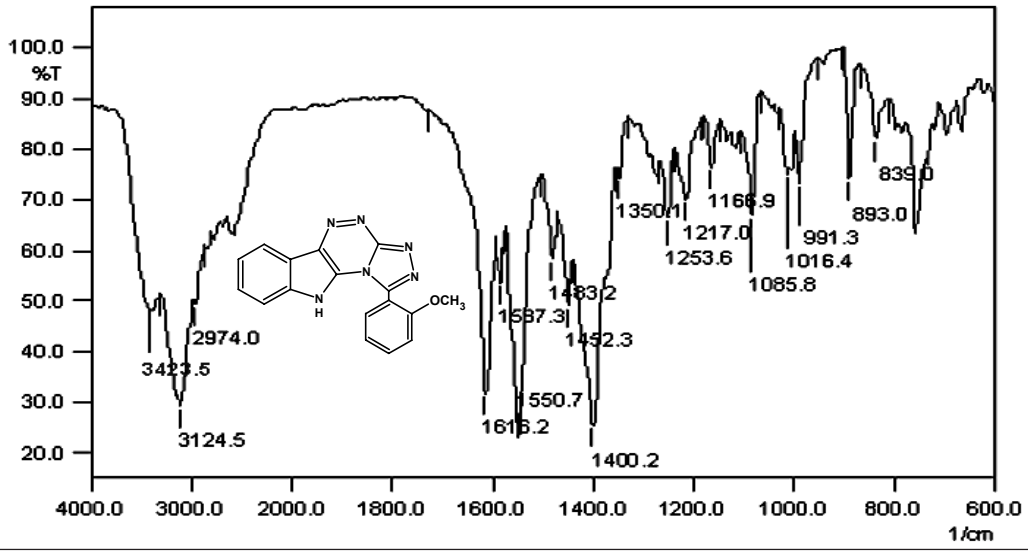


Table 1.2 :IR Spectral data of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles

Code	Substitution (R)	-NH Str.	Ar-H Str.	>C=N Str. / -N=N- str / Aromatic Region (>C=C< str)				>C-N< Str. (3° amine)	>C-NH< Str. (2° amine)	Phenyl Substitution	Substitution
KUITT-1	3,4-diOCH_3	3352	3128	1611	1517	1487	1463	1400	1298	744 (1:3:4 tri sub)	3016,1263,1234 (C-H,C-O-C str)
KUITT-2	4-OCH_3	3412	3128	1612	1581	1558	1504	1400	1257	840 (1:4 di sub.)	2920, 1267 (C-H, C-O-C str)
KUITT-3	4-SCH_3	3396	3124	1618	1548	1600	1589	1400	1367	891 (1:4 di sub.)	2953, 709 (C-H, C-S str)
KUITT-4	3-Br	3409	3101	1616	1535	1500	1465	1384	1299	898 (1:3 di sub.)	748 (C-Br str)
KUITT-7	4-Cl	3410	3118	1618	1537	1535	1470	1308	1238	829 (1:4 di sub)	750 (C-Cl str)
KUITT-8	3-NO_2	3409	3116	1620	1523	1508	1455	1353	1299	875 (1:3 di sub)	1353 (-N=O str., merge)
KUITT-9	4-NO_2	3394	3116	1620	1523	1504	1461	1400	1299	856 (1:4 di sub.)	1350 (-N=O str.)
KUITT-10	H	3425	3114	1614	1587	1537	1485	1386	1230	750 (mono sub)	-
KUITT-11	2-NO_2	3404	3124	1610	1587	1523	1465	1400	1220	846 (1:3 di sub)	1344 (-N=O str.)

Table 1.2 :IR Spectral data of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indoles

Code	Substitution (R)	-NH Str.	Ar-H Str.	>C=N Str. / -N=N- str / Aromatic Region (>C=C< str)				>C-N< Str. (3° amine)	>C-NH< Str. (2° amine)	Phenyl Substitution	Substitution
KUITT-12	3-OC_6H_5	3434	3126	1610	1583	1562	1506	1400	1225	750 (1:3 di sub)	1267 (Ar-O-Ar str.)
KUITT-14	3-OCH_3	3404	3143	1608	1554	1565	1456	1400	1315	885 (1:3 di sub)	2949,1222 (C-H, C-O-C str)
KUITT-16	3-Cl	3419	3132	1608	1585	1522	1477	1400	1232	896 (1:3 di sub)	758 (C-Cl str)
KUITT-17	2-OH	3404	3120	1614	1589	1562	1539	1400	1253	740 (1:2 di sub)	3423, 1230 (O-H merge, C-O str.)
KUITT-18	4-OH	3406	3136	1608	1554	1500	1458	1377	1272	844 (1:4 di sub.)	3423, 1230 (O-H merge, C-O str.)
KUITT-19	3-OH	3304	3136	1610	1554	1490	1458	1400	1319	877 (1:3 di sub.)	3305, 1215 (O-H merge, C-O str.)
KUITT-20	3-OCH_3, 4-OH	3404	3128	160	1560	1502	1460	1400	1288	788 (1:3:4 tri sub.)	3404,2927,1197 (O-H merge, C-H, C-O str.)
KUITT-21	4-CH_3	3419	3126	1610	1554	1498	1460	1373	1220	844 (1:4 di sub)	2922,1400 (C-H str., C-H i.p. def.)
KUITT-22	9-Anthracyl	3404	3128	1614	1554	1537	1480	1400	1271	759 (mono sub)	-

^{1}H NMR Spectral Study

^{1}H NMR Spectrums of the 1-substituted phenyl-10*H*-[1,2,4]triazolo [3',4':3,4][1,2,4]triazino[5,6-*b*]indoles show signals relevant to the number of protons and their electronic environment known as chemical shift. Chemical shift may move to either upfield (Shielding) or down field(deshielding), according to the electronic environment of the corresponding proton. In addition to this, each ^{1}H NMR signal further splitted into number of subpeaks according to the number of neighbouring protons present in the skeleton of molecule. The splitting of the NMR signal further provides signification about the degree of interaction between neighbouring protons by means of spin-spin coupling constant J.

Instrument : BRUKER AC 300MHz FT-NMR

Internal Reference : TMS Solvent : $CDCl_3$ or DMSO d_6

1-(3,4-Dimethoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino [5,6-*b*]indole(KUITT-1)

^{1}H NMR Spectrum of KUITT-1 shows two singlets, each relevant to three protons of two methoxy substituents occupied at 3,4-position of phenyl residue at chemical shift δ 3.88 and δ 3.92 respectively. While a singlet for the proton of an amine group appears at δ 12.28. Protons $H_{(c),}$ $H_{(b)}$ & $H_{(a)}$ of the phenyl residue shows a singlet at δ 7.995 , a doublet at δ 7.219 with J value of 8.4Hz and a doublet at δ 8.084 with J value of 6Hz respectively, which reveals that $H_{(b)}$ & $H_{(a)}$ are in ortho position to each other where as $H_{(a)}$ & $H_{(c)}$ are relatively in meta position.. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ 8.229 with J value 7.5Hz, a triplet at δ 7.37 with J value of 7.8 & 7.2Hz, a triplet at δ 7.74 with J value of 7.2 & 7.2Hz and a doublet at δ 7.461 with J value 8.1Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

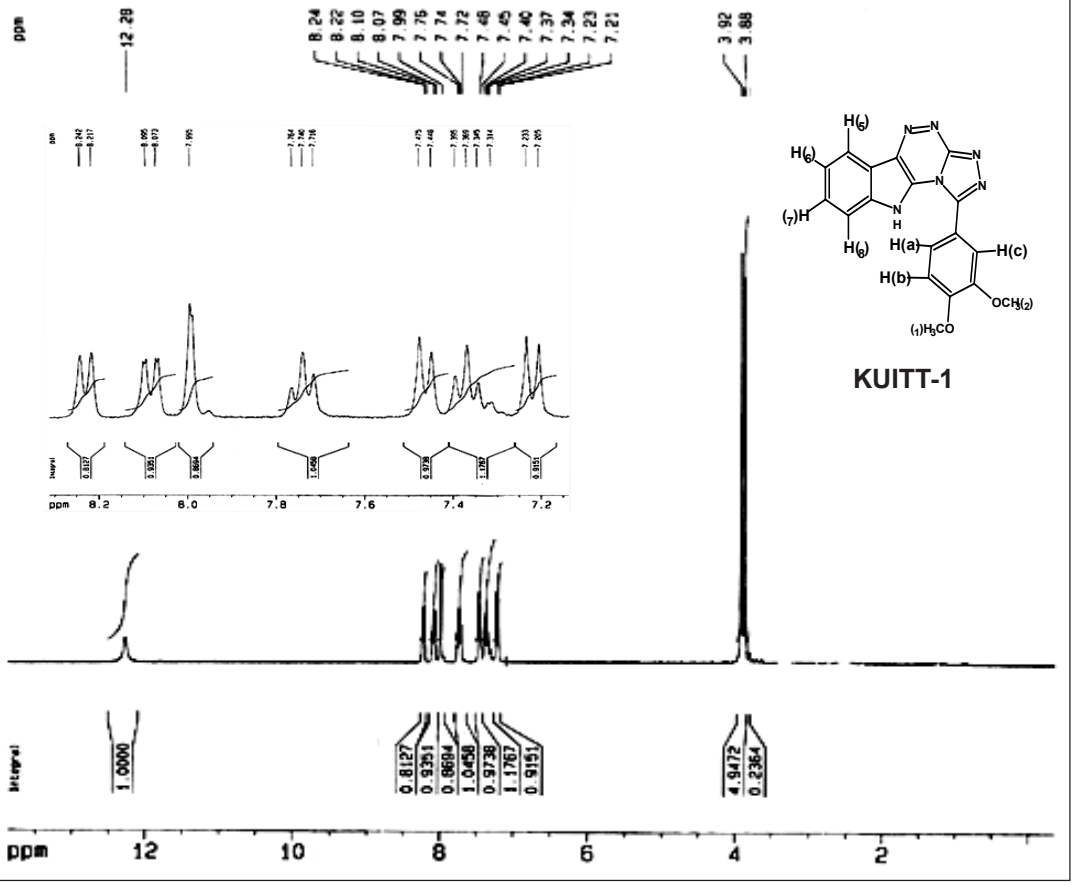
1-(4-Methoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole(KUITT-2)

^{1}H NMR Spectrum of KUITT-2 shows a singlet relevant to three protons of a methoxy substituent at chemical shift value δ 3.9 ppm. A singlet due to secondary amine proton appears at δ13.04. Phenyl residue substituted at triazole ring possess two pair of chemically equivalent but magnetically non-euivalent protons viz. $H_{(d)}$ & $H_{(e)}$ appear as two doublet at δ 8.389 with J value of 9Hz and 7.248δ ppm with J value of 8.7Hz respectively. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ 8.337 with J value 7.8Hz, a triplet at δ 7.464 with J value of 7.5 & 7.5Hz, a triplet at δ 7.827with J value of 7.5 & 8.1Hz and a doublet at δ 7.584δ with J value 8.1Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

1-phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (KUITT-10)

^{1}H NMR Spectrum of KUITT-10 shows a singlet the proton of an amine group appears at δ13.03. Phenyl residue substituted at triazole ring possess five protons among which proton $H_{(b)}$ & $H_{(c)}$ signals with integration value of three protons, were merge due to close electronic environment, hence appears as multiplet at δ 7.677, while $H_{(a)}$ shows a doublet at δ 8.436 with J value of 6.6Hz. Protons $H_{(5)}$, $H_{(6)}$,$H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ 8.348 with J value 7.8Hz, a triplet at δ 7.454 ppm with J value of 7.5 & 7.5Hz, a triplet at δ7.823 with J value of 7.5 & 7.8Hz and a doublet at δ 7.583 with J value 7.8Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

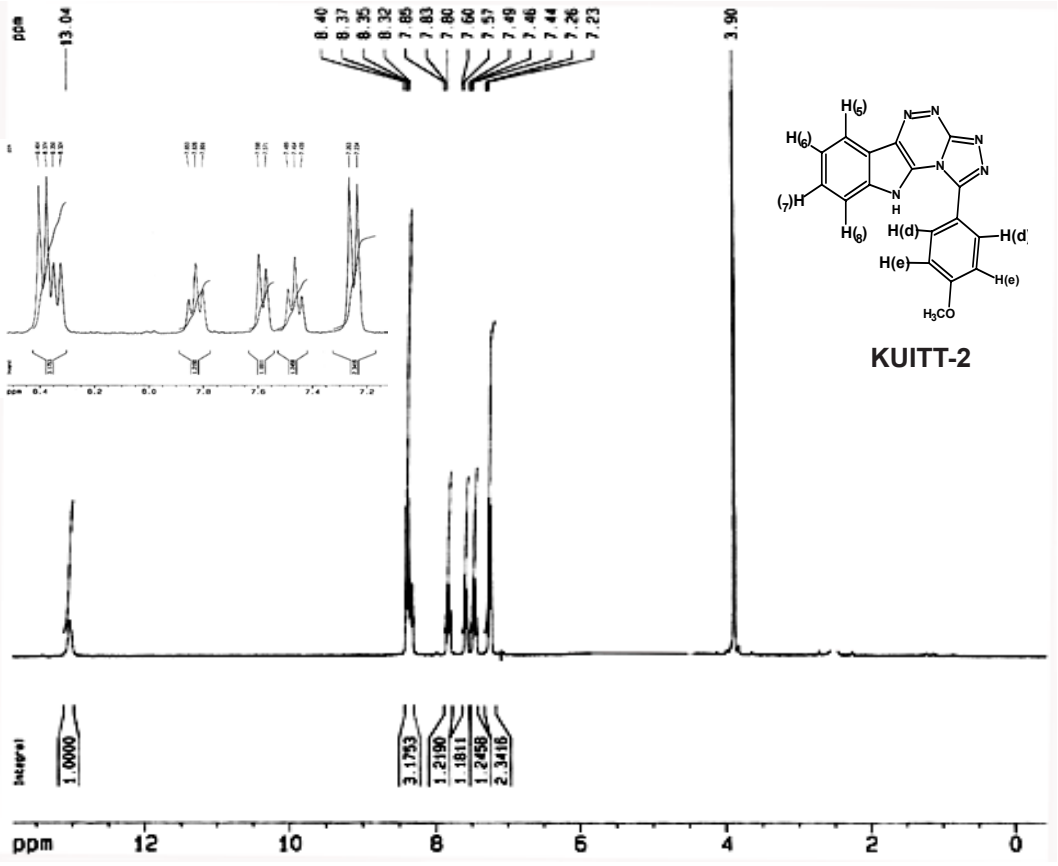
^{1}H NMR Spectrum of 1-(3,4-dimethoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (KUITT-1)



KUITT-1

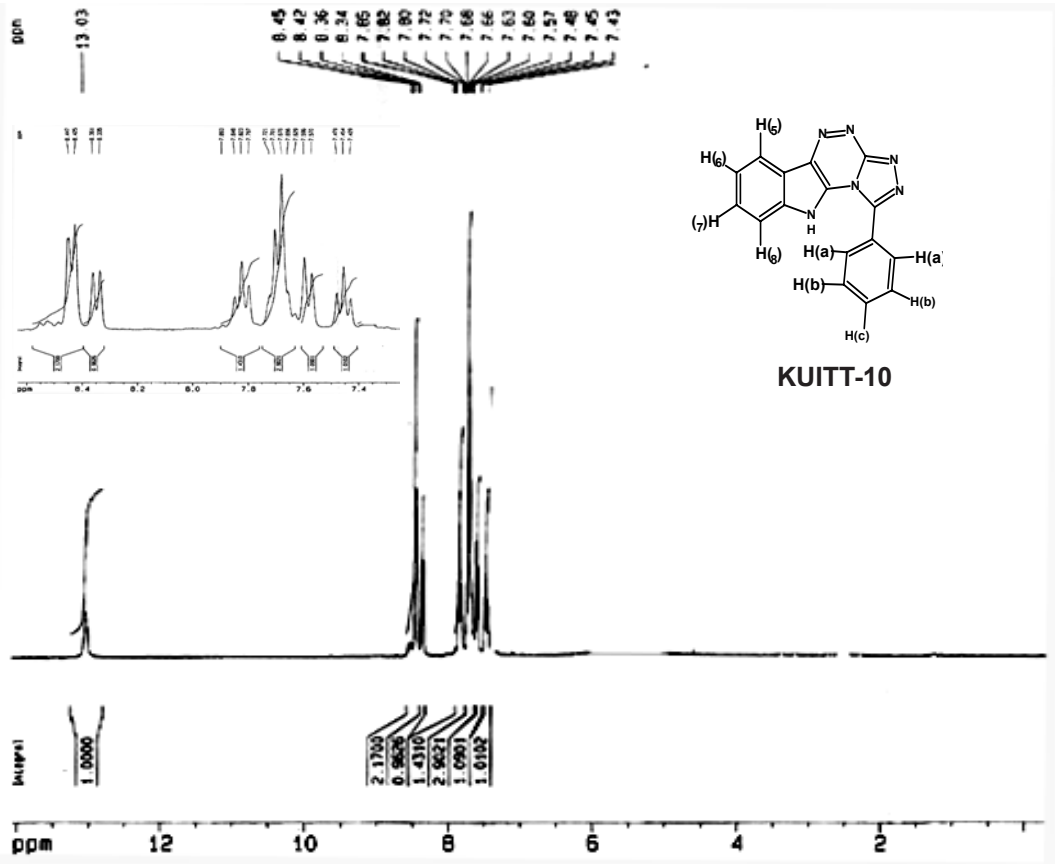
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $OCH_{3(1)}$	3.32	3	s	-
- $OCH_{3(2)}$	3.9	3	s	-
- NH	12.28	1	s	-
Ar-$H_{(c)}$	7.995	1	s	-
Ar-$H_{(a)}$	8.084	1	d	6
Ar-$H_{(b)}$	7.219	1	d	8.4
Ar-$H_{(6)}$	7.37	1	t	7.8 & 7.2
Ar-$H_{(8)}$	7.461	1	d	8.1
Ar-$H_{(7)}$	7.74	1	t	7.2 & 7.2
Ar-$H_{(5)}$	8.229	1	d	7.5

^{1}H NMR Spectrum of 1-(4-Methoxy)phenyl-10*H*-[1,2,4]triazolo[3',4':3,4][1,2,4]triazino[5,6-*b*]indole (KUITT-2)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- OCH_3	3.9	3	-	-
- NH	13.04	1	s	-
Ar-$H_{(d)}$	8.389	2	d	9
Ar-$H_{(e)}$	7.248	2	d	8.7
Ar-$H_{(6)}$	7.464	1	t	7.5 & 7.5
Ar-$H_{(8)}$	7.584	1	d	8.1
Ar-$H_{(7)}$	7.827	1	t	7.5 & 8.1
Ar-$H_{(5)}$	8.337	1	d	7.8

^{1}H NMR Spectrum of 1-Phenyl-10*H*-[1,2,4]triazolo[3',4':3,4] [1,2,4]triazino[5,6-*b*]indole (KUITT-10)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- NH	13.03	1	s	-
Ar-$H_{(a)}$	8.436	2	d	6.6
Ar-$H_{(b,c)}$	7.677	3	m	-
Ar-$H_{(6)}$	7.454	1	t	7.5 & 7.5
Ar-$H_{(8)}$	7.583	1	d	7.8
Ar-$H_{(7)}$	7.823	1	t	7.5 & 7.8
Ar-$H_{(5)}$	8.348	1	d	7.8

EI Mass Spectral Study.

Instrument: SHIMADZU GCMS-QP-2010

Sample technique: Direct injection probe

m/z range: 40-400

EI Mass spetrums of 1-substituted phenyl-10*H*-[1,2,4]triazolo[3',4':3,4] [1,2,4]triazino[5,6-*b*]indoles become the most important tool to characterize the structure of the molecule as the signals correspond to m/z value of the relevant fragments suggest very important structural feature like mode of cyclization and aromatic nature of the molecule. which lead this analytical technique advantageous over NMR, IR and UV spectroscopy in this perticular case.

1-(3,4-dimethoxy)phenyl-10H-[1,2,4]triazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole(KUITT-1)

In the EI Mass spectrum of 1-(3,4-dimethoxy)phenyl-10H-[1,2,4]triazolo [3',4':3,4][1,2,4] triazino[5,6-b]indole, the appearance of an intense Molecular Ion Peak at m/z 346, aromatic nature of the triazolotriazinoindole fused system, a signal at m/z value 101relevent to benzonitrile suggest the loss of benzonitrile fragment exhibits foolproof evidence of angular cyclization. More over, an ion corresponds to m/z 331 suggest loss of methyl group from the molecular ion, subsequently ions relevant to m/z 304, m/z 174, m/z 126 were also detected.

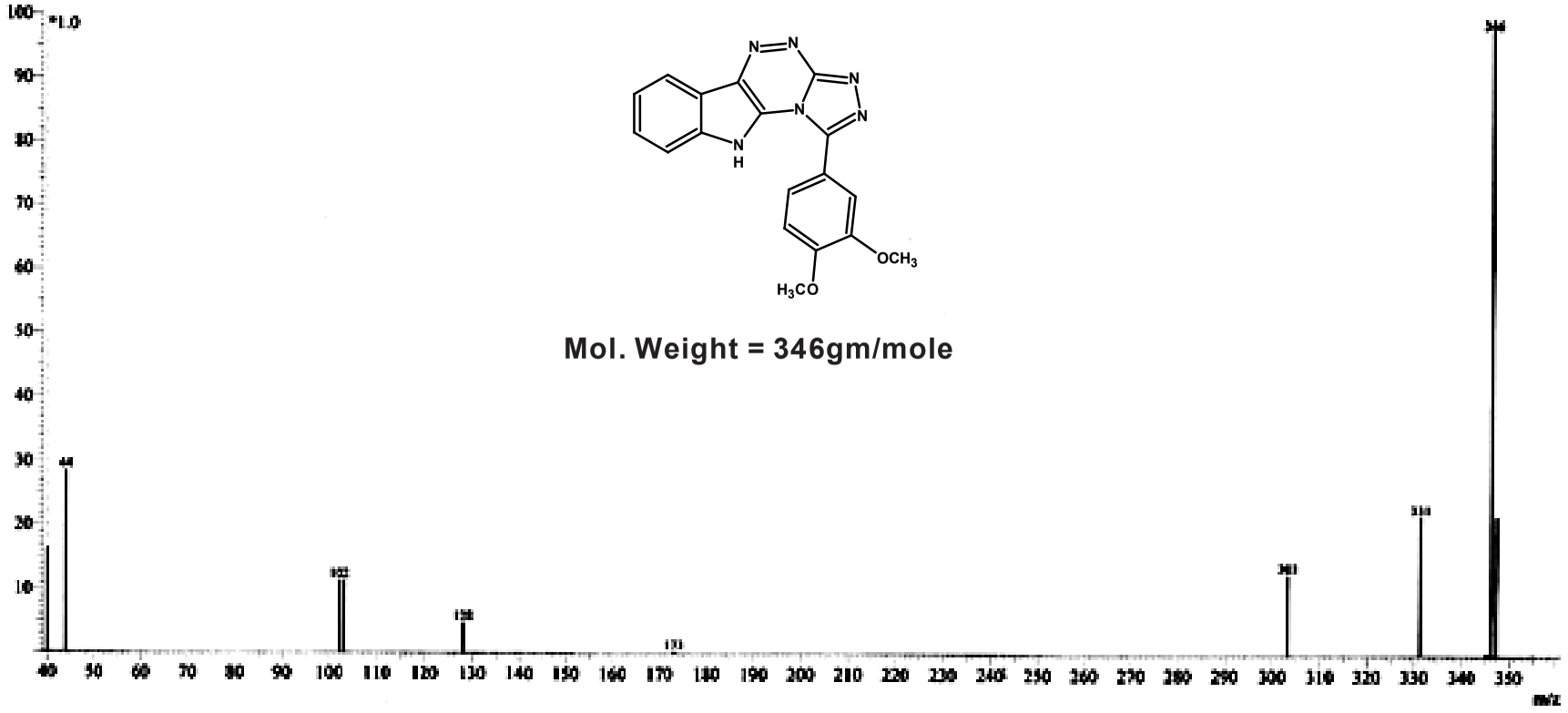
1-(4-methoxy)phenyl-10H-[1,2,4]triazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole(KUITT-2)

In the EI Mass spectrum of 1-(4-methoxy)phenyl-10H-[1,2,4]triazolo [3',4':3,4][1,2,4] triazino[5,6-b]indole, the appearance of an intense Molecular Ion Peak at m/z 316, aromatic nature of the triazolotriazinoindole fused system, a signal at m/z value 101 relevent to benzonitrile suggest the loss of benzonitrile fragment exhibits foolproof evidence of angular cyclization. More over, an ion corresponds to m/z 301 suggest loss of methyl group from the molecular ion, subsequently ions relevant to m/z 273, m/z 158, m/z 128 were also detected.

1-(4-methyl)phenyl-10H-[1,2,4]triazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole(KUITT-21)

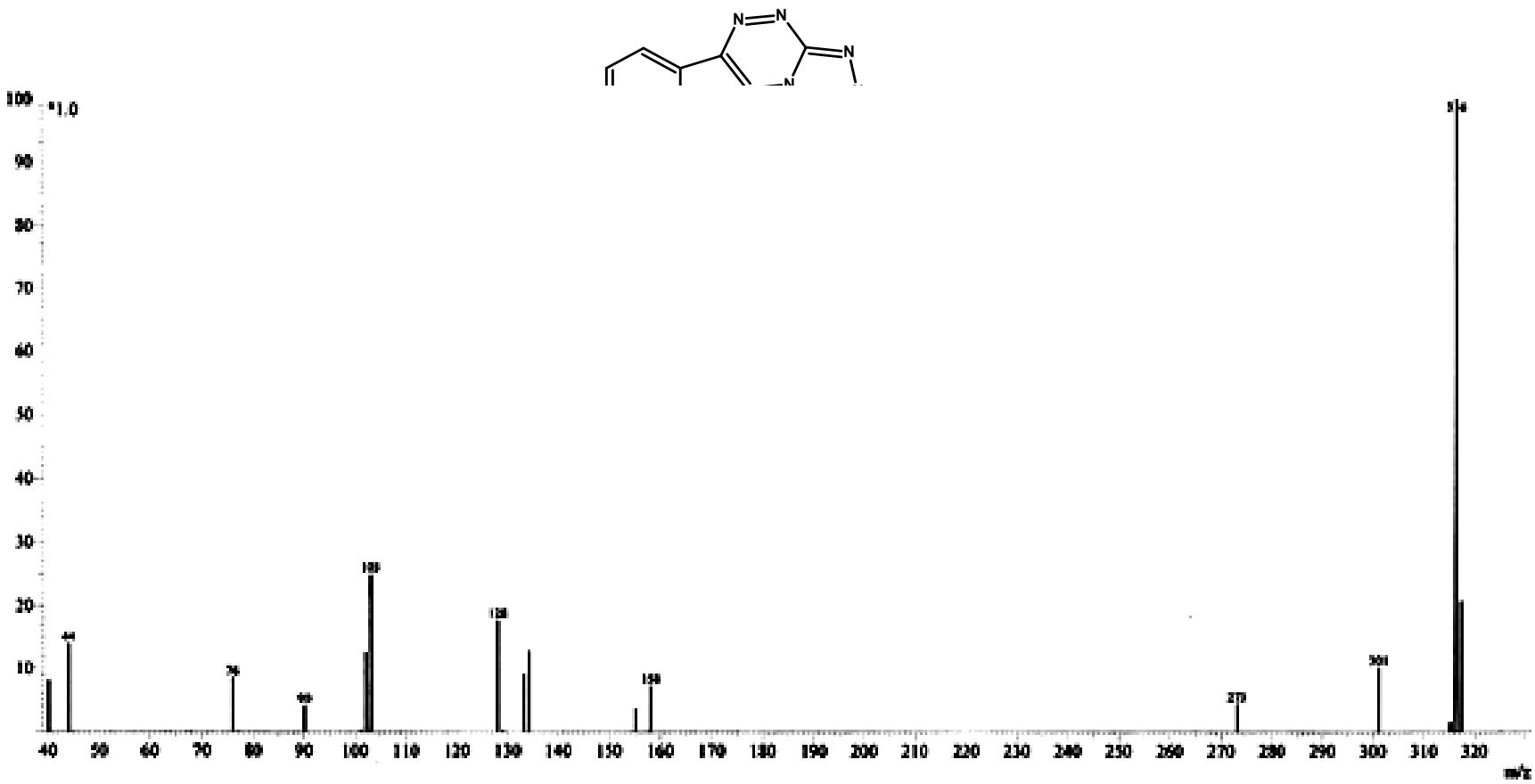
In the EI Mass spectrum of 1-(3,4-dimethoxy)phenyl-10H-[1,2,4]triazolo [3',4':3,4][1,2,4] triazino[5,6-b]indole, the appearance of an intense Molecular Ion Peak at m/z 300, aromatic nature of the triazolotriazinoindole fused system, a signal at m/z value 101relevent to benzonitrile suggest the loss of benzonitrile fragment exhibits foolproof evidence of angular cyclization. More over, ions relevant to m/z 271, m/z 155, m/z 128, m/z 118 were also detected.

EI MASS Spectrum of 1-(3,4-Dimethoxy)Phenyl-10*H*-[1,2,4]triazolo[3',4':3,4] [1,2,4]triazino[5,6-*b*]indole (KUITT-1)

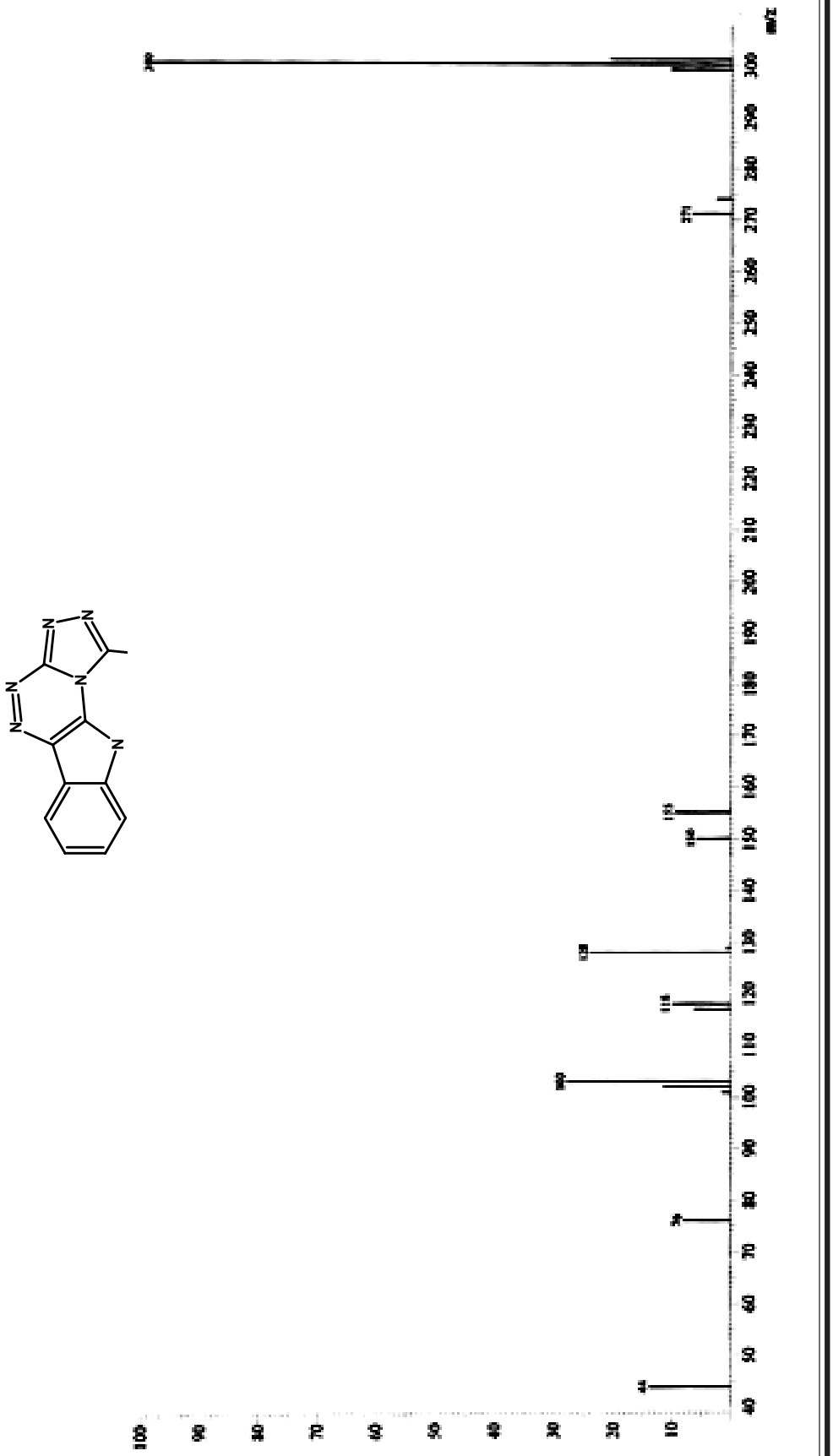


Mol. Weight = 346gm/mole

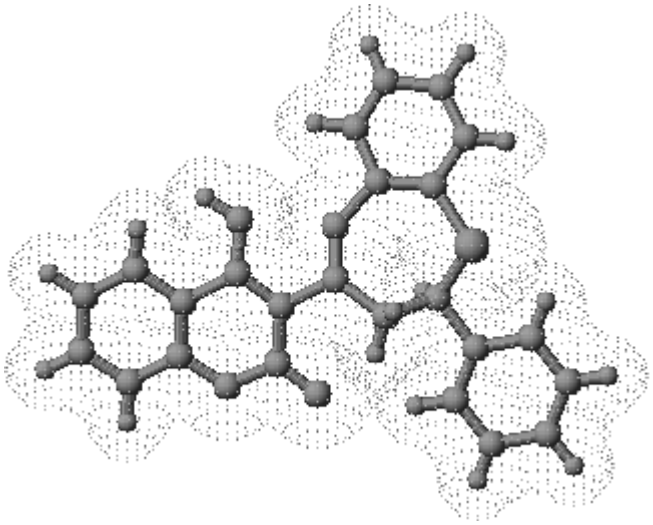
EI MASS Spectrum of 1-(4-Methoxy)Phenyl-10*H*-[1,2,4]triazolo[3',4':3,4] [1,2,4]triazino[5,6-*b*]indole (KUITT-2)



EI MASS Spectrum of 1-(4-Methyl)-10*H*-[1,2,4]triazolo[3',4':3,4] [1,2,4]triazino[5,6-*b*]indole (KUITT-21)



CHAPTER-2



Synthesis and Characterization of Some 4-Hydroxy-3-(2(un)substituted phenyl-2,3-dihydro -1,4-benzothiazepine-4-yl)-2H-chromen-2-ones

Introduction

1,5-Benzothiazepine refered as benzo fused seven membered heterocylic ring with two hetero atoms *viz.* sulphur and nitrogen occupied by 1and 5 position of the seven membered cyclic system. 1,5-benzothiazepine is one of three possible benzo condensed derivatives, *viz.* 1,4-, 4,1- and 1,5-benzo thiazepines of the 1,4-thiazepine[1]. The parent 1,5-benzothiazepine itself has not hitherto been described in the literature. However, its derivatives are the most frequently studied-benzothiazepines. This may be due to their easy accesibility and wide range of therapeutic activities like cardiovascular[2], vasodilator[3,4] and antiarrythmic[5]. Actions of some 2,3-dihydro-1,5-benzothiazepin-4(5H)-ones have already been published in the early seventies. Later on haemodynamic effects[6], antiulcer activity[7,8], calcium antagonistic and spasmolytic activities[9-13], angiotensine converting enzyme inhibition[14,15] etc have been described as their major activities. More over antihypertensive drugs with 1,5-benzothiazepine active ingredients have already been marketed. Thus, the 1,5-benzothiazepines are useful compounds in the drug research which stimulated the invention of various synthetic procedures for their preparation and chemical transformations[16].

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The importance of the 1,5-benzothiazepine nucleus has been well proved and illustrated by large number of patents filed, as chemotherapeutic agents. A number of therapeutic activities have been associated with it, such as antihypertensive[17,18], antiasthmatic[18,19], analgesic[20], cardio vascular[21], platelet aggregation inhibitor and calcium channel antagonists[18] Ahmed et al[22,23] patented 1,5-benzothiazepine derivatives as potential anticancer drugs.

1,5-Benzothiazepine derivatives are of particular interest for lead discovery because they have been shown to have activity against different families of targets[24]. The 1,5-Benzothiazepine scaffold has been used as a dipeptide mimic not only in protease inhibitors, but also interleukin-1â converting enzyme inhibitors[25], elastase[26] or angiotensin-converting enzyme inhibitors[27], and as in antagonists of several G-protein coupled receptors such as cholecystokinin[29] receptor or the angiotensin II receptor[30], too.

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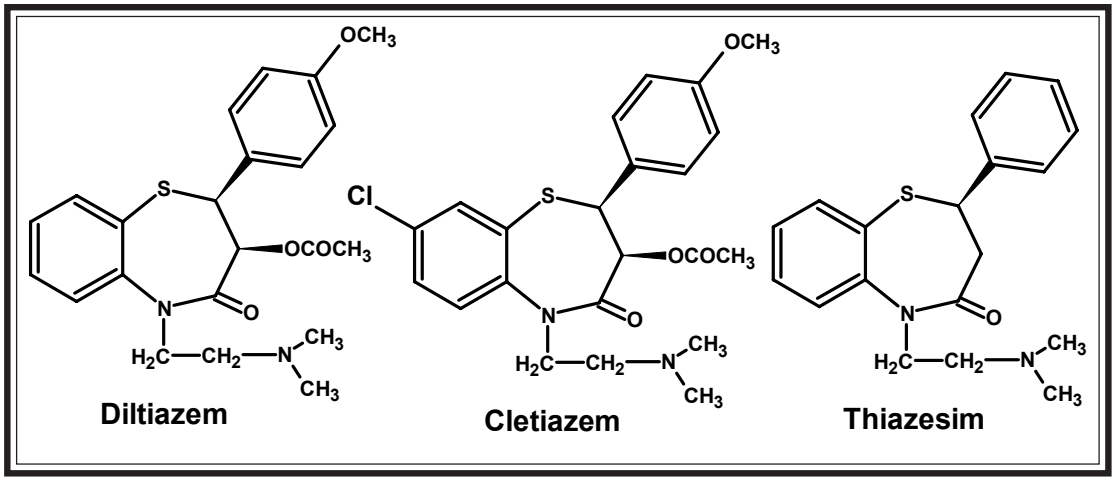
Pharmacology

1,5-Benzothiazepines are widely used in different therapeutic areas and therefore an interesting scaffold for De Novo exploration. The 1,5-Benzothiazepine scaffold is extremely versatile and widely used in a number of famous drugs. Currently 1,5-Benzothiazepin-2-ones are being clinically used as coronary vasodilators (e.g. Diltiazem), as calcium antagonists (e.g.Clentiazem) and as antidepressant (e.g. Thiazesim)[30]



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Atta-ur-Rahman et al[31] have reported that Benzothiazepine derivatives may act as calcium antagonists[32], angiotensin converting enzyme inhibitors[33], anticonvulsant and tranquilizers[34], anticancer drugs[35,36] and endogenous natriuretic activities[37]. The coveted drug *diltiazem*, for example is being used as calcium channel blocker[38], calcium channel modulator[39-43], blood platelet aggregation inhibitors[44], antiarrhythmic[45], antithrombotic[46], antianginal[47], and antiischemic[48] and also for other cardiovascular activities. The literature survey reviewed that the pharmacological profile of 1,5-benzothiazepine can classified into following categories.

❖Calcium channel antagonist and Vasodilator

❖ Peripheral and Mitochondrial Benzodiazepine receptor antagonist / Anti convulsant / CNS depressant

❖ Antiplatelet aggregation ❖ Anticancer

❖ Anti-HIV ❖ Antimicrobial ❖ Miscellaneous

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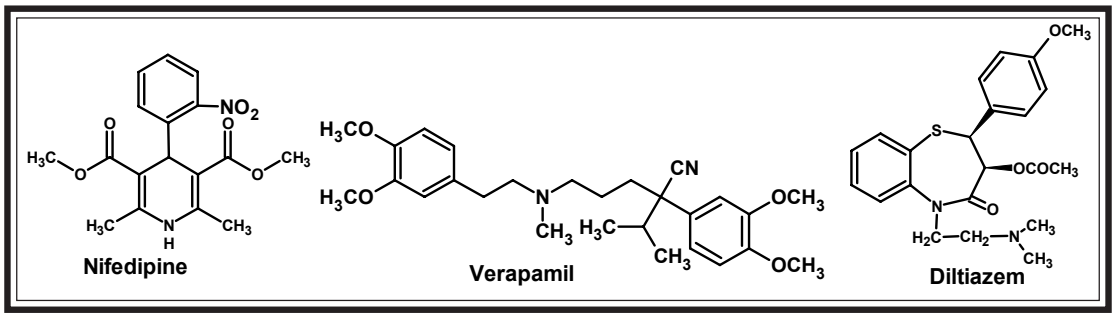
❖ Calcium channel antagonist

Ca^{+2} channel antagonists are classified into three major chemical classes, the 1,4-dihydropyridines (Nifedipine), phenylalkylamines(Varapamil) and 1,5-benzothiazepines (Diltiazem). In addition, several unrelated new chemicals have been identified as Ca^{+2} channel antagonists. It is widely accepted that these chemical classes of Ca^{+2} channel antagonists have distinct but allosterically interacting binding sites within the á$_1$ subunit of the L-type Ca^{+2} channel[49]. Each class of Ca^{+2} channel antagonists suppresses the L-type Ca^{+2} channel current ($I_{Ca(L)}$) in a distinctive manner, and has unique tissue selectivity [50-52]



Hagiwara et al[53] have introduced a novel 1,5-benzothiazepine derivative, (+)-*cis*-3- acetyloxy -5- 2- 2- 3,4-dimethoxyphenyl ethyl -methylaminoethyl -2,3-dihydro-2- 4-methyoxyphenyl -1,5-benzothiazepine-4 5*H* -one (DTZ323), In rabbit crude T-tubule membranes, DTZ323 has been shown to produce complete inhibition of [^{3}H] diltiazembinding with a Hill coefficient close to unity, indicating competitive inhibition. DTZ323 *(K_i*= 6.6×10^{-9} M) was 48 times more potent than diltiazem *(K_i*= 3.1×10^{-7} M) and nine times more potent than clentiazem *(K_i*= 6.1×10^{-8} M). Thus, DTZ323 has the highest affinity for 1,4-dihydropyridine receptors of skeletal muscle membrane of all the 1,5-benzothiazepine derivatives studied to date.

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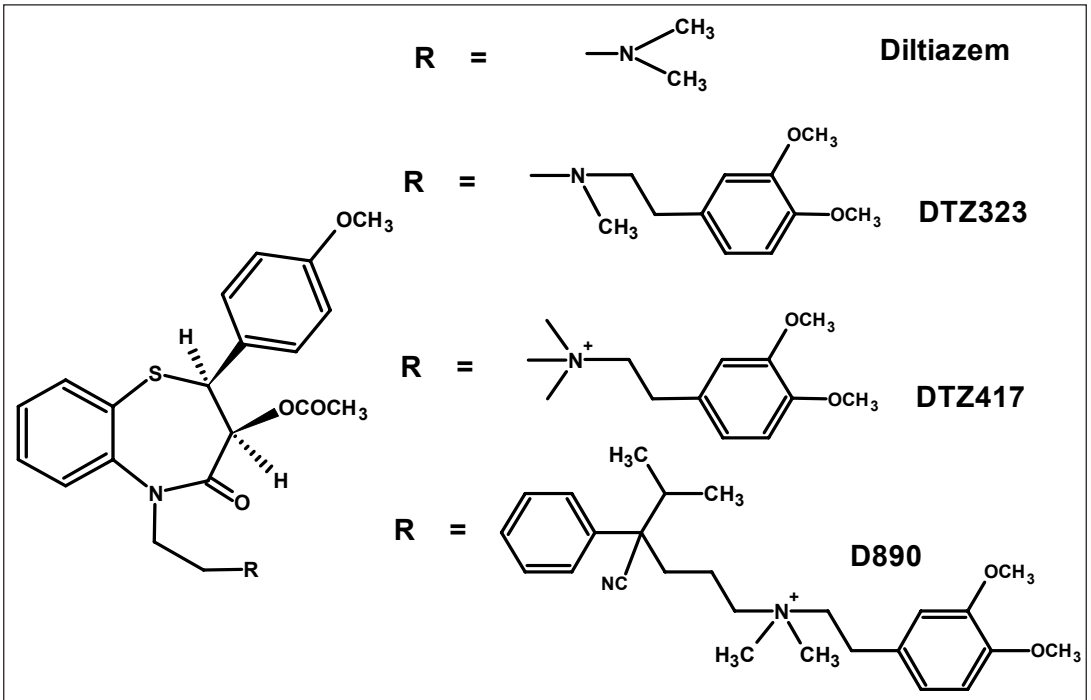

Structures of 1,5-Benzothiazepine derivatives studied

Junko Kurokawa et al[54] have characterized pharmacological effects of DTZ323 on membrane currents[54] in guinea-pig ventricular myocytes using the whole-cell patch-clamp technique. DTZ323 suppressed the L-type Ca^{+2} channel currents($I_{Ca(L)}$) more selectively than the T-type Ca^{+2} channel and the Na^{+} channel currents. DTZ323 inhibited ($I_{Ca(L)}$) in a use- and a voltage-dependent manner with 24 times higher potency than that of diltiazem. Rate of recovery of ($I_{Ca(L)}$) from the conditioned block by DTZ323 was faster compared with diltiazem and verapamil, and was steeply dependent on the holding potential at resting membrane potential range in ventricular myocytes (-90mV to -60 mV). The result suggested that DTZ323 is a selective Ca^{+2} channel antagonist, the most potent among the 1,5-benzothiazepine Ca^{+2} channel antagonists and that the voltage- and use-dependent effect of DTZ323 on ($I_{Ca(L)}$) is due to the steep voltage dependence of the rate of dissociation from the cardiac L-type Ca^{+2} channels.

55.J. Kurokawa, S. Adachi-Akahane, T. Nagao, *Mol. Pharmacology,* **51,** 262(1997)

In addition to this, by using same technique Taku Nagao et al[55] have tested this novel potent 1,5-benzothiazepine derivative (DTZ323) and its quaternary ammonium derivative (DTZ417) on guinea pig ventricular myocytes to determine whether 1,5-benzothiazepine Ca^{+2} channel blocker approaches its binding domain within the cardiac L-type Ca^{+2} channel from inside or outside of the membrane. The extracellular application of DTZ417 suppressed the L-type Ca^{+2} channel currents($I_{Ca(L)}$) with an IC50 value of 1.2 ± 0.02 ì M, which was close to the IC50 value of diltiazem (0.63 ± 6 0.01 ì M). The suppression of ($I_{Ca(L)}$) by DTZ417 was voltage and use dependent but lacked tonic which has allowed to investigate the onset of the effect on ($I_{Ca(L)}$) by changing the holding potential (HP) from 290 to 250 mV in the presence of DTZ417. DTZ417 did not have significant effects on ($I_{Ca(L)}$) at an HP of 290 mV. At 250 mV, DTZ417 (50 ì M) applied from the extracellular side completely suppressed ($I_{Ca(L)}$), whereas it had no effect from the intracellular side.DTZ323 (1 ì M) also inhibited ($I_{Ca(L)}$) only from the extracellular side, without any effects by the intracellular application of less than or equal to 10ì M. However, a quarternary phenylalkylamine derivative, D890(0.1 ì M), acted only from the intracellular side. These results suggest that in contrast to the phenylalkylamine binding site, in cardiac myocytes, the 1,5-benzothiazepine binding site is accessible from the extracellular side of the L-type Ca^{+2} channel.

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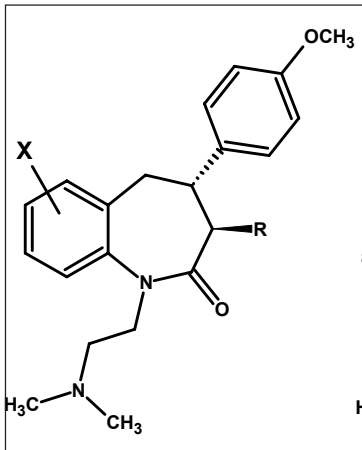
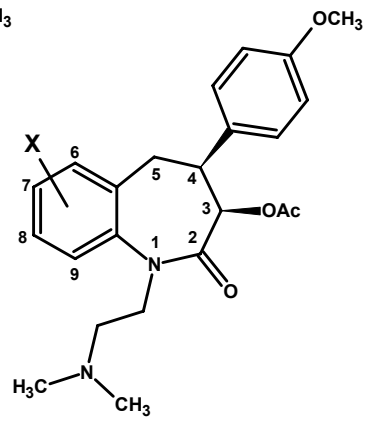
Jagbandhu Das et al[56] have indentified 3-alkylbenzazepinones as novel analogues of diltiazem. Structure-activity relationship studies in this series have led to identification of several analogues as potent calcium channel blocking agents, both *in vitro* and *in vivo*. Analogues containing a 6-trifluoromethyl substituent (1a and 1b) are the most potent vasorelaxants *in vitro*. The oral antihypertensive activity of these compounds is comparable to its 3-acetoxy derivative 1 (X = 6-CF_3) and 8-chlorodiltiazem. The 3-allyl analogue 1c is a more potent antihypertensive agent than 1a, 1b or 8-chlorodiltiazem and has a longer duration of action *in vivo*. The present study demonstrated that like the benzothiazepinone (diltiazem) series, both the absolute and relative stereochemistry of the C3 and C4 (benzazepinone numbering)substituents are important to calcium channel blocking activity in the benzazepinone series. The general structure activity relationship elicited that in the analogous series of 3-acetoxybenzazepinones, the 6-trifluoromethyl substituent appears to be important to maximal potency *in vitro* and antihypertensive activity.These studies have resulted in the identification of some of the most potent benzazepinone/benzothiazepinone calcium channel blocking agents reported to date.



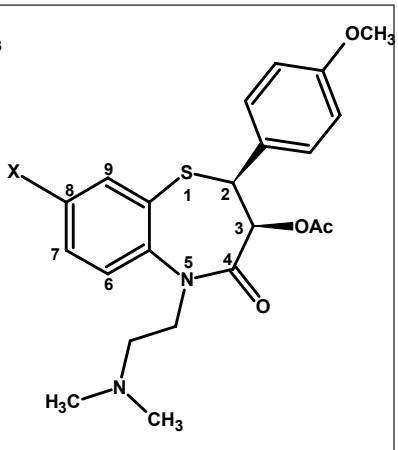
1a **R = CH_3 , X = 6-CF_3**

1b **R =C_2H_5 , X = 6-CF_3**

1c **R =allyl , X = 6-CF_3**



3-Acetoxy-1-benzazepine-2-one



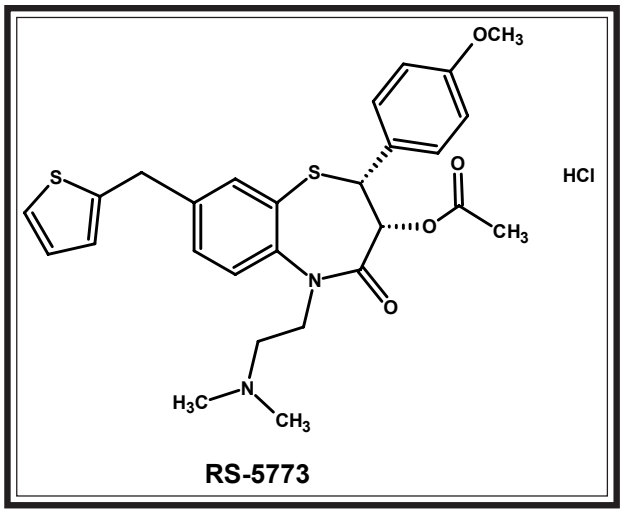
Diltiazem (X = H)

Chlorodiltiazem (X = Cl)

Schleifer and Tot[57] have attempted molecular modeling study of diltiazem mimics at L-type calcium channels, to generate a pharmacophore model for chemically diverse structures that specifically interact with the diltiazem binding site of L-type calcium channels by using molecular mechanics and quantum chemical methods solvation energies, logP values, conformational and electronic features of classical 1,5-benzothiazepine-4(5H)one (BTZ, e.g.,diltiazem),1-benzazepin-2-one(BZ),pyrrolo[2,1- d][1,5] benzothiazepine, pyrrolo[2,1-c][1,4]benzothiazine & benzobicyclo[2.2.2]octyl amines derivatives were determined. Furthermore, the molecular electrostatic potentials (MEPs) and common interaction fields derived from use of the GRID programme were compared. A pharmacophore model with three crucial pharmacophoric characteristics, identified with (1) two aromatic ring systems in a distance of about 6.7 A, (2) a basic side chain with pK(a) in the physiological range, and (3) a 4'-methoxy moiety. In addition, a strong negative MEP in 4-position (carbonyl oxygen) and hydrophobic electron-rich features in the position equivalent to the sulphur atom of BTZ derivatives were explored to be favourable for receptor binding and calcium antagonistic effect. Moreover, the stabilizing effect of substituents in 3-position of BZs on the bioactive 'M' twist-boat conformation of the heptagonal ring could be demonstrated by molecular dynamics simulations. Based on these molecular descriptors, the quinazolinone derivative MCI-176 is predicted to be a potential ligand of the diltiazem binding site.

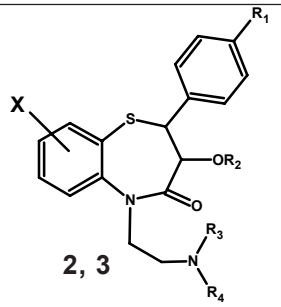
Ueyama, N. et al[58] have studied a series of 2,3,4,5-tetrahydro-1,5-benzothiazepine for the intracellular Ca^{2+} inhibitory effects using methoxamine- or caffeine-induced contraction of isolated rabbit arteries. Structure-activity relationship studies of these compounds are discussed and the results suggest that novel 5-[3-[2-(3,4- dimethoxyphenyl) ethyl]aminopropionyl]-2,3,4,5-tetrahydro-1,5-benzothiazepine fumarate showed the most potent inhibitory action on the intracellular Ca^{2+} release.

Shiga, H and coworkers[59] have examined *in vitro* cardiovascular effects of a new 1,5-benzothiazepine derivative, RS-5773 ((2S,3S)-3-acetoxy-8-benzyl-2,3-dihydro-5-[2- (dimethylamino) ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4-(5H)-one hydrochloride, CAS 129173-57-5), in isolated rat aorta and isolated guinea pig heart preparations. Like diltiazem or nifedipine, RS-5773 preferentially relaxed K^+-contracted aorta rather than phenylephrine-contracted aorta, suggesting that the agent interfered with calcium channels. Vasorelaxant effects of RS-5773 on K^+-contracted aorta was about 5 times more potent than those of diltiazem. The vasorelaxation with RS-5773 developed very slowly and was resistant to washout. The negative effects of the agent on contractile force of guinea pig papillary muscles and beating rate of guinea pig atria were not much different from those of diltiazem, although the actions of RS-5773 developed more slowly than those of diltiazem. These results indicated that RS-5773 is a diltiazem congener with vascular preference and long-lasting actions. In this respect, RS-5773 resembled clentiazem. But vascular and cardiac effects of RS-5773 developed more slowly than those of clentiazem and they were more resistant to washout.



RS-5773

Nagao T. et al[60] described that the structure activity relationships(SAR) of some 40 derivatives of diltiazem made clear the effect of substituents at the position 2,3 and 5 and their stereochemical requirements for the activity[60]. Prior to this study, the effect of substitution on the fused benzene ring of diltiazem remain uncertain since only 7-chloro derivative (2, X=7-Cl, R^1=OCH_3, R_2=Ac, R_3, R_4=Me) has been synthesized[61]. In an attempt to improve the effectiveness and duration of action of diltiazem and to gain further insight into the SAR, Hirozumi Inoue et al[62] have introduced halogen substituents at the position 6-9(i.e.halogen substituents on the fused benzene ring) of diltiazem. These compounds were evaluated for their effects on vertebral and coronary blood flows and antihypertensive activity. The 8-chloro derivative ((+)-2b), the ,most potent compound in this series was selected for clinical evaluation as a cerebral vasodilating and antihypertensive agent(Table 1).



2, 3

Table-1 : Account of substitutioents applied to study VBF, CBF & antihypertensive effect of Halogenated-1,5-Benzothiazepines

Compds	X	R_1	R_2	R_3	R_4	Stereo Isomer
2q	8-Cl	OMe	Ac	Me	CH_2-CH=-CH_2	cis
2r	8-Cl	OMe	Ac	Me	CH_2-C_2H	cis
2s	8-Cl	OMe	Ac	Me	Bzl	cis
2t	8-Cl	OMe	Ac	Et	Et	cis
2u	8-Cl	OMe	Ac	Me	Me	trans
2v	8-Cl	Me	Ac	Me	Me	cis
2w	8-Cl	SMe	Ac	Me	Me	cis
(-)-2x	9-Cl	OMe	Ac	Me	Me	cis
(+)-2x	9-Cl	OMe	Ac	Me	Me	cis
2y	8-F	OMe	Ac	Me	Me	cis
2z	9-F	OMe	Ac	Me	Me	cis
2aa	7,8 -Cl_2	OMe	Ac	Me	Me	cis
2bb	8,9 -Cl_2	OMe	Ac	Me	Me	cis
(+)-2cc	7-Cl	OMe	Ac	Me	Me	cis
2cc	7-Cl	OMe	Ac	Me	Me	cis
(+)-3a	8-Cl	OMe	H	Me	Me	cis
3b	8-Cl	OMe	H	Me	Et	cis
(+)-3c	9-Cl	OMe	H	Me	Me	cis
3d	8,9-Cl_2	OMe	H	Me	Me	cis

Arround 75compoundsof the titled series were tested for their effects on vertebral blood flow(VBF) in anesthetized dogs and coronary blood flow(CBF) in isolated guinea pig hearts and their antihypertensive activity in SHR. The core structure activity relationship of the present study was summerized below in correlation with the compounds listed in the table.

Effect on Vertebral Blood flow(VBF).

Introduction of a chloro substituent at the 8-position of diltiazem confers increased activity with longer duration of action ((+)-2b). The activity of the 7-Cl ((+)-2cc) and 9-Cl((+)-2x) derivatives is comparable to that of diltiazem. In spite of being racemic modifications, the 6-Cl (2a) and 7,8-Cl_2(2aa) derivatives exhibit moderate activity. The fluoro (2y and 2z) and 8,9-Cl_2 (2bb) substitution result in a decrease in activity. Generally, the 3-OH derivatives are less potent than the corresponding acetoxy derivatives, except for the 9-Cl and 8,9-Cl_2 derivatives (2x vs 3c and 2bb vs 3d).

Duration of the action of the 3-OAc derivatives, however,is usually longer than that of the 3-OH derivatives.Therefore, when compared, the total increase in blood flow,which is calculated by multiplying the potency ratio(maximum increase) by half duration, the 3-OAc derivatives (2x and 2bb) are more potent than the 3-OH congeners 3c and 3d. The 2,3-trans isomer of the 8-Cl derivative (2u) and the levorotatory isomers of 2b, 2x and 2cc are only marginally active. The importance of 2S,3S stereochemistry in this series of derivatives has already been reported[62].

In view of the good activity of the 8-Cl derivative (2b), the effect of modifying the substituents at the 2**,** 3, and 5 position was further examined. With regard to the effect of the length of the 3-acyloxy group on activity, maximum activity was seen in the acetoxy (2b) and propionyloxy (2d) derivatives. Gradual decrease in activity was observed with the lower (2c) or higher (2e and 2f)analogues. The methoxy (2l), carbamate, carbonate(2g) and various benzoyl (2h-k) derivatives exhibit decreased activity. When the dimethylamino

group in the side chain of 2b is replaced by larger amino groups (2m-t), a significant decrease in potency is observed. Only the methylallylamino derivative (2q) exhibits good potency with short duration of action. Replacement of the 4-Me0 group in the 2-phenyl moiety of 2b with MeS (2w) and Me (2v) groups results in a considerable decrease in activity.

Effect on Coronary Blood flow(CBF).

The most effective compounds in this test are (+)-2e,(+)-2f, (+)-2x and (+)-2o. No clear relationships are observed between the effects on CBF and VBF

Hypotensive Effect in SHR

Some of the compounds showed interesting effects on VBF and CBF were tested for their hypotensive activity in SHR.The hypotensive activity of the compounds is roughly parallel with their effect on VBF. Thus, the 3-acyloxy derivatives ((+)-2b, (+)-2x, (+)-2o), (+)-2c, and (+)-2d with strong increasing effect on VBFexhibit long-lasting and potent hypotensive action. The most active compound in this series,(+)-2b, was found to be 3 or 4 times as potent as diltiazem.The corresponding carbinols ((+)-3a, & (-)-3b and(+)-3c) of these o-acyl derivatives exhibit much-reduced activity. Compounds (+)-2l, 2y, and 2z show good activity in spite of their moderate effect on VBF. As a consequence of the above SAR, (+)-(2S,3S)-3-acetoxy-8-chloro-5- [2-(dimethylamino)ethyl]-2,3-dihydro-2- (4-methoxyphenyl)-l,5-benzothiazepin-4(5H)-one((+)-2b) was selected for further study. The maleate of(+)-2b is currently under clinical trial as a cerebral vasodilating and antihypertensive agent under the code name of TA-3090.

63(a). T.Nagao,M. Sato, H.Nakajima, A.Kiyomoto,*Chem.Pharm. Bull.,***21**, 92(1973).
(b) T.Nagao, M.Sato, H.Nakajima, A.Kiyomoto, *Jpn. J. Pharmacol.*,**22,**1(1972)
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64a.M.Masui, S.Funakawa, O.Uno, S.Mihara, Y.Takahara, K.Matsunaga, K.Iwaki,*J Cardiovasc Pharmacol.,***28**(4):526(1996)
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67. E.Costa, A.Guidotti, *Annu. Rev. Pharmacol. Toxicol.*, **19,** 531(1979)

Iwaki K et al[64,64a] have studied on a new antihypertensive agent, S-2150, a benzothiazepine derivative for its hypotensive, antimyocardial-stunning effects in dogs as well as antinecrotic and antiarrythmic effects in reperfused rat hearts. S-2150 (30 mg/kg, p.o.) decreased the blood pressure in conscious renal hypertensive dogs. Although the maximal hypotensive effect of S-2150 was observed at 5-9 h after administration, the effect of diltiazem was seen at 2.0 h. Arrhythmia was not observed as a hypotensive effects of S-2150 but was markedly induced by diltiazem. In anesthetized open-chest dogs, S-2150 (20 micrograms/kg/min, i.v.) caused by hypotensive effect similar to that of diltiazem but decreased myocardial work (double product) by much less than did diltiazem. S-2150 more promptly improved the local myocardial stunning caused by occlusion of the left anterior descending coronary artery and its reperfusion. This effect did not accompany the energy-sparing action in ischemic/reperfused myocardium, which was different from the case of diltiazem. Blockage of both Ca2+ channels and alpha 1-adrenoceptors by S-2150 seems to lead to cardiovascular effects different from those of diltiazem.

The effects of S-2150 on ischemia/reperfusion injury revealed that S-2150 reduced the myocardial infarct size (IS) induced by 20-min coronary artery occlusion followed by reperfusion. The incidence of arrhythmia was blocked by S-2150, and this effect offered protection against cardiac death. Prazosin did not modify the IS or incidence of reperfusion arrhythmias, but combined treatment with a noneffective dose of diltiazem showed significant cardioprotective effects.Both drugs decreased mechanical function and increased coronary flow, with S-2150 being less cardiodepressive and more vasodilatory. S-2150 is cardioprotective at doses comparable to hypotensive doses even though its cardiodepressant effect is much weaker than that of diltiazem. This effectiveness may be partly explained by its dual characteristics: blocking the Ca channel and the alpha 1-adrenoceptor.

❖ **Peripheral / Mitochondrial Benzodiazepine receptor antagonist / Anti convulsant / CNS depressant**

There were numerous studies directed towards the development of new annelated 1,5-benzothiazepine derivatives owing to their remarkable pharmacological properties on CNS[65].

Benzodiazepine interacts with two major classes of recognitions sites, the "central" and "peripheral" types.

The central benzodiazepine receptors(CBR), located in the neuronal tissues[65a,65b], more specifically on the synaptic membranes[65c].

The principal site action of benzodiazepines in the central nervous system is believed to be a domain that regulates chloride channel gating activated by ã-aminobutyric acid(GABA) on $GABA_A$ receptors[66-68]. CBR mediate classical pharmacological properties like anxiolytic, anticonvulsant, sedative and muscle relaxant of the clinically widely used benzodiazepine[65a].

By contrast, the GABA-independent[69,71] peripheral or "mitochondrial" benzodiazepine receptors(MBR) have been identified in a wide range of peripheral tissues as well as in central nervous system[88,89] and their subcellular location has been reported to be mainly on mitochondrial[68a,68b,82,84], nuclear[69,71] and in the plasma membrane[87].

Benzodiazepine peripheral type receptors have been identified in nearly all the mamalian tissues, including heart[73-76], endocrine glands[73,74,77], kidney[71,76] and erythrocytes[78,79]. In the CNS they are essentially located on glial cells, mainly astrocytes[80,81].

Subcellular fractionation studies demonstrated that they are principally associated with the outer mitochondrial membranes [82-84]. Hence termed as mitochondrial benzodiazepine receptors or MBR. Thus the 'peripheral-type' benzodiazepine receptor (PBR) is pharmacologically distinct from the central-typebenzodiazepine receptor (CBR), and in the last decade has been the object of extensive studies in order to elucidate its biological and pharmacological role. This receptor protein, expressed in central and peripheral

tissues, is located in the outer mitochondrial membrane [83-90]. The physiological role of MBR is still not clear; they are involved in various cellular functions[68c-68h], including regulation of mytochondrial oxidative phosphorylation[68i,68j], inhibition of cell proliferation[70], immune response modulation and most importantly steroidogenesis[68k,68l].

They might be useful in the diagnosis of central nervous system tumors and infact, glial tumors selectively concentrate MBR[72].

In rodents, diazepam is rather nonselective, while its 4-chloro derrivative designated as R_o - 5-4864, shows high affinity for peripheral and very low affinity for $GABA_A$ receptors[91,92].

Subsequently other classes of organic compounds were found to have high affinity and specificity for peripheral benzo diazepine receptors. Some isoquinolinecarboxamides (e.g.PK 11195) have shown high affinity and great selectivity for peripheral receptors[73,74,93].

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65b.J.Tallmann, S. Paul, P..Skolnick, D.Gallager, *Science*,**207**,274(1980)

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Among these derivatives, PK11195 was the most widely used specific probe for peripheral benzodiazepine receptors.

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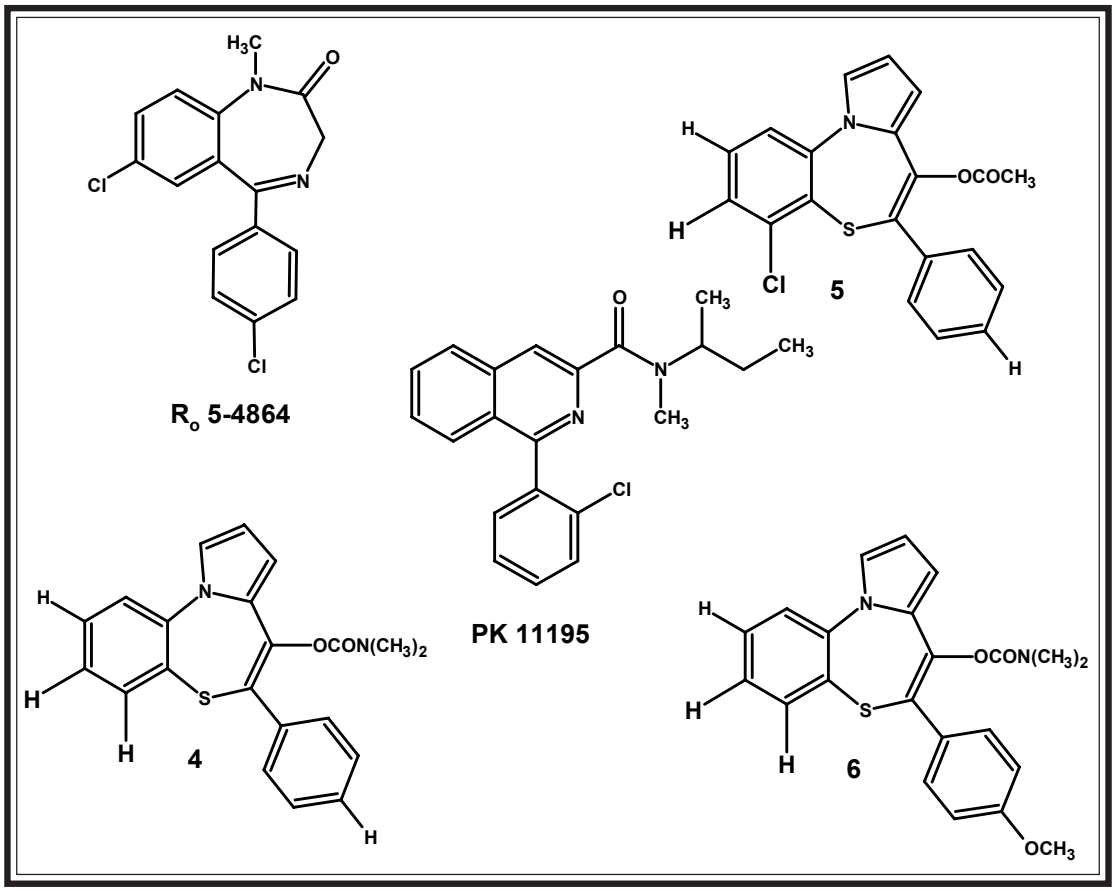
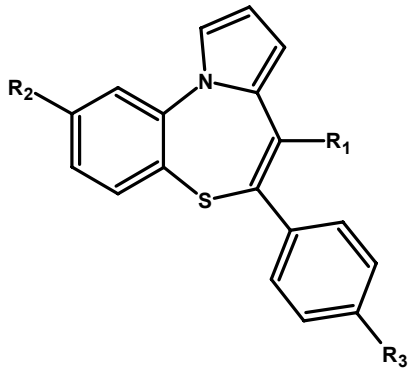
Fiorini and Greco et al[94-95] have proposed Pyrrolobenzothiazepine derivatives as a new class of ligand specific for mitochondrial benzodiazepine receptor. The majority of newly synthesized compounds showed micro or nanomolar affinity for PK11195 binding inhibition. A structure activity relationships study on 42 compounds and a molecular modeling approach led to a preliminary structural selectivity profile: 6,7-double bond, the carbamoyloxy, alkanoyloxy and mesyloxy side chains at the 7-position and prospective chloro substitution at the 4-position seemed to be the most important structural features improving affinity. Therefore, 7-[(dimethylcarbamoyl)oxy]- and 7-acetoxy-4-chloro-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine (4 and 5) were synthesized along with 7-[(dimethylcarbamoyl)oxy]-6-(p-methoxyphenyl)pyrrolo [2,1-d][1,5] benzothiazepine(6). These were the most promising compounds with IC_{50} values of 9,8 and 9 nM respectively, under conditions where PK11195 had an IC_{50} of 2 nM.





Co-mp	R_1	R_2	R_3
7	$OCON(CH_3)_2$	H	H
8	$OCOCH_3$	H	Cl
9	OSO_2CH_3	H	OCH_3
10	OCO-3-pyridyl	H	OCH_3
11	$OCOC_6H_2$ -3,4,5-tri(OMe)$_3$	Cl	OCH_3
12	OCH_3	H	H
13	H	H	H

7-(acyloxy)-6-arrylpyrrolo[2,1-d][1,5]benzothiazepines (7-13)

In continuation with the previous work, Alessandro Dalpiaz et al[96] reported the X-ray crystallographic structure of potent (7-13) and two inactive(10 and 11) 7-(acyloxy)-6-arrylpyrrolo[2,1-d][1,5]benzothiazepine derivatives, as well as binding affinity constants for two newly assayed analogs in which the acyloxy side chain was replaced by a methoxy group(12) or removed (13). Structure-affinity relationships and molecular mechanics calculations performed using crystal structures as references led to a revised 3D pharmacophore model accounting for all the data available. Interestingly the hypothetical receptor-bound conformations of 7-9 displayed a considerable degree of similarity with their crystal geometries. Additional calculations have confirmed that the poor affinities of benzothiazepines bearing an aroyloxy group (10 and 11) should be ascribed to the steric and/or electronic features of the side chain aryl moieties rather than unfavorable conformational properties.

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Nunzia Cinone and coworkers[97] afforded to develop a unique 3D interaction model of endogenous and synthetic peripheral benzodiazepine receptor ligands. Different classes of Peripheral-type Benzodiazepine Receptor (PBR) ligands were examined and common structural elements were detected and used to develop a rational binding model based on energetically allowed ligand conformations. Two lipophilic regions and one electrostatic interaction site were suggested as essential features for high affinity ligand binding, while a further lipophilic region expressed as important for modulator role. A comparative molecular field analysis, performed over 130 PBR ligands by means of the GRID/GOLPE methodology. The outcome from the 3D QSAR model and the GRID interaction fields computed on the putative endogenous PBR ligands DBI (Diazepam Binding Inhibitor) and TTN (Tetracontatetraneuropeptide) was used to identify the amino acids most probably involved in PBR binding. Three amino acids, bearing lipophilic side chains, were detected in DBI (Phe49, Leu47 and Met46) and in TTN (Phe33, Leu31 and Met30) as likely residues underlying receptor binding. Moreover, a qualitative comparison of the molecular electrostatic potentials of DBI, TTN and selected synthetic ligands indicated also similar electronic properties. Convergent results from the modeling studies of synthetic and endogenous ligands suggest a common binding mode to PBRs. This may help the rational design of new high affinity PBR ligands.

The compounds studied so far belong to approximately three different classes of PBR ligands, which are structurally unrelated:

(1) pyrrolobenzothiazepine[99, 101,102,104] & pyrrolobenzoxazepine derivatives[103],

(2) quinoline and isoquinoline derivatives [100],

(3) pyrrolopyridin-5-one and pyrroloquinolin-1-one derivatives [98].

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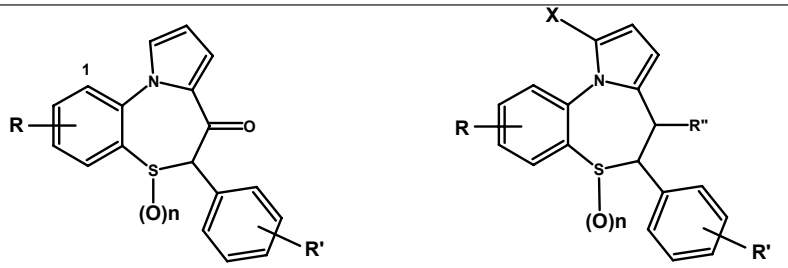


Chemical structures of pyrrolo[2,1-d][1,5]benzothiazepine derivatives

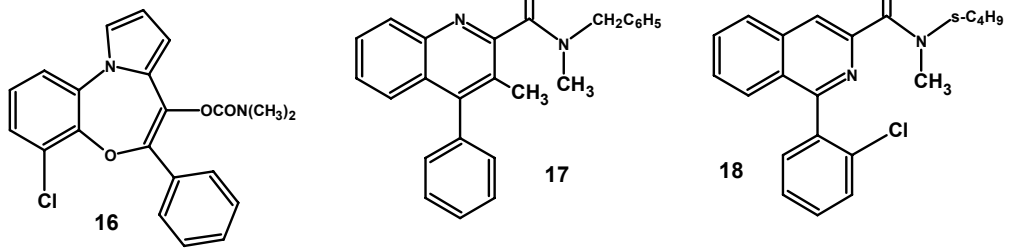
R = H, 1-Cl, 2-Cl, 3-Cl, 4-Cl, 3-CF_3, 2-OCH_3,1-CH_3, 2-CH_3, 3-CH_3

R' = H, 2-F, 4-Cl, 4-OCH_3, 4-F n = 0,1,2

R"= H, OSO_2CH_3, OCH_3, $OCOCH_3$, $OCOC_2H_5$, OCO-n-C_3H_7, OCO-n-C_4H_9, OCO-n-C_5H_{11}, $OCON(CH_3)_2$, $OCOC_6H_2$-3,4,5-$(OCH_3)_3$, OCO-3-pyridyl, OCO-4-pyridyl, $OCON(C_2H_5)_2$, $OCO(CH_2)_5CH_3$, $OCO(CH_2)_6CH_3$, $OCOC_6H_{11}$, $OCON(i\text{-}C_3H_7)_2$, $OCON(CH_2\text{-}CH_2)_2O$,

X= H, $CH_2N(CH_3)_2$

	R	R'	R"	n
14	H	4-OCH_3	OCO-3-pyridyl	0
15	H	H	$OCOC_6H_{11}$	0



16 **17** **18**

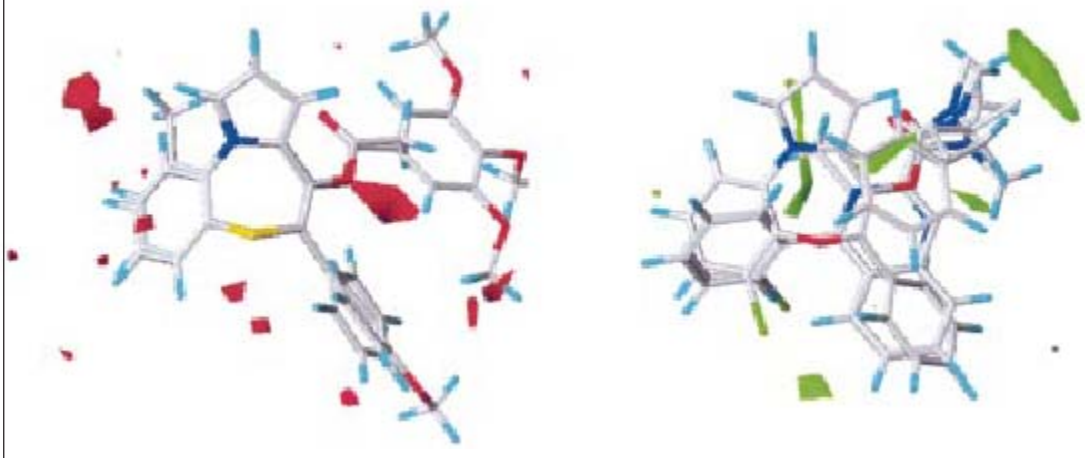
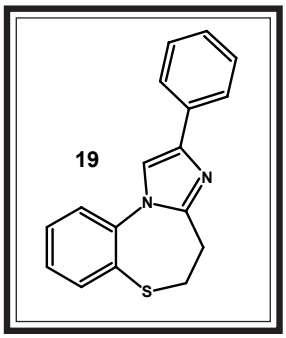


Figure-1. Non cross-validated GRID/GOLPE contour plots (lipophilic DRY probe). Red contours represent regions of interaction decreasing binding affinity. Molecules shown to help interpretation are: **15** (pIC_{50}= 5.30); **14** (pIC_{50}=4.00) (contour level –0.13). Green contours represent regions of interaction enhancing binding affinity. Molecules shown to help interpretation: **18** (pIC_{50}=8.70); **16** (pIC_{50}=8.89); **17** (pIC_{50}=8.77) (contour level 0.12).

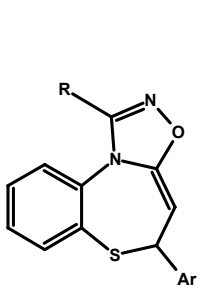
Figure-1 reveals that close to position 1 of the arylpyrrolobenzothiazepine 15, there is no space for substituents, thus the occupation of this space by even a methyl substituent produces unfavourable effects on binding. The amide nitrogen of compound 14 bears a bulky substituent, whose interaction with the probe is also unfavourable for binding. The green areas in Figure indicate the regions where lipophilic interactions enhance binding affinity. In fact, on the amide nitrogen atom of compounds 16, 17 and 18 lipophilic substituents favour the receptor binding. It is worthy to note that green and red polyhedra occupy regions where additional lipophilic interactions may take place. This could be due to the presence of a region of limited accessibility, where substituents of small size can be accommodated, but where repulsive interactions can occur as the substituent size increases. In addition, halogen atoms, especially the chloro substituent of arylpyrrolobenzoxazepine (16) and isoquinoline derivatives (18), enhance the receptor affinity, as demonstrated by the presence of an additional green region close to it.

In contrast to these, Ambrogi V. et al[105], have evaluated imidazo[2,1-d][1,5]benzothiazepine derivatives for their affinity for the benzodiazepine receptor and tested their ability to displace [^{3}H]Flunitrazepam from bovine brain membrane protein. A few of the tested compounds showed good affinity among which (19) found to be most potential with the dissociation constant K_i = 0.043ì M with refernce to that of Chlordiazepoxide(K_i = 0.72) as a standard. The GABA(γ -amino butyric acid) ratio of the active compound suggested that it could act as an antagonist or an inverse agonist. The binding results showed the importance of presence of the lipophilic phenyl substituent at the 2-position on imidazole nucleus and a proton acceptor (nitrogen at 3-position) in the 5-membered ring and was devoid of proton donor group. As the similar structural features found with benzodiazepine ligands, these structural requirment considered to be essential for the Benzodiazepine receptor antagonist or inverse agonist activities.

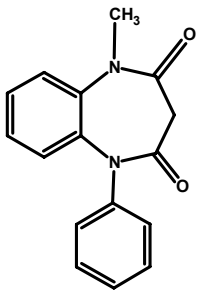


Prior to this the same author have studied annelated[1,5]benzothiazepines *viz.* N-2 alkyl amino derivatives of 4,5-dihydro-s-triazolo[3,4-d][1,5] benzothiazepines and screened for their CNS activity in mice and several of them showed interesting activity[106]. In addition to this, the tricyclic 1,5-benzothiazepine derivatives containing tetrazole and triazine nucleus were described[107], among which a compound of triazinothiazepine series expressed significant antimicrobial activity while another compound of tetrazolothiazepine series showed moderate affinity for the benzodiazepine receptor.

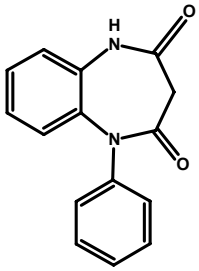
De Sarro and coworkers[108] have studied a series of 5H-[1,2,4]oxadiazolo [5,4-d][1,5]benzothiazepine and screened as anticonvulsant agents in DBA/ 2 mice. The 5-(4-bromophenyl)-1,3-diphenyl derivative, the most active compound of the series was 20 times more potent that the parent benzothiazepine and shown an activity comparative to clobazam and better than desmethylclobazam.



5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepines



Clobazam



Desmethylclobazam

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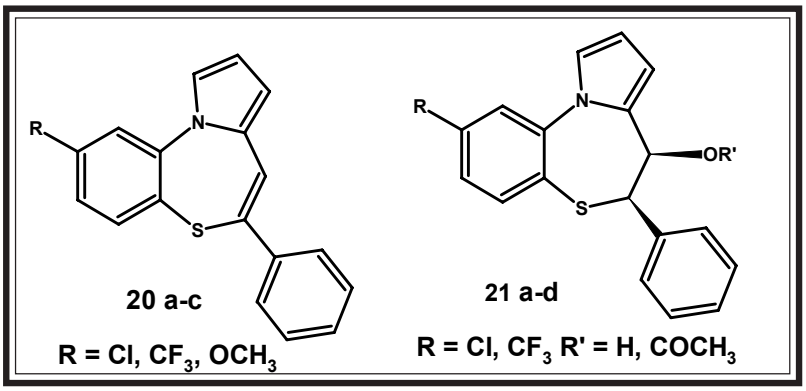
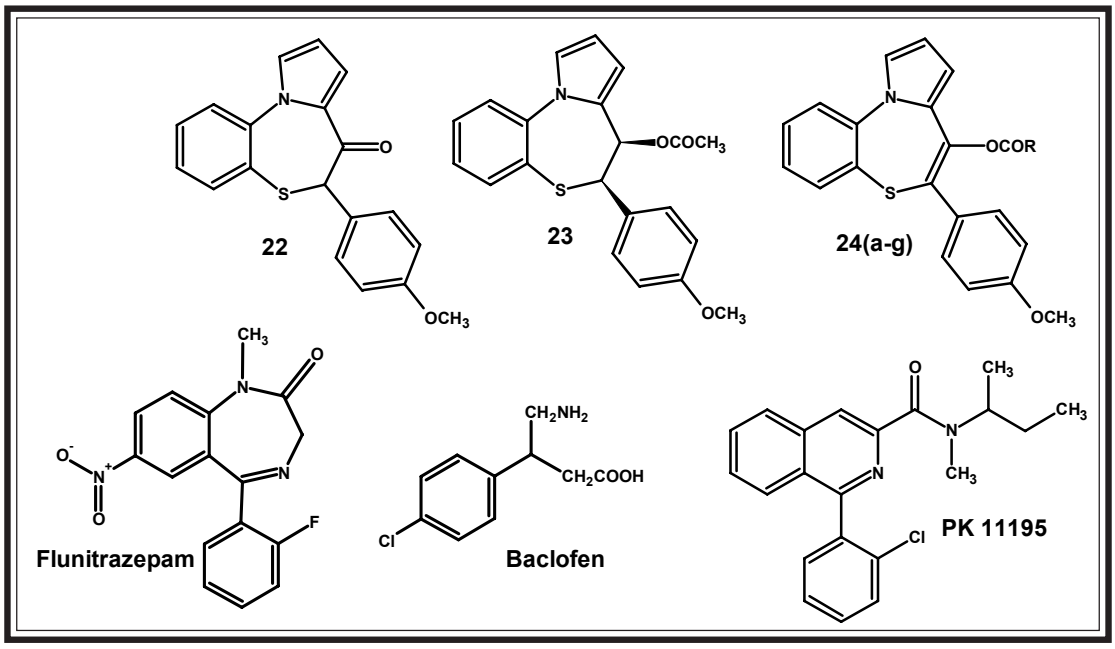


In 1984, Nacci and Fironi[109] have studied 9-chloro-, 9-trifluoromethyl- and 9-methoxy-5- phenylpyrrolo [2,1-d] [1,5] benzothiazepine (20 a-c) and cis-9-chloro- and cis-9-trifluoromethyl-4,5-dihydro-4-hydroxy-5- phenylpyrrolo [2,1-d] [1,5] benzothiazepine with the respective acetyl derivatives (21a-d) as sedative agents and were tested against the anti-amphetamine activity in the rat. The pyrrolo [2,1-d] [1,5] benzothiazepine -5-carboxamide showed sedative activity similar to that of diazepam.

In continuation with this, 6-p-methoxyphenylpyrrolo[2,1-d][1,5] benzothiazepine derivatives were evaluated for their affinities for Benzodiazepines and GABA receptor subtypes. Under present study 6-p-Methoxyphenylpyrrolo[2,1-d][1,5]benzothiazepin-7(6H)-one (22), cis-7-acetoxy-6,7-dihydro-6-p-methoxyphenylpyrrolo[2,1-d] [1,5]benzothiazepine (23) and some significant 7-acyloxy-6-p-methoxyphenylpyrrolo[2,1-d][1,5]benzothiazepines (24 a-g) were synthesized and tested *in vitro* for inhibition of the specific binding of 3H-Flunitrazepam, 3H-PK 11195, 3H-Muscimol and 3H-(-)Baclofen to central and peripheral benzodiazepine, GABA-A & GABA-B receptors, respectively. The compounds (22), (24 a) and (24 c) were active on the peripheral benzodiazepine receptor; in particular (24 a) and (24 c) were very active. The compound (24 g) showed an affinity, even though scanty, for the central benzodiazepine receptor[109a].

109(a).V. Nacci, I.Fiorini, A.Garofalo, A.Cagnotto, *Farmaco*., **45**(5)**,** 545(1990)



❖ **1,5-Benzothiazepine derivatives as Platelet aggregation inhibitors.**

It is generally accepted that platelets play a pivotal role in the progress and development of thrombotic disorders[110-114] especially cerebral vascular diseases such as transient ischemic attack,[115] ischemic heart diseases such as myocardial infarction,[116-118] and peripheral vascular diseases.[119] Recently, the inhibition of platelet functions by drugs is thought to be a useful therapeutic means for the prophylaxis and treatment of these diseases. For this purpose, many inhibitors of platelet aggregation such as Acetyl Salicylic Acid, Sulfinpyrazone, Dipyridamole, and Ticlopidine have been used clinically.[120-129]

As discussed earlier, diltiazem, a Ca^{2+} antagonist of the 1,5-benzothiazepine structure, has been developed, and its efficacy on angina pectoris, hypertension, and myocardial infarction has been well established.[130] It has already been reported that diltiazem shows antiplatelet action,[131-135] and it has also been reported that diltiazem,[136-137] its derivative clentiazem, and their basic metabolites[138] have inhibitory effects on platelet aggregation.

Similarly, Akio Odawara et al[139] have suggested, TA-993, an *l-cis* 4',8-dimethyl derivative of the Ca^{2+} antagonist diltiazem, and some of its metabolites inhibited platelet aggregation induced by collagen, ADP, epinephrine, platelet activating factor, arachidonic acid, and U-46619 in human platelets *in vitro*. Among the metabolites, MB3 was the most potent (IC_{50} <1 µmol/L; several hundred times more potent than the parent compound). The *d* isomer of MB3 was >100 times less potent than the *l* isomer. Unlike acetylsalicylic acid (ASA), TA-993 inhibited both primary and secondary phases of ADP-induced platelet aggregation and also exhibited a disaggregating effect on human platelet aggregates. The inhibitory effect of TA-993 was enhanced when used in combination with ASA. In ex vivo studies involving rats, TA-993 (0.3 to 100 mg/kg PO) dose-dependently inhibited collagen-induced platelet aggregation (ED_{50} 3 mg/kg po). In the whole-blood platelet aggregation system in rats, orally administered TA-993 was also inhibitory in single (3 to 30 mg/kg) or repeated daily (10 mg/kg per day for 10 days) dosage. Orally administered TA-993 dose-dependently inhibited ADP-induced platelet aggregation ex vivo in dogs (0.3 to 10 mg/kg), significantly protected mice against collagen+epinephrine–induced thromboembolic death (10 mg/kg), and inhibited thrombus formation in an arteriovenous shunt in rats (30 mg/kg). The Ca^{2+}-antagonistic action of TA-993 was very weak in depolarized canine basilar arteries: the potency was 1/10 that of diltiazem (*d-cis*) and *d*-TA-993. These results suggest that antiplatelet action is more characteristic of the *l-cis* than the *d-cis* 1,5-benzothiazepine structure and that TA-993 may become a clinically useful antiplatelet agent of this structure series.

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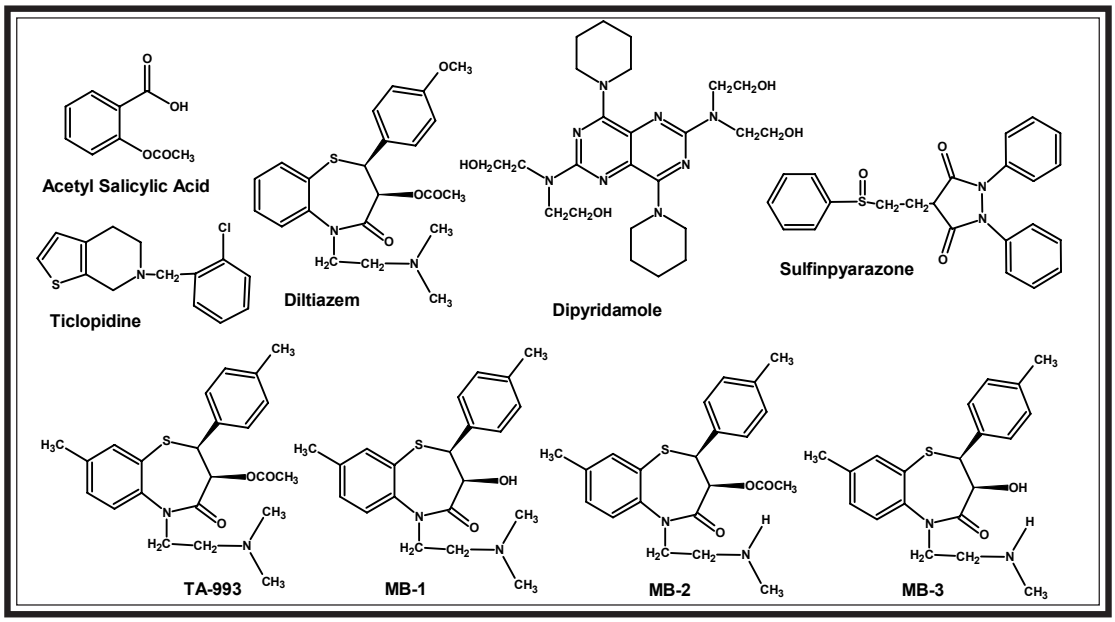

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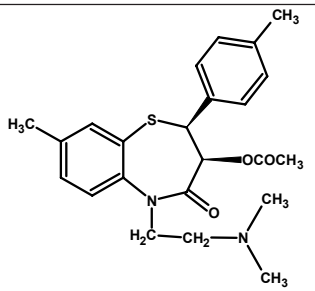
In continuation with this, Doi H. and co-researchers[140] have suggested that TA-993 (cis-(-)-2-(4-methylphenyl)-3-acetoxy-2,3-dihydro-5-(2-dimethylamino ethyl)-8-met hyl-1,5-benzothiazepine-4(5H)-one maleate), has a selective increasing action on limb blood flow in addition to an antiplatelet action. In this report the effect of TA-993 on a time dependent decrease in developed tension of electrically-induced contraction of tibialis anterior muscle in a rat model of peripheral circulatory insufficiency induced by occlusion of abdominal aorta was studied. The developed tension decreased by 20-30% in a sham-operated group and 30-40% in an abdominal aorta-occluded group at the end of the experimental period of 60 min. Intraduodenal administration (i.d.) of TA-993 (10 mg/kg) to the abdominal aorta-occluded rats ameliorated the decrease in developed tension to the level of the sham-operated group. Moreover, TA-993 at 10 mg/kg, i.d. significantly increased femoral arterial blood flow supplied through collateral circulation and decreased the whole blood viscosity in this model. These results suggest that TA-993 improves dysfunction of skeletal muscle contraction due to peripheral circulating insufficiency through an increase in collateral blood flow and an improvement of red blood cell deformability.

In addition to this, Minako Kaburaki et al[141] have stated that TA-993 has unique and selective increase action on limb blood flow with little arterial pressure besides antiplatelet action, and described the mechanism of the increase action on limb blood flow caused by TA-993 in anasthetized dogs. In a canine blood-perfused hindlimb preparation with a donor dog, TA-993(100 mg/kg i.v.) did not increase femoral blood flow when administered to the donor dog, but did when administered to a recipient dog. TA-993 did not show the increasing action on femoral blood flow in the presence of hexamethonium or phentolamine, where as it did in the presence of propranolol or atropine. TA-993 also showed a weak increasing effect on heart rate, which was inhibited by any one of these blockers. TA-993

(300 mg/kg i.v.) did not alter the phenylephrine (1–100 mg/kg i.a.)- or the talipexole (3–100 mg/kg i.a.)-induced increase in perfusion pressure in an autoperfused hindlimb. These results suggest that the increasing action of TA-993 on limb blood flow is mediated by the sympathetic nervous system but that the adrenergic receptors are not likely to be the central point of action of this new agent. There is a possibility that the mechanism of the increasing action on heart rate is different from that of its increasing action on limb blood flow.

Inoue, H et al[142] have studied 2,3-Dihydro-1,5-benzothiazepin-4(5H)-ones substituted with an alkyl, alkoxy, alkylthio, hydroxy, or amino group on the fused benzene ring of the 1,5-benzothiazepine skeleton and evaluated for vasodilating, antihypertensive, and platelet aggregation-inhibitory activities. (-)-cis-3-Acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-8- methyl-2-(4-methylphenyl)-1,5-benzothiazepin-4(5H)-one (25) was selected for further studies as a potent inhibitor of platelet aggregation.



25: (-)-cis-3-Acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-8- methyl-2-(4-methylphenyl)-1,5-benzothiazepin-4(5H)-one

❖ **Anticancer agents/Apoptotic agents.**

The regulation of apoptosis or programmed cell death is critical to the normal development and maintenance of tissue homeostasis. The molecular machinery of apoptosis has been studied extensively, and many components have been identified. Two main pathways of cell death have been elucidated (1)a death-receptor mediated pathway and (2)a mitochondrial pathway. During receptor mediated apoptosis, ligand interaction at the cell surface initiates signal transduction cascades by adaptor complexes that lead to activation of downstream substrates such

as caspases.[143,144] Activation of the mitochondrial apoptotic pathway occurs in response to various stimuli including cellular stress, DNA damaging agents, and UV irradiation results in the release of apoptogenic proteins, which activate downstream substrates[143,145,146]. Signaling cascades that regulate mitochondrial apoptotic pathways are incompletely defined, but some have been shown to involve mitogen activated protein (MAP) kinases and members of the Bcl-2 protein family.[145,147]

MAP kinases are a group of serine/threonine protein kinases that control a wide variety of cellular processes including cell growth and differentiation. In mammalian cells, three MAP kinases have been characterized in detail, the c-Jun N-terminal kinase (JNK), the p38 MAP kinase, and the extracellular signal regulated kinase (ERK). The JNK and p38 kinases are activated primarily by cellular stress such as heat shock, osmotic shock, and DNA damaging drugs, whereas the ERK subgroup is primarily activated by mitogens such as growth factors.

The consequence of JNK activation in cells is dependent on a number of factors including the death-inducing stimulus and the cell type, and these have been shown to mediate both pro- and antiapoptotic responses.[148] Proapoptotic JNK signaling cascades are mediated in response to a variety of stimuli such as the TNFR death receptor and the DNA damaging agent etoposide[149] and in some cases have been shown to regulate apoptosis by a direct interaction with a number of regulators such as members of the Bcl-2 family.[147,150]

Margaret Mc Gee et al[151] have developed five novel pyrrolo-1,5-benzoxazipines as proapoptotic agents. Their JNK-dependent induction of apoptosis in tumor cells suggested their potential as novel anticancer agents. The core structure of the apoptotic agent 6 was investigated, and the SARs were expanded with the design and synthesis of several analogues including pyrrolo-1,5-benzoxazipines and pyrrolo-1,5-benzothiazipines. The apoptotic activity of

the compounds reported demonstrated that the presence of an oxygen or a sulfur atom does not significantly influence potency and efficacy.Therefore, SAR analysis was performed by considering the effects of the fused A-ring and R' and R" substituents placed on both pyrrolobenz(pyrido)oxazepine and pyrrolobenzothiazepine skeletons.

To rationalize the structural parameters responsible for apoptotic activity, a systematic conformational search on compounds 26, 28, 29, 32, 33, and 34 was performed. Each generated conformation was subjected to a geometry optimization. Conformers were energetically selected and then grouped into families by fitting the atoms of the tricyclic ring system. Structural and conformational features of the active compounds were compared to the ones of the inactive pyrrolobenzoxa(thia)zepines, allowed to identify three main areas of interaction with the molecular target, schematically represented in Figure-2.

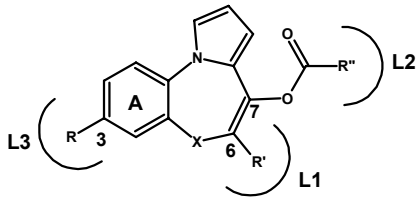


Figure - 2

X = O or S

A = Benzo, Naptho[2,3], Pyrido[3,2-b]

R = H, COOEt, COOH

R' = Ph, 4-MePh, 4-MeOPh, 1-naphthyl, 2-napthyl

R" = Me, $N(Me)_2$, n-butyl, n-pentyl, n-$(Et)_2$, 4-pyridyl

Comp.	X	A	R	R'	R"
26	O	Naptho[2,3]	H	Ph	$N(Me)_2$
27	O	Benzo	H	1-naphthyl	Me
28	O	Benzo	H	1-naphthyl	$N(Me)_2$
29	O	Benzo	H	2-naphthyl	$N(Me)_2$
30	S	Benzo	H	1-naphthyl	Me
31	S	Benzo	H	1-naphthyl	$N(Me)_2$
32	S	Benzo	H	1-naphthyl	$N(Et)_2$
33	S	Benzo	H	4-MeOphthyl	4-pyridyl
34	S	Benzo	H	1-naphthyl	4-pyridyl
35	O	Benzo	COOEt	1-naphthyl	$N(Me)_2$
36	O	Benzo	COOH	1-naphthyl	$N(Me)_2$

The R' group could be accommodated into the L1 region of the putative binding site. The potent apoptic agents 27 and 28 demonstrated that an extension of the aromatic ring system is responsible for the activity. It was clearly indicated that a shape dependant effect of the substituent placed at C-6 on the pyrrolooxa(thia)zepines-induced apoptotic event (Figure 3). In particular, the additional aromatic ring is able to favorably interact with L1

if it protrudes along the *X* direction of the Cartesian axes displayed in Figure 3 (1-naphthyl derivatives 28, 34; Figure 3A) while it is not tolerated along the *Y* direction (29, Figure 3B).

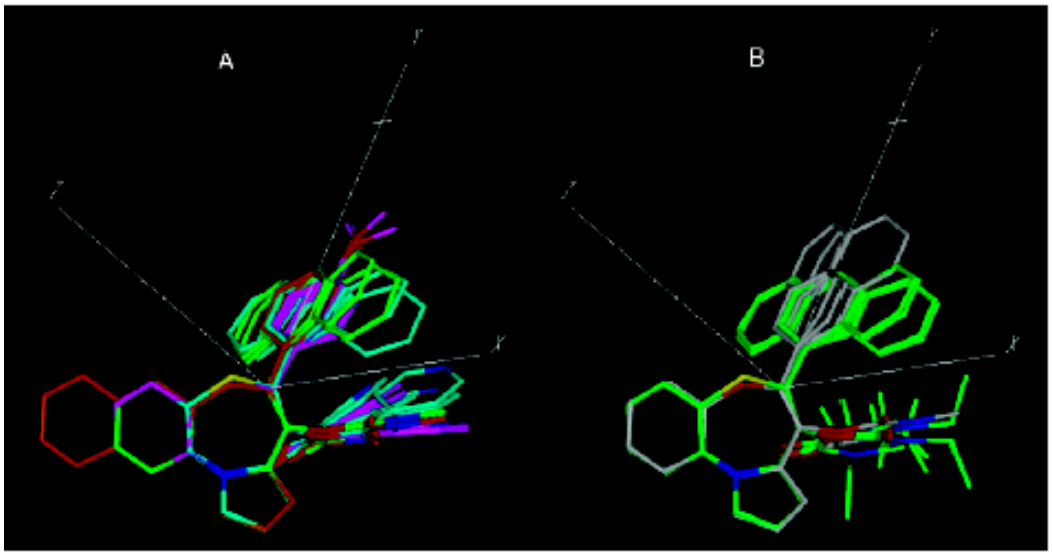


Figure 3. (A) Superimposition of the conformers of active pyrrolobenzooxa(thia)zepines (26, orange; 28, green; 33, violet; 34, cyan).
(B) Superimposition of the conformers of inactive compounds (29, white; 32, green-blue). Heteroatoms are colored by atom type:O, red; N, blue. Hydrogens are omitted for clarity.

The R" substituents interact with a second region in the putative binding site (L2, Figure 2).Accordingly the combination of a 1-naphthyl group at C-6 with a 4-pyridyl at C-7 led to one of the most potent apoptotic agent of the series (34). The apoptotic activity shown by 34 on HL-60 cells indicated that a planar aromatic substituent (i.e., 4-pyridyl group) could be well-accommodated in the L2 pocket of the putative receptor binding site.

In the pharmacophore hypothesis, the aromatic ring **A** may establish interactions in the putative binding site pocket L3 (Figure 2). In this region, polycyclic aromatic systems such as a naphtho-fused group strengthen apoptotic activity, and the introduction of a polar ethoxycarbonyl function in the benzo-fused system of 6 led to a potent, cell-penetrating apoptotic agent (35), probing tolerance at the L3 site. The cell-penetrating compound 35 induced apoptosis in tumor cells, while its nonpenetrating analogue 36, was incapable of inducing apoptosis or activating JNK. Plasma membrane

permeabilization of tumor cells resulted in 36-induced JNK activation, suggesting that the pyrrolo-1,5-benzoxazepine molecular target is intracellular. Interestingly, compound 26 displayed cytotoxic activity against a panel of human tumor cell lines but demonstrated negligible toxicity *in vivo* with no effect on the animal's haematology parameters.

❖ **Non nucleoside Reverse Transcriptase Inhibitors/Anti-HIV agents.**

Roberto De Santo and Roberta Costi[152] have designed , synthesized and tested 2H-Pyrrolo[3,4-b][1,5]benzothiazepine derivatives(PBTAs) and the related intermediates 3-pyrrolyl aryl sulphones(PASs) as potential anti HIV-1 agents targeted at the reverse transcriptase(RT). The PBTAs were conceived as tricyclic analogs of nevirapine, pyrrolo[1,2-*b*] [1,2,5]benzo thiadiazepine 37(PBTD) and pyrrolo[2,1-*d*] [1,2,5]benzothiadiazepine38, NNRTIs endowed with potent anti-HIV-1 activities. The majority of tested PBTAs were active against HIV-1-induced cytopathicity in MT-4 cells at concentrations ranging from 0.3 to 40 μM. In particular, compound 39 was the most potent derivative with EC_{50} = 0.3 μM, comparable to that of nevirapine used as reference drug. In the 3-pyrrolyl aryl sulfones series only three sulfones were found active against HIV-1 replication cycle. The following preliminary SAR could be depicted for the title derivatives:

i) the conformationally restrained PBTAs are more potent than the

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(e) R.Mane, D. Ingle, *Indian J. Chem.*, *Sect. B* , **21B**, 973(1982)

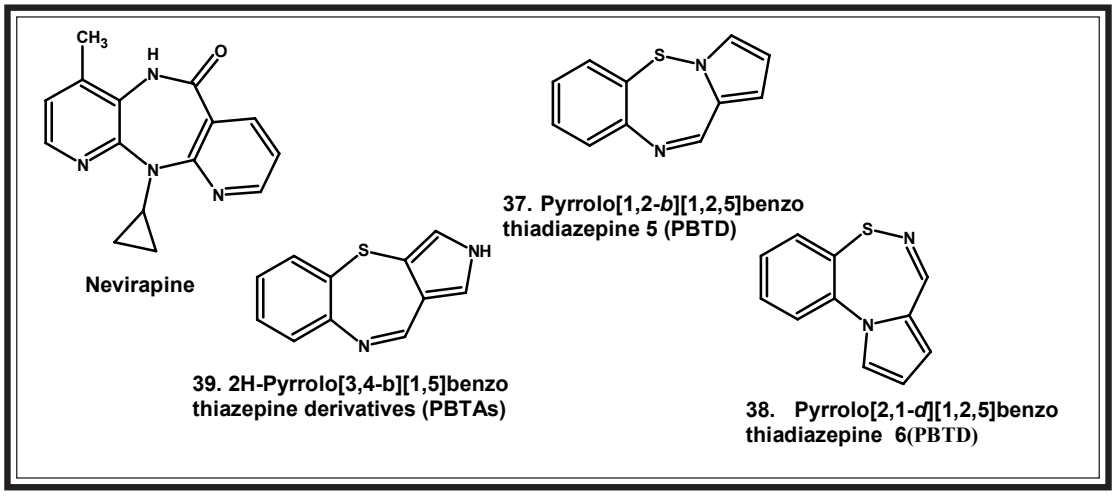
corresponding open counterparts (PASs);

ii) the DMA group give the highest anti-HIV-1 potency in the PBTAs series;

iii) PBTAs and the corresponding thiones are equipotent;

iv) an unsubstituted amino group, as part of *p*-chloroanilino moiety, is a strong determinant for the antiviral activity in the PASs series.

The most potent derivatives in cell-based assays were proven to target the RT in enzyme assays. Unfortunately, none of the test compounds inhibited the multiplication of clinically relevant drug-resistant viruses (mutants of HIV-1 carrying K103N and Y181C mutations) at concentrations lower than 30 μM. However, the good results obtained against replication of wt HIV-1, lead us to consider compound 39 as a lead compound for further investigation in this field. In particular, efforts were directed to modifications of compound 39 devoted to obtain new derivatives active against HIV-1 mutant strains.



❖ Antimicrobial agents.

The [1,5]benzothiazepine scaffold is extremly versatile, features in a great number of famous drugs and are widely used in a number of different therapeutic areas and therefore represent an interesting scaffold for De Novo exploration. Recent literature report suggested their value as antimicrobial agents. In addition to this, some authors shed light on the possibility to elicit antibacterial properties within this class[153]

Combinatorial chemistry has recently been highlighted as an important tool for drug discovery activities.[154] As a part of this, Fabrizio et al[155] afforded to exploit a novel combinatorial approach to the[1,5]benzothiazepine scaffold in order to investigate its antibacterial properties.

Diversity is obviously the driver to explore compounds potentially endowed with pharmacological activity and the [1,5]benzothiazepine scaffold affords the possibility to introduce three points of diversity quite readily as depicted in Figure-4.

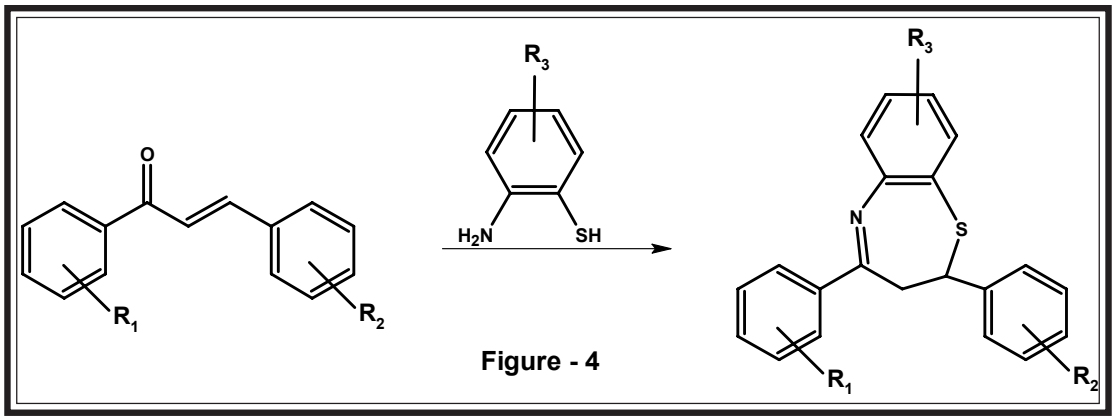
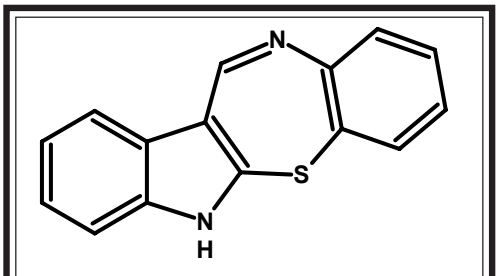


Figure - 4

V. Ambrogi and co-workers[156] have studied antimicrobacterial and cytostatic activities of Indolo[2,3-b][1,5]benzothiazepines and its 5,6-dihydro derivatives and several of them showed good activity against Gram possitive bacteria and *Cryptococci.*



Indolo[2,3-b][1,5]benzothiazepine

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Bonsignore L. et al[157] have studied *in vitro* antimicrobial activity of benzo fused seven membered heterocycles viz. 2,4-dione derivatives of 1,5-benzodithiepine, 1,5-benzodiazepine and 1,5-benzothiazepine along with analogous of 1,5-benzodioxepine, 1,5-benzoxathiepine and 1,5-benzoxazepine. Some of these compounds showed a good activity against some Gram positive micro organisms and blastomycetes.

❖ **Miscellaneous activities.**

Bradykinin (B_2) receptor agonist activity.

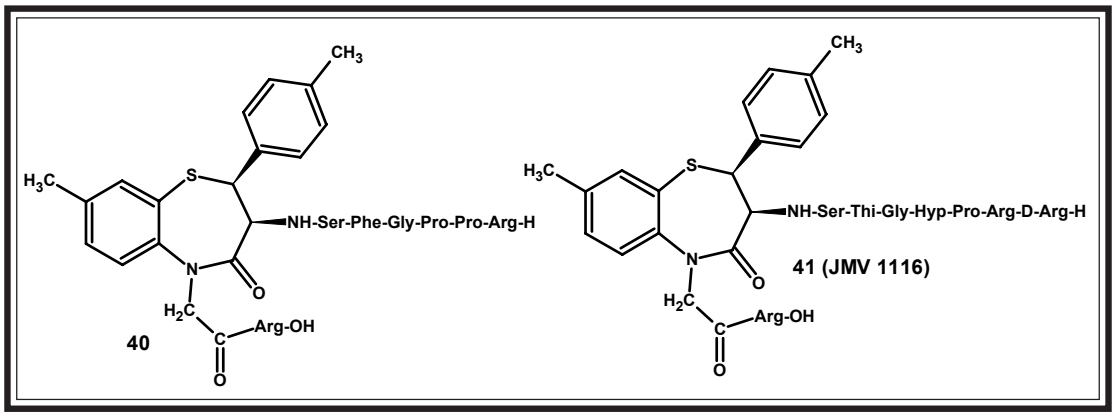
Bradykinin (BK), a linear nonapeptide hormone (HArg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH), and related kinins are thought to be involved in a wide variety of physiological and pathophysiological responses through activation of two types of receptors named B_1 and B_2 that have been cloned.[158,159] Bradykinin induces vascular and bronchial smooth muscle contraction and causes vasodilation and microvascular leakage.[160,161] After local injection into the skin, bradykinin produces all of the classical signs of inflammation: pain, swelling, redness, and heat.[159,162,163] Due to the pathophysiological role of bradykinin, considerable effort toward the development of bradykinin receptor antagonists as potential therapeutic agents has been carried out for several years. Since the initial discovery of bradykinin, a large number of peptidic and pseudopeptidic antagonists of bradykinin receptors have been described.[164,165] One of the most potent and selective bradykinin B2 receptor antagonists described so far is HOE 140, a decapeptide (H-D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-D-Tic^7-Oic^8-Arg^9-OH)[166] containing several unusual amino acids. This antagonist has sufficient high receptor affinity and *in vivo* life time to be investigated as a potential drug. On the other hand, it has been suggested that the cardioprotective effects of angiotensin-converting enzyme (ACE) inhibitors are due, at least in part, to a metabolic protection of bradykinin[167-169] and are therefore resulting from bradykinin B2 receptor activation. These findings along with the studies indicating that potent bradykinin pseudopeptide

agonists were useful in the delivery of anticancer drugs into brain tumors by increasing the permeability of the blood-brain barrier[170] suggest that the development of agonists having improved metabolic stability and oral bioavailability might be of great interest. Among them, one of the most studied bradykinin agonist, RMP-7[170] (H-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-Pro^7-4-Me-Tyr^{8}Ø(CH_2NH)-Arg^9-OH), was shown to enhance penetration of various anticancer drugs into human brain tumors[171,172] and of antiviral drugs through the blood-ocular barrier in the guinea pig.[173,174]

Extensive spectroscopic studies have shown that bradykinin possesses a high degree of conformational flexibility in solution, although evidence of a â-turn-like structure spanning the residues Ser^6-Pro^7-Phe^8-Arg^9 has been reported in DMSO and SDS micelles.[175,176] Based on 1H NMR and computational studies performed on several bradykinin analogues, it has been suggested that the high affinity of bradykinin antagonists, including HOE 140, for the B_2 receptor was related to their propensity to adopt C-terminal â-turn conformations.[177-179] However, ACE, a major bradykinin-degrading enzyme,[180] cleaves its substrate at Pro7-Phe8 and Phe5-Ser6 amide bonds suggesting that ACE inhibitors may display features complementary to the bradykinin receptor.

In an attemp to design bradykinin and HOE 140 analogues peptidic moieties at positions 7 and 8 since these positions are thought to be important for the agonist/antagonist activity and represent the cleavage site for ACE, because of design of peptidomimetics, incorporation of nonpeptide scaffolds into bioactive molecules has been the focus of extensive research over the last 10 years[181]. Muriel Amblard and coworkers[182] have synthesized bradykinin analogue (H-Arg-Pro-Pro-Gly-Phe-Ser-D-BT-Arg-OH, 40) in which the Pro-Phe dipeptide was replaced by the (3*S*)[amino]-5-(carbonylmethyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one (D-BT) moiety. The same modification was performed on the potent bradykinin B2 receptor antagonist HOE 140 (H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH),

in which the -D-Tic-Oic- moiety was replaced by D-BT to yield H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-BT-Arg-OH, 41(JMV1116). These compounds were examined *in vitro* for their binding affinity toward bradykinin B1 and B2 receptors as well as for their ability to interfere with bradykinin-induced contraction of both human umbilical vein and rat uterus. The two compounds 40 and 41 competed with [^{3}H]bradykinin binding to the human cloned B_2 receptor giving *K*i values of 13± 2 and 0.7± 0.1 nM, respectively. Unexpectedly, both compounds were full bradykinin B2 receptor agonists on the human umbilical vein (p*D*2 = 6.60± 0.07 for 40 and 6.80± 0.08 for 41) and rat uterus (p*D*2=7.20± 0.09 for 40 and 7.50± 0.09 for 41) preparations with the same efficacy as bradykinin. In addition 41 induced a concentration dependent phosphoinositide production in CHO cells expressing the human cloned B2 receptor. These data provide evidence for a bioactive conformation of bradykinin constrained at the dipeptide Pro-Phe.



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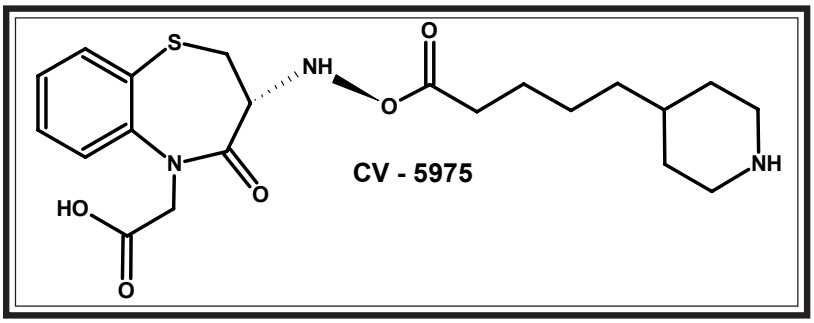
Angiotensin converting enzyme inhibitors

Inada Y et al[183] have described (R)-3-amino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid derivatives as structurally new angiotensin converting enzyme(ACE)inhibitors. A number of compounds has shown potent ACE inhibion *in vivo* and *in vitro.* Structure activity studies indicated that a piperidinyl moiety on the amino group at 3-position conferred long lasting ACE inhibitory activity and that the duration of activity on the length of the carbon chain in the 1-carboxy-ù -(4-piperidyl) alkyl group. (R)-3-[(S)-1-carboxy-5-(4-piperidyl)pentyl]amino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid(CV-5975) was selected as most promising ACE inhibitor for further studies because of its marked inhibitory activity.



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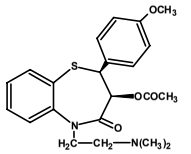
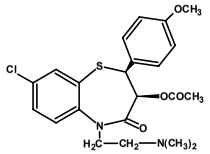
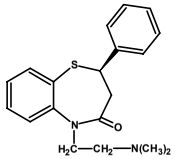
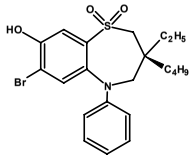
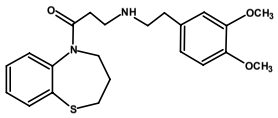
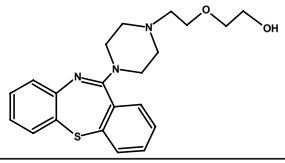
Some 1,5-Benzothiazepine drugs and derivatives under preclinical/Phase clinical trials.

Drug Name / Code	CAS Number	Structure	Chemical Name	Phase	Activity	Originator
Diltiazem			(+)(2S,3S)-3-acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one	preregistered	calcium channel antagonist, antihypertensive, antiagninal	
Clentiazem	96128-92-6, 96125-53-0 (free base)		(+)(2S,3S)-3-acetoxy-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one	preregistered	calcium channel antagonist, antihypertensive, antiagninal	Tonabe seiyaku
Thiazesim			(2R)-5-[2-(dimethylamino)ethyl]-2-phenyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one	preregistered	antidepresant	
GW-577	179410-97-0		7-bromo-3(s)butyl-3-ethyl-8-hydroxy5-phenyl-2,3,4,5-tetrahydro1,5-benzothiazepine-1,1-dioxide	preclinical	Treatment of Lipoproptein disorder, Inhibition of Ileal bile acids	Glaxo-welcome
KT-363	105394-80-7		5-[N-[2-(3,4-dimethoxyphenyl)ethyl]-ß-alanyl]-2,3,4,5-tetrahydro1,5-benzothiazepine	Phase-II	Antihypertensive, antiarrhythmic, calcium channel antagonist	Kotabuki
Quetiapine fumarate, ZD-5077,ICI-204636, Zm-204636,Seroquel	111974-72-2, 111974-69-7 (free base)		11-[4-[2-(2-hydroxyethoxy)ethyl]-1-piperazinyl]dibenzo[b,f][1,4]thiazepine	Launched (1997)	Antipsychotic, Dopamine D_2 antagonist, 5HT2A antagonist	Astrazeneca, Fujisawa

Synthetic Approaches

1,5-benzothiazepine are useful compounds in the drug research which stimulated the invention of various synthetic procedures for their preparation and chemical transformations[1].

There were number of synthetic approaches proposed in literature for the development of different 1,5-benzothiazepine derivatives among which important aspects discussed are as per following.

❖ Isolated approaches

❖[4+3] Cycloaddition reaction.

❖Glycidic ester condensation with 2-amino thiophenol.

❖Solid phase reactions, microwave assisted &/or one pot synthesis and Green chemistry approach &/or solvent free synthesis.

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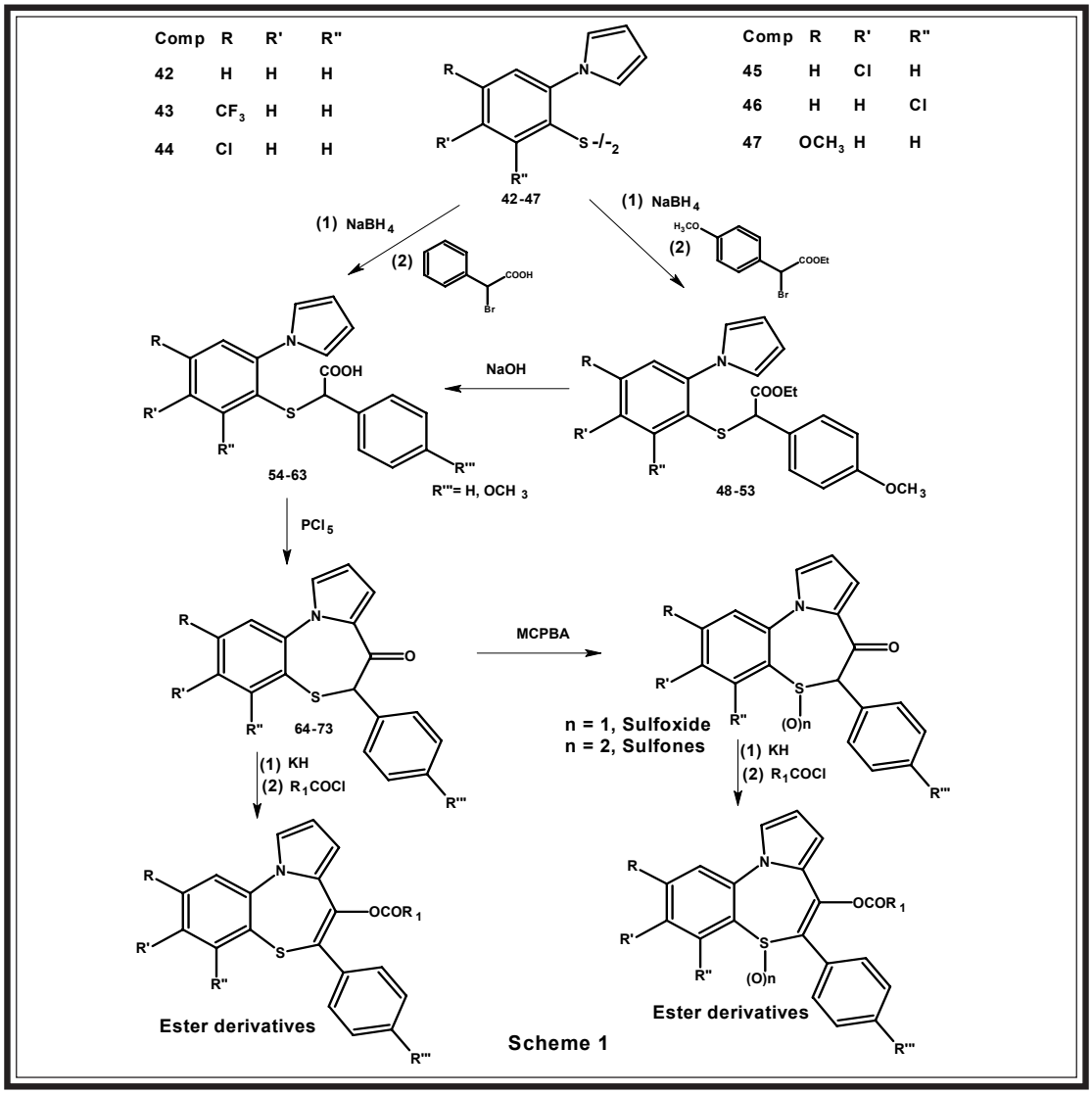
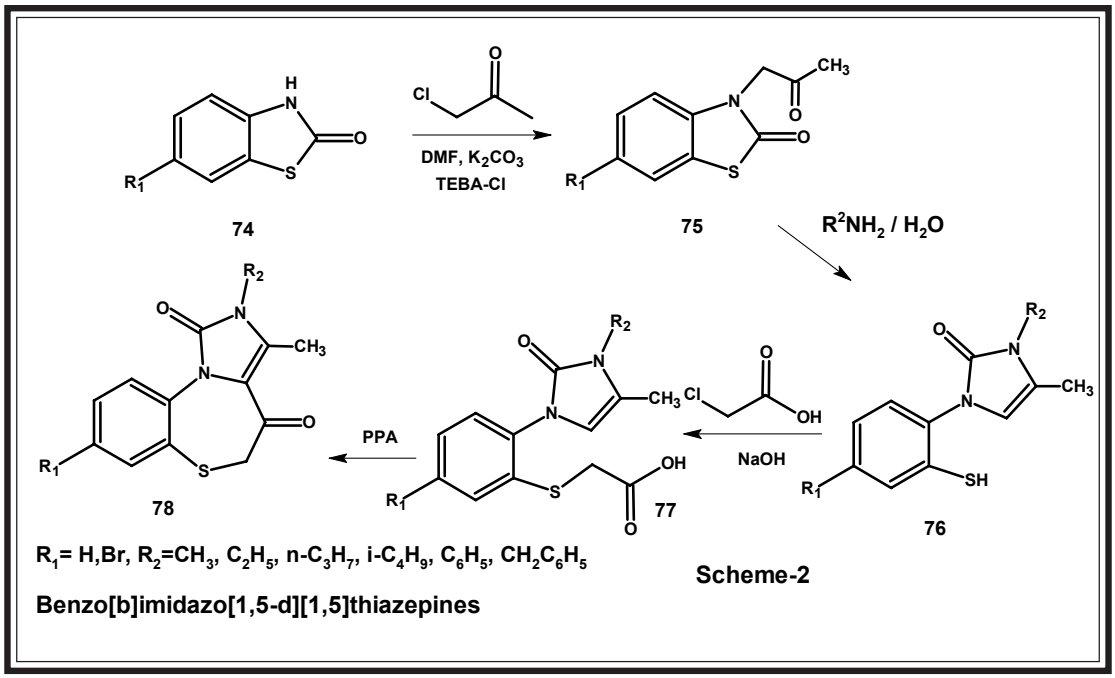
❖ **Isolated approaches.**

Fiorini et al[94] have synthesized 6-arylpyrrolo[2,1-d][1,5]benzothiazepine derivatives as shown in scheme-1. Esters(48-53) were obtained in good yield starting from bis(2-N-pyrrolylphenyl)disulphides(42-47) by reduction with sodium borohydride[184] followed by treatment with ethyl-á-bromo-p-methoxyphenylacetate[109a]. Hydrolysis of esters 48-53 with sodium hydroxide afforded acids 55[109a],56,58,60,62 and 63. Acids 54[186],57[185],59 and 61 were one step prepared starting from the appropriate disulphide, sodium hydroxide and commercially available á-bromo acetic acid. Intramolecular cyclization of acids 54-63 using phosphorus pentachloride gave ketones 64-73. Oxidation of 64 and 65 with m-chloroperbenzoic acid (MCPBA) afforded sulfoxides and sulphones. Finally sulfoxides and sulphones were converted to the desired esters using potassium hydride and the selected acid chlorides.



Scheme 1

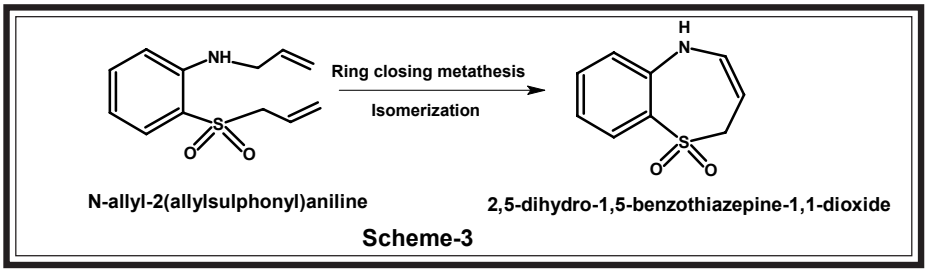
K.Petrova et al[187] have described method of synthesis of novel tricyclic ring system Benzo[b]imidazo[1,5-d][1,5]thiazepine. A method for synthesis of the title compounds 78, consisted of an intramolecular cyclization of 1-(2-carboxymethylthiophenyl)-2H-imidazol-2-ones 77 in poly phosphoric acid. Compound 77 was prepared by a reaction of 1-(2-mercaptophenyl)-2H-imidazol-2-one 76 with chloroacetic acid. 2H-Imidazolones 76 were obtained by a ring transformation of substituted 2(3H)-benzothiazolones 75 in reactions with primary amines. The method is convenient because of its simplicity and good yields(Scheme-2).



Scheme-2

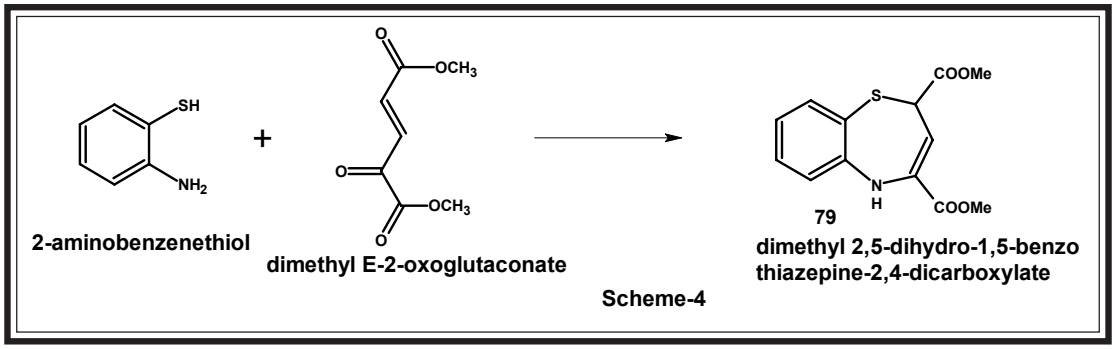
Benzo[b]imidazo[1,5-d][1,5]thiazepines

An isomerization ring closing metathesis(RCM) strategy afforded 2,5-dihydro-1,5-benzothiazepine-1,1-dioxide, from the protected N-allyl-2-(allylsulfonyl)aniline[188]. (Scheme-3)

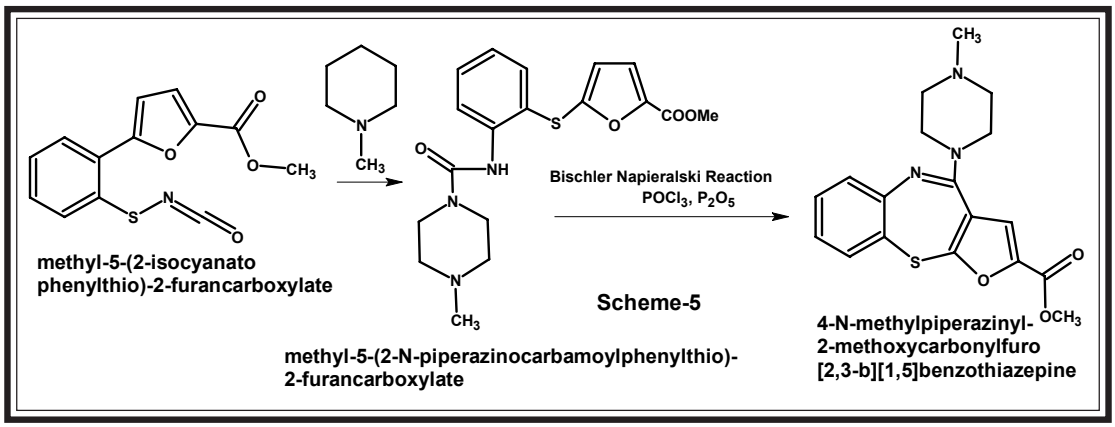


Scheme-3

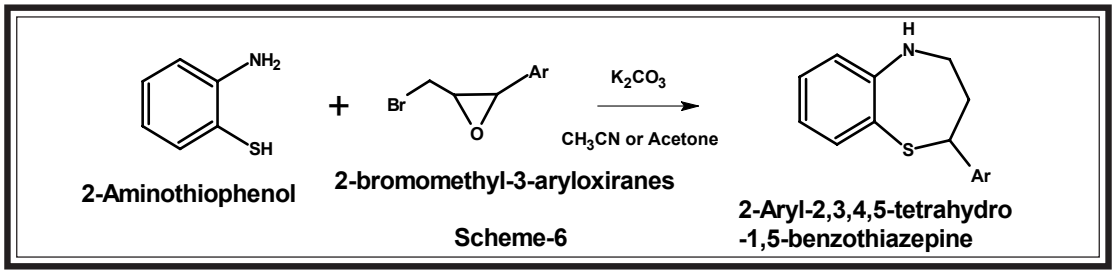
Blitzke T. et al[189] have synthesized 2,5-dihydro-1,5-benzothiazipine(79) by incorporating heterocycles segments originating from the precursor dimethyl(E)-2-oxoglutaconate by cyclocondensation with 2-aminobenzenethiol(Scheme-4).



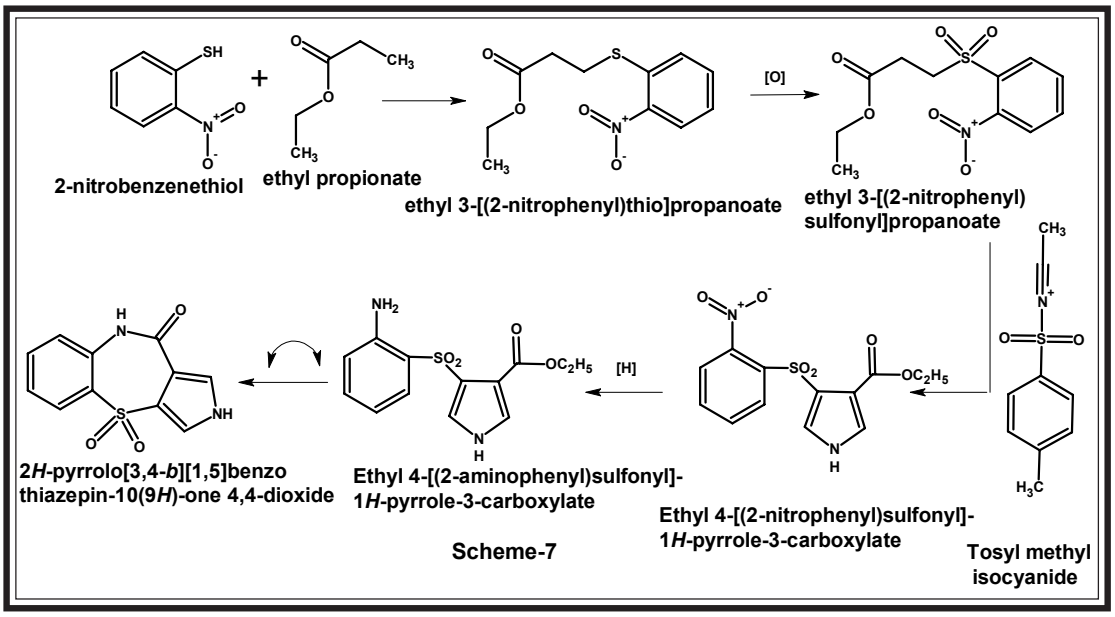
Ogawa M. and coworkers[190] have demonstrated facile synthesis of furobenzothiazepines. Thus, The reaction of methyl 5-(2-isocyanatophenylthio)-2-furancarboxylate with N-methylpiperazine gave 5(2-N-piperazinocarbamoylphenylthio)-2- furancarboxylate. Furthermore, 4-N-methylpiperazinyl-2- methoxycarbonylfuro[2,3-b][1,5]benzothiazepine was obtained by the Bischler-Napieralski reaction of with phosphorus oxy-chloride in the present of phosphorus pentoxide. Three furobenzothiazepines could be obtained using the same method. Based on the pharmacological studies of these compounds, it was found that 4-morpholinyl-2-methoxycarbonylfuro[2,3- b][1,5]benzothiazepine 4b had anti-inflammatory activity similar to flufenamic acid (Scheme-5).



Ganesh T & Krupadanam G.[191] have synthesized 1,5-benzothiazepine derivatives by the reaction between 2-aminobenzene thiol and 2-bromomethyl-3-aryloxirane in acetone or acetonitrile and potassium carbonate.(Scheme-6)

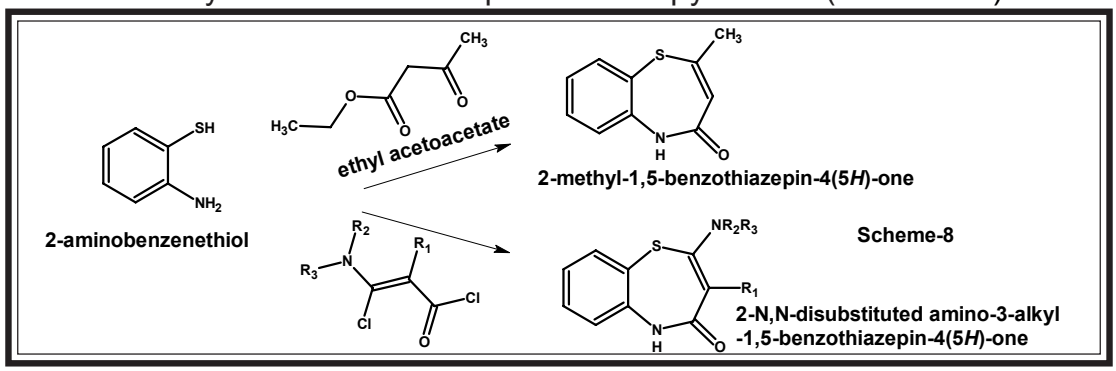


Santo et al[192] have synthesized pyrrole annulated 1,5-benzothiazepines. The synthetic stretegy involved condensation of 2-nitrothiophenol with ethyl propiolate afforded 3-(2- nitrophenylthio)propenoate. Oxidation of sulfur atom to sulfone group gave ethyl 3-(2-nitrophenylsulfonyl)propenoate, which underwent condensation with tosyl methylisocyanide (TosMIC) to yield ethyl 4-(2-nitrophenylsulfonyl)pyrrole-3-carboxylate. Reduction of nitro group afforded ethyl 4-(2-aminophenylsulfonyl)-1H-pyrrole-3-carboxylate, which was cyclized to 2H-pyrrolo[3,4-b][1,5] benzothiazepin-10(9H)-one 4,4-dioxide. Similar procedure was used for the synthesis of 9,10-dihydro-10-methyl-2H- pyrrolo[3,4-b][1,5]benzothiazepine 4,4-dioxide (Scheme-7).



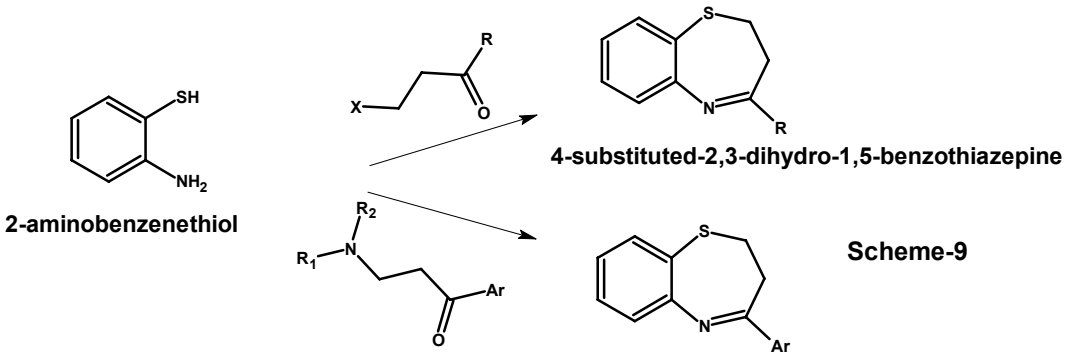
Scheme-7

2-methyl-1,5-benzothiazepine-4(5H)-one has been prepared by the reaction of 2-aminothiophenol with ethylacetoacetate in xylene at reflux temperature[193,194]. While 2,3-disubstituted 1,5-benzothiazepine-4(5H)-ones were synthesized by the reaction of 2-aminothiophenol with á-chloro-â-chlorocarbonyl enamines in the presence of pyridine[195](Scheme-8).



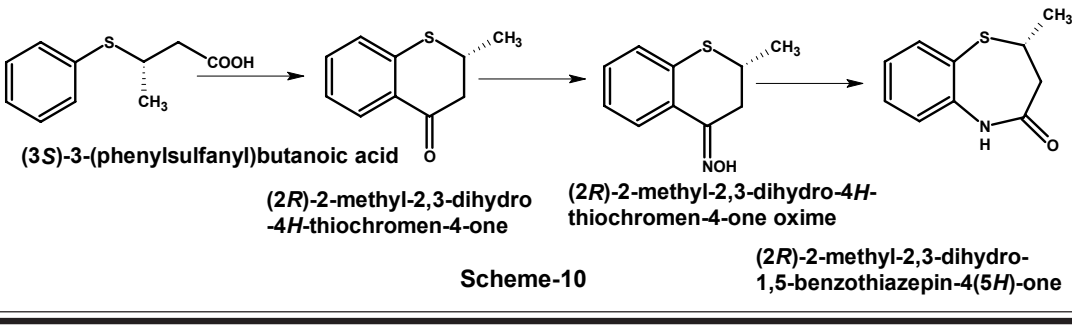
Scheme-8

A series of 4-aryl-2,3-dihydro-1,5-benzothiazepine was synthesized by the reaction of 2-aminothiophenol with N,N-disubstituted(2-aminoethyl)aryl ketones by Hideg and Hankovszky[196-198]. Similarly 4-substituted-2,3-dihydro-1,5-benzothiazepines have been synthesized by the reaction of 2-aminothiophenol with â-haloketones[199,200](Scheme-9).



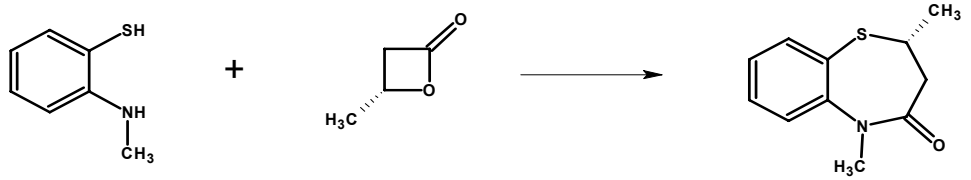
Scheme-9

A chemoenzymatic enatioselective synthesis of the (R)-2,3-dihydro-2-methyl-1,5-benzothiazepine-4(5H)-one has been propossed by Dike et al[201]. The procedure starts with Baker's yeast reduction of ethylacetoacetate. The optically active ester obtained in this was used for the preparation of the (R)-3-(phenylmercapto)butyric acid as key intermediate of this synthesis. The later was then cyclized into (R)-2-methyl-1-thiochromone, oxime of which yielded the (R)-2,3-dihydro-2-methyl-1,5-benzothiazepine-4(5H)-one by Beckmann rearrangement (Scheme-10).



Scheme-10

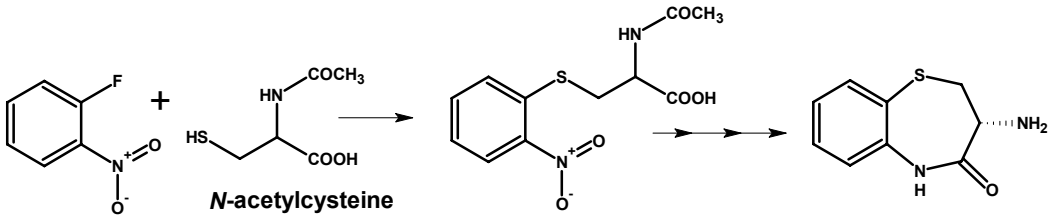
An efficient one pot synthesis of the (R)-2,3-dihydro2,5-dimethyl-1,5-benzothiazepin-4(5H)-one, has been described by Breitschuh and Seebach[202]. (S)-â-Butyrolactone was allowed to react with 2-(N-methylamino)thiophenol to yield the taget compound without isolation of the appropriate carboxyllic acid intermediate (Scheme-11).



2-(methylamino)benzenethiol (4*R*)-4-methyl-2-oxetanone (2*R*)-2,5-dimethyl-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one

Scheme-11

Optically active 3-amino-2,3-dihydro-1,5-benzothiazepine-4(5H)-ones are very interesting and useful benzothiazepine owing to their angiotensin converting enzyme(ACE)inhibition activity. It was first synthesized by Slade et al[203]. 2-Fluoronitrobenzene was allowed to react with N-acetylcysteine to afford corresponding nitrocarboxylic acid derivative which afforded the target compound on several steps (Scheme-12).

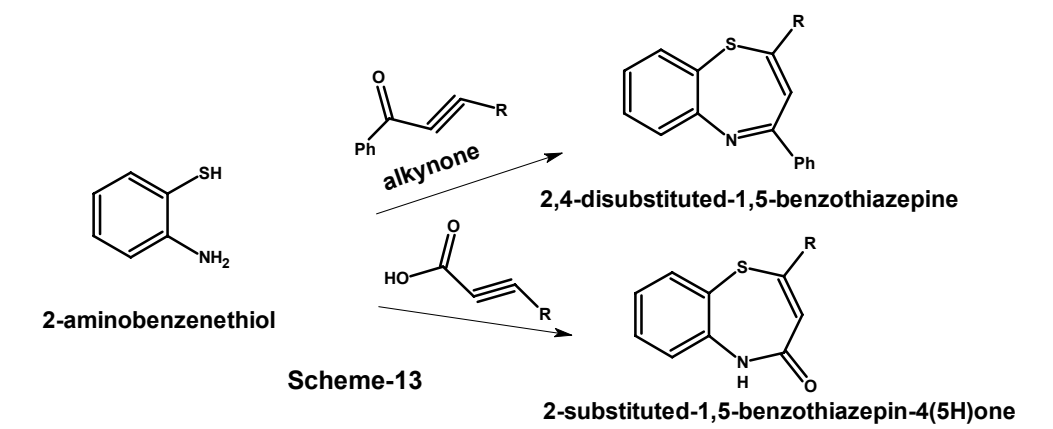


2-Fluoronitrobenzene *N*-acetylcysteine *N*-acetyl(2-nitrophenyl)cysteine

Scheme-12 (3*R*)-3-amino-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one

❖ 4+3 cycloaddition reaction

2,4-disubstituted-1,5-benzothiazepine derivatives were synthesized from 2-aminobenzenethiol, allowed to react with alkynones in a mixture of hot methanol and acetic acid[204,205]. General procedure for 1,5-benzothiazepin-4(5H)-ones followed 2-aminothiophenol heated either with propiolic acid (R=H) or its â-substituted derivatives[206-208] (Scheme-13).



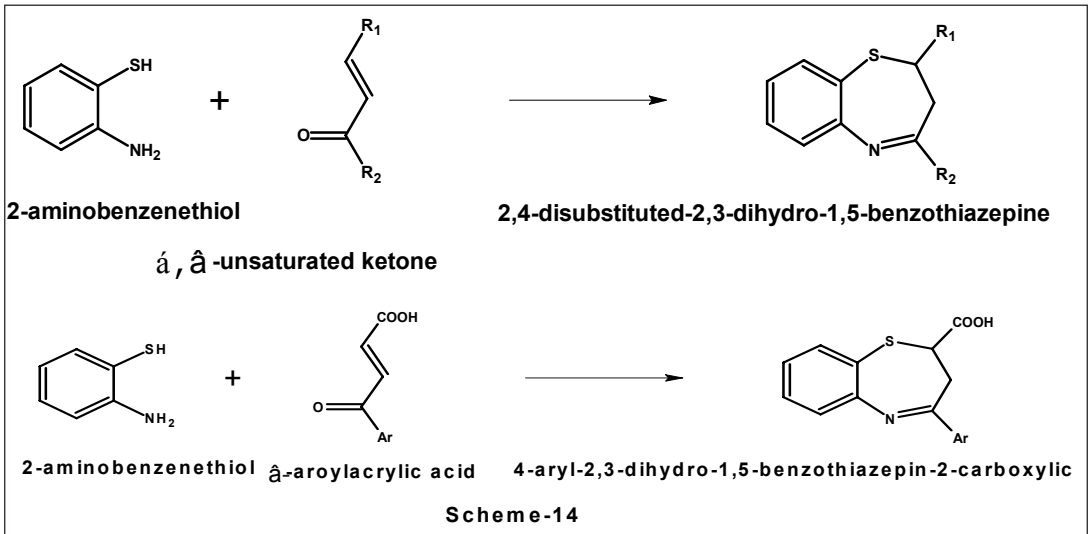
2-aminobenzenethiol

Scheme-13

2,4-disubstituted-1,5-benzothiazepine

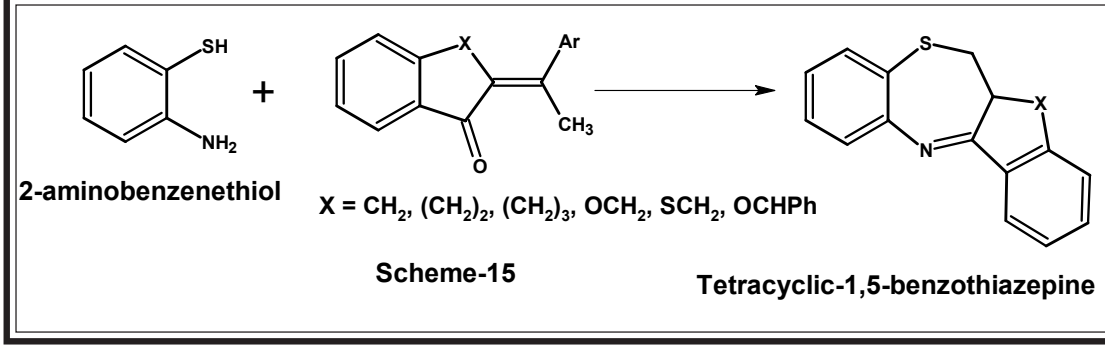
2-substituted-1,5-benzothiazepin-4(5H)one

The 2,4-disubstituted 2,3-dihydro-1,5-benzothiazepines can easily be synthesized by the reaction of 2-aminothiophenol with á,â-unsaturated ketones.These synthetic procedures have been thoroughly investigated in numerous laboratories. The intense studies resulted in the better understanding of various steric and electronic factors influencing the formation of such 1,5-benzothiazepines[209]. More over 4-aryl-2,3-dihydro-1,5-benzothiazepin-2-carboxylic acids have been synthesized in the late eighties by the reaction of 2-aminothiophenols with â-aroyl acrylic acids[210,211].



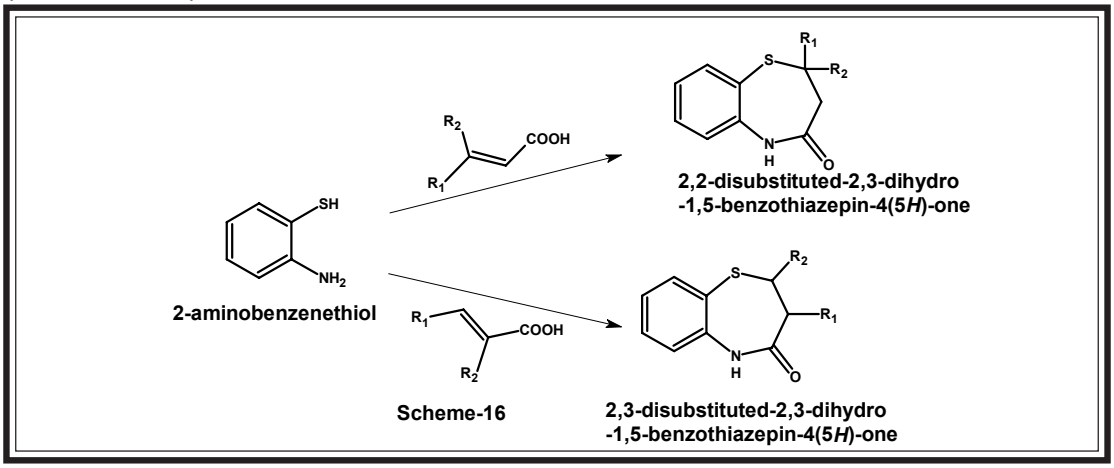
Scheme-14

Similar reaction of exocyclic á,â-unsaturated ketones with 2-aminothio phenol afforded tetracyclic benzothiazepines[212-215]. Stereochemistry of the targeted compounds has been elucidated by a combined utilization of various techniques. The spectroscopic measurments unequivocally proved that only one diastereomer was obtained in each case which revealed a completely diastereoselective formation of such benzothiazepines under the reaction conditions used for their synthesis (Scheme-15).



Scheme-15

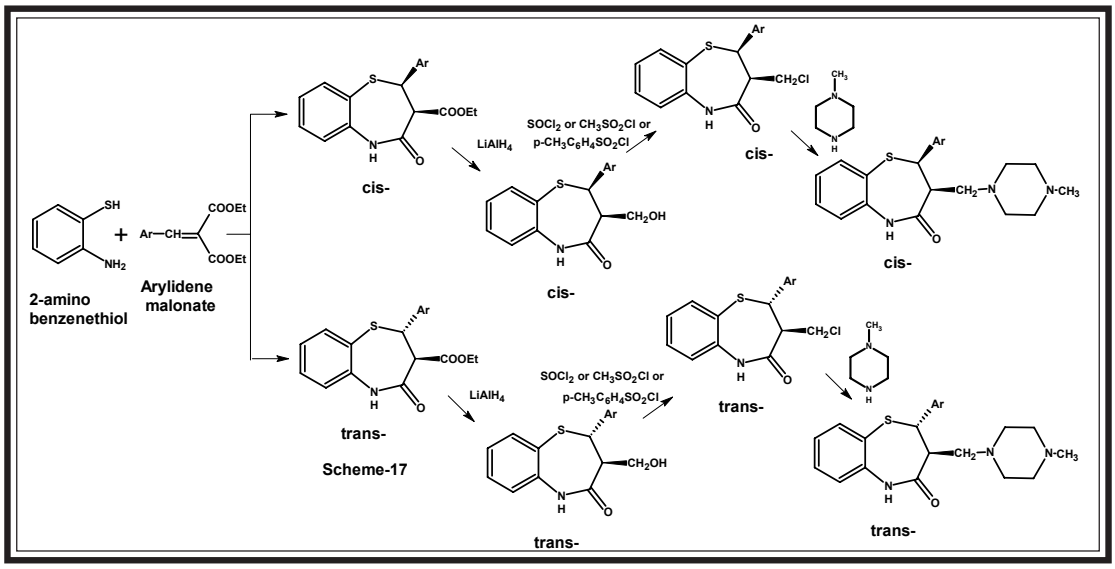
2,2-disubstituted-2,3-dihydro-1,5-benzothiazepin-4(5H)ones were synthesized by reaction between 2-aminothiophenol and 3,3-disubstituted-á,â-unsaturated carboxylic acids[216]. While, the simplest method for synthesis of 2,3-disubstituted-2,3-dihydro-1,5-benzothiazepin-4(5H)ones based on reaction between 2,3-disubstituted-á,â-unsaturated carboxylic acids[208,217,218] (Scheme-16).



Scheme-16

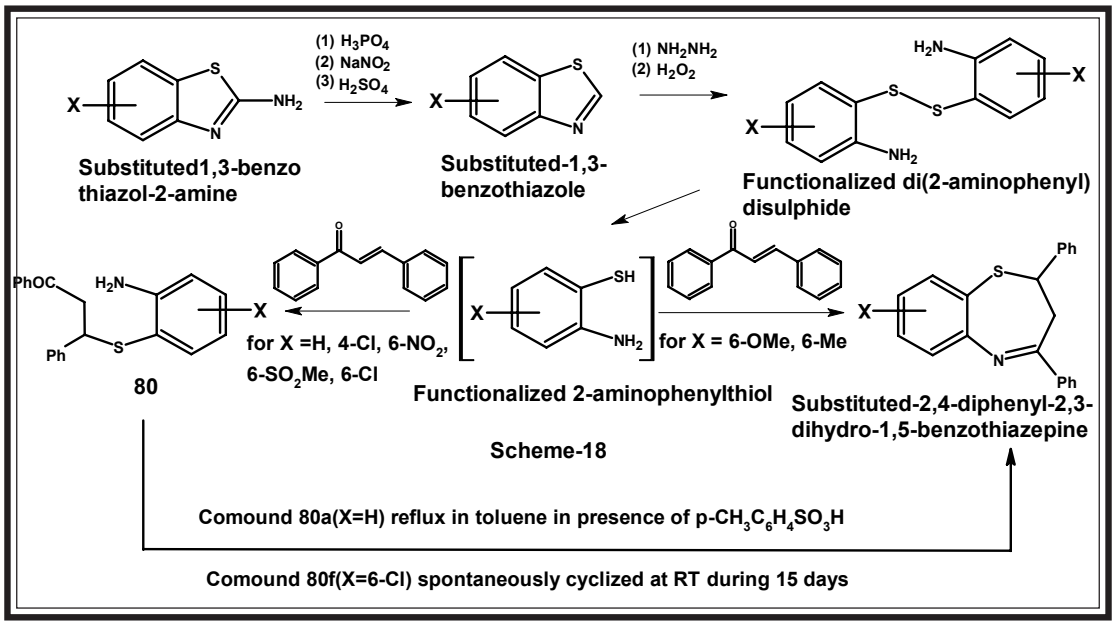
2,3-disubstituted-2,3-dihydro-1,5-benzothiazepin-4(5H)ones comprises the ethyl-2-aryl-2,3-dihydro-1,5-benzothiazepine-4(5H)-one-3-carboxylates[7,8,219-221]. Both their *cis*- and *trans*- isomers have been synthesized by the reaction of arylidene malonates and 2-aminothiophenol. The resulting ethyl-2-aryl-2,3-dihydro-1,5-benzothiazepine-4(5H)-one-3-carboxylates cam be reduced to corresponding alcohol by lithium aluminum hydride in tetrahydrofuran. The alcohols afforded the appropriate chlorides on treatment with thionyl chloride, methanesulfonylchloride or p-toluenesulfonylchloride, which on heating with N-methylpiperazine yielded2-aryl-2,3-dihydro-3-(4-methylpiperazinylmethyl)-1,5-benzothiazepin-4(5H)-ones. Some of these benzothiazepines were found to possess considerable antiulcer and gastric disorder inhibitors (Scheme-17).

212.G.Toth, A.Szollosy, A.Levai, H.Duddeck, *Org.Magn.Reson.,***20**,133(1982)
213.G.Toth, A.Szollosy, A.Levai, H.Duddeck, *Magy.Kem.Foly,* **89**,274(1983)
214.A.Levai, *Sci.Pharm.*,**64**,523(1996)
215.A.Levai, *Heterocyclic Commun.*,**3**,211(1997)
216.W.H.Mills, J.B.Whitworth, *J.Chem.Soc.*,2738(1927)
217.J.Krapcho, E.Spitzmiller, C.Turk, *J.Med.Chem.*,**6**,544(1963)
218.A.Lavai, H.Duddeck, *Pharmazie*, **38**, 827(1983)
219.S.Ohno, K.Izumi, K.Mizukoshi, K.Kato, M.Hori, *Chem.Pharm.Bull.*, **31**,1780(1983)
220.S.Ohno, K.Izumi, K.Mizukoshi, K.Kato, M.Hori, *Chem.Pharm.Bull.*, **36**,551(1988)
221.B.Letois, J.Lancelot, C.Saturrnino, M.Robba, P.De-Capraiis, *J.Heterocycl.Chem.*, **29**, 1769(1992)

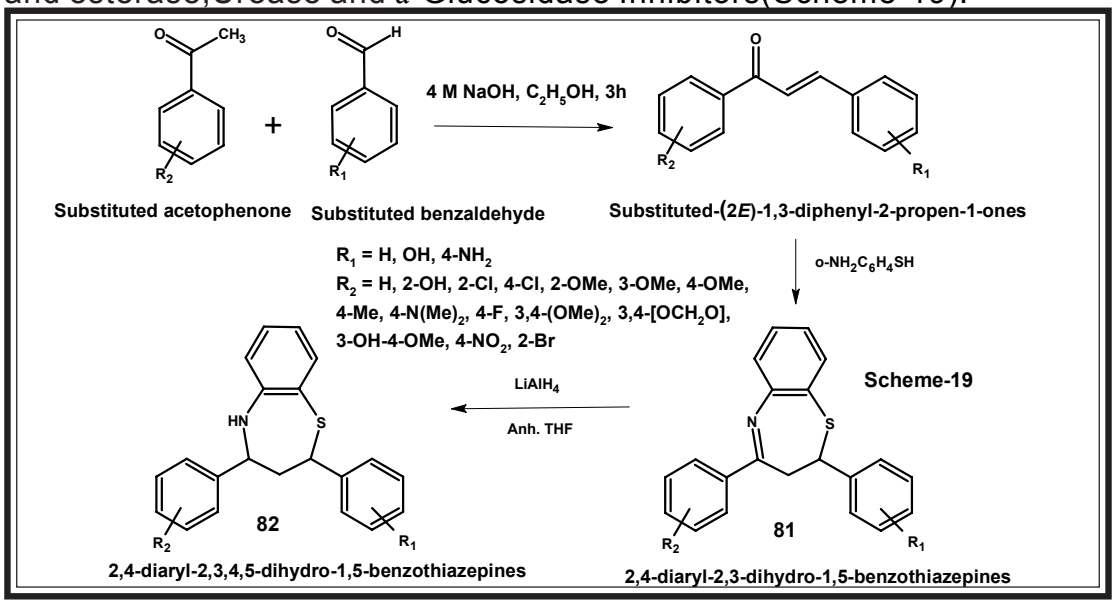


Scheme-17

2,4-diaryl-2,3-dihydro-1,5-benzothiazepine were usually prepared by [4+3]cyclocondensation between 2-aminothiophenol with á,â-unsaturated ketones in the presence of catalytic amount of acid[222] or under neutral conditions[223,224]. A.R.Katritzky et al[225] have described that reductive cleavage of benzothiazoles provides good yield of functionalized 2-aminothiophenols or the stable functionalized di(2-aminophenyl)disulphides as 2-aminobenzenethiols precursors. In an attempt to reduce disulfides in presence of chalcone with hydrazine hydrate gave 3,5-diarylpyrazoline, while reduction of disulfides by using triphenylphospine in 20% of water/acetone mixture with Chalcones (2 equivalent) in the presence of a slight excess of triphenylphosphine (1.1 equivalents) gave 46% ring opened product (80). During this reaction, the fast and complete cleavage of disulphide was progressively followed by reverse oxidation of the yielded o-aminothiophenol. By repeating the condensation under argon or nitrogen the air oxidation of the intermediate 2-aminothiophenol was avoided and compound 80 was obtained in 89% yield. Compound 80a (X = H) was cyclized to corresponding benzothiazepine by refluxing in toluene in catalytic amount of p-toluenesulphonic acid, while intermediates 80b(X = 6-OCH_3) and 80g((X = 6-CH_3) occured in situ to obtain appropriate benzothiazepine. Surprisingly analogous self cyclization was observed with compound 80f (X = 6-Cl)upon conservation at room temperature for 15 days (Scheme-18).



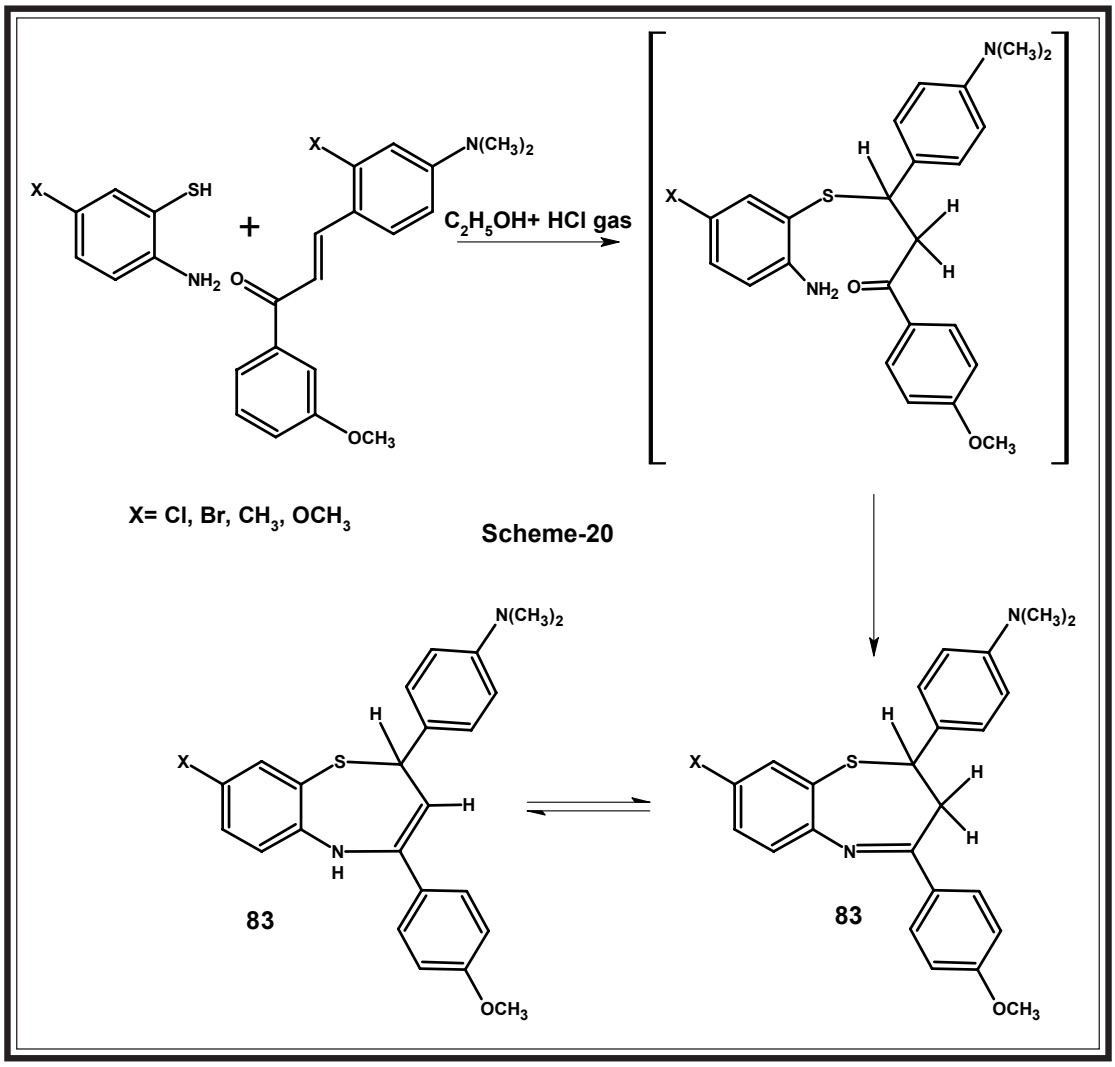
Atta-ur-Rahman et al[31] have synthesized 2,4-diaryl-2,3-dihydro-1,5-benzothiazepines(81) by cycloaddition reaction between substituted chalcones and o-aminothiophenol in acidic methanol, among which compounds having substitutions(R_1=2-OH, R_2=H), (R_1=2-OH, R_2=4-F), (R_1=2-OH, R_2=4-Cl), (R_1=2-OH, R_2=4-OMe) and (R_1=2-OH, R_2=4-Me) were reduced with lithiumaluminumhydride in anhydrous tetrahydrofuran afforded diastereomeric mixture of corresponding tetrahydro analogues(82). All the synthesized compounds *viz.*chalcones, 2,4-aryl-2,3-dihydro-1,5-benzo thiazepines(81) and 2,4-diaryl-2,3,4,5-tetrahydro-1,5-benzothiazepines(82) were screened as DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavengers and esterase,Urease and á-Glucosidase Inhibitors(Scheme-19).



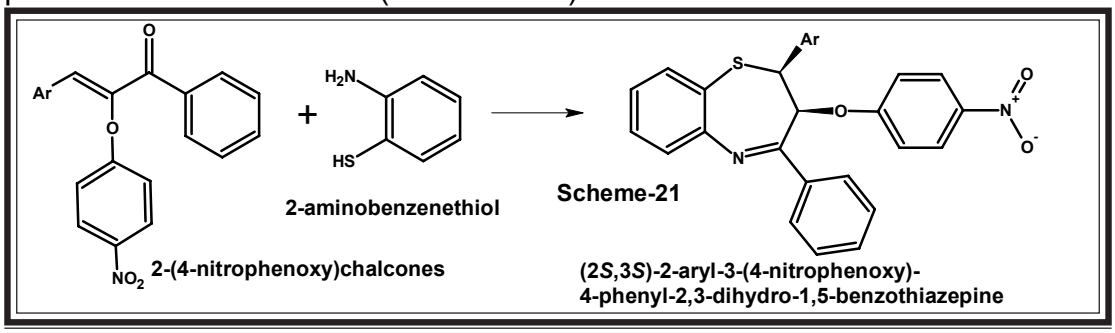
U.C.Pant and coworkers[239] have studied the reactions between á,â-unsaturated carbonyl compounds and 2-amino benzenethiols under various reaction conditions by using i) acidic medium-methanol/ethanol containing glacial acetic acid [226,227], ethanol saturated with hydrogen chloride gas [228], toluene containing traces of trifluoroacetic acid [229] ii) basic medium-pyridine [230] or toluene containing piperidine [231] and iii) neutral medium-anhydrous toluene [232,233] or o-xylene [234,235]. Since the yields of the products were found satisfactory in acidic medium containing hydrogen chloride gas [236,237], this method was used for the syntheses of 2-(p-dimethylaminophenyl)-4-methoxyphenyl-8-substituted-1,5-benzothiazepines(83).(Sceme-20)

In the literature, 2-amino benzenthiol has been reported to react with á,â-unsaturated ketones or chalcones to give a Michael addition type adduct [238], formed by the nucleophilic attack of the electron rich S of the thiol on the â-carbon atom of the chalcone, rendered electrophilic by a carbonyl group, when the reaction is carried out under milder conditions by using a neutral medium such as toluene [232,233]. It has also been reported that a mixutre of intermediate and final products or only final products are obtained under acidic or basic reaction conditions. The Michael addition products, when isolated, were cyclized to obtain the final product thereby establishing the reaction product as formed in two steps, the Michael addition product is an intermediate which is subsequently cyclized to give the final product.

222.W.Stephens, L.Field, *J.Org.Chem.*, **24**, 1576(1959)
223.U.Pant, M.Chugh, S.Pant, C.Modwel, *J.Indian.Chem.Soc.*, **69**, 342(1992)
224.U.Pant, M.Chugh, S.Pant, C.Modwel, *J.Indian.Chem.Soc.*, **68**, 418(1980)
225.A.Katritzky, B.Rogovoy, C.Chassaing, V.Vvedensky, B.Forood, B.Flatt and H.Nakai, *J.Heterocycl.Chem.*, **37**, 1655(2000)
226.U.Pant, A.Gupta, V.Singh, *Indian J. Chem.*, **22(b)**, 1057(1983).
227.S.Pant, B.Sharma, C.Sharma, U.Pant, *J.Indian. Chem. Soc.*, **73**, 83(1996).
228.S.Pant, U.Pant, *Indian. J. Chem.*, **33 b,**988(1994).
229.S.Pant, B. Joshi, U.Pant, *Indian J. Chem.,* **32 B**, 869(1993)
230.S.Pant , A.Sharma, C.Sharma,U.Pant, A.Goel , *Indian J. Chem.* , **35B**, 794(1996)
231. M.Upreti, S.Pant, A.Dandia, U.Pant, *Indian J.Chem.*, **36B**, 1181(1997)
232.U.Pant, M. Chugh, S.Pant, C.Modwel, *J.Indian Chem.Soc.*, **68,** 418(1991)
233.U.Pant, M. Chugh, S.Pant, C.Modwel, *J.Indian Chem Soc.*, **69**, 342(1992)
234.U.Pant, A. Gupta, *Indian. J. Chem.*, **20**, 157(1981)
235.S.Pant, A.Bhatia, A.Sharma, U.Pant, *Indian J. Chem.*, **33B,** 885(1994)

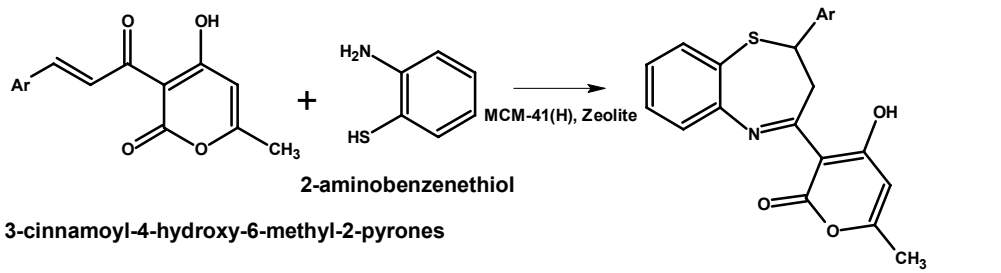


V.S.Rao et al have utilized á-(4-Nitrophenoxy)chalcones as intermediate for the synthesis of some new cis- (±)-2-aryl-3-(4-nitro phenoxy)-4-phenyl-1,5- benzothiazepine by cyclocondensation of 2-amino thiophenol in the presence of mild base[240] (Scheme-21).



236.M.Khanna, D. Kumar, C.Garg, R.Kapoor, *Indian J. Chem.*, **34B**, 333(1995)
237.M.Mushfiq, G.Mudgal, *J. Chem. Res. Synop.*,**5**, 168(1992)
238.J.Lancelot, B.Letois, C.Saturnino, P.Caprariis, M.Robba, *Org. Prep. Proceed. Int.*, **24**, 204(1992)
239.S.Pant, B.Singhal, M.Upreti, U.C.Pant, *Molecules*, **3**,159(1998)
240.V.Rao, A.Gupta, C.Gupta, *Syn.Commun.*,**30**(15), 2763(2000).
241.L.Nagarapu, R.Narendar, K.Sucheta, *Heterocyc.Commun.*, **9**(1), 35(2003)

Nagarapu et al[241] have developed convenient procedures for the synthesis of 2-aryl-4-(4-hydroxy-6-methyl-2-pyrone-3-yl)-2,3-dihydro-1,5-benzothiazepines by the condensation reaction between 2-aminothiophenol and 3-cinnamoyl-4-hydroxy-6-methyl-2-pyrones with MCM-41(H) Zeolite in ethanol (Scheme-22).



2-aminobenzenethiol

3-cinnamoyl-4-hydroxy-6-methyl-2-pyrones

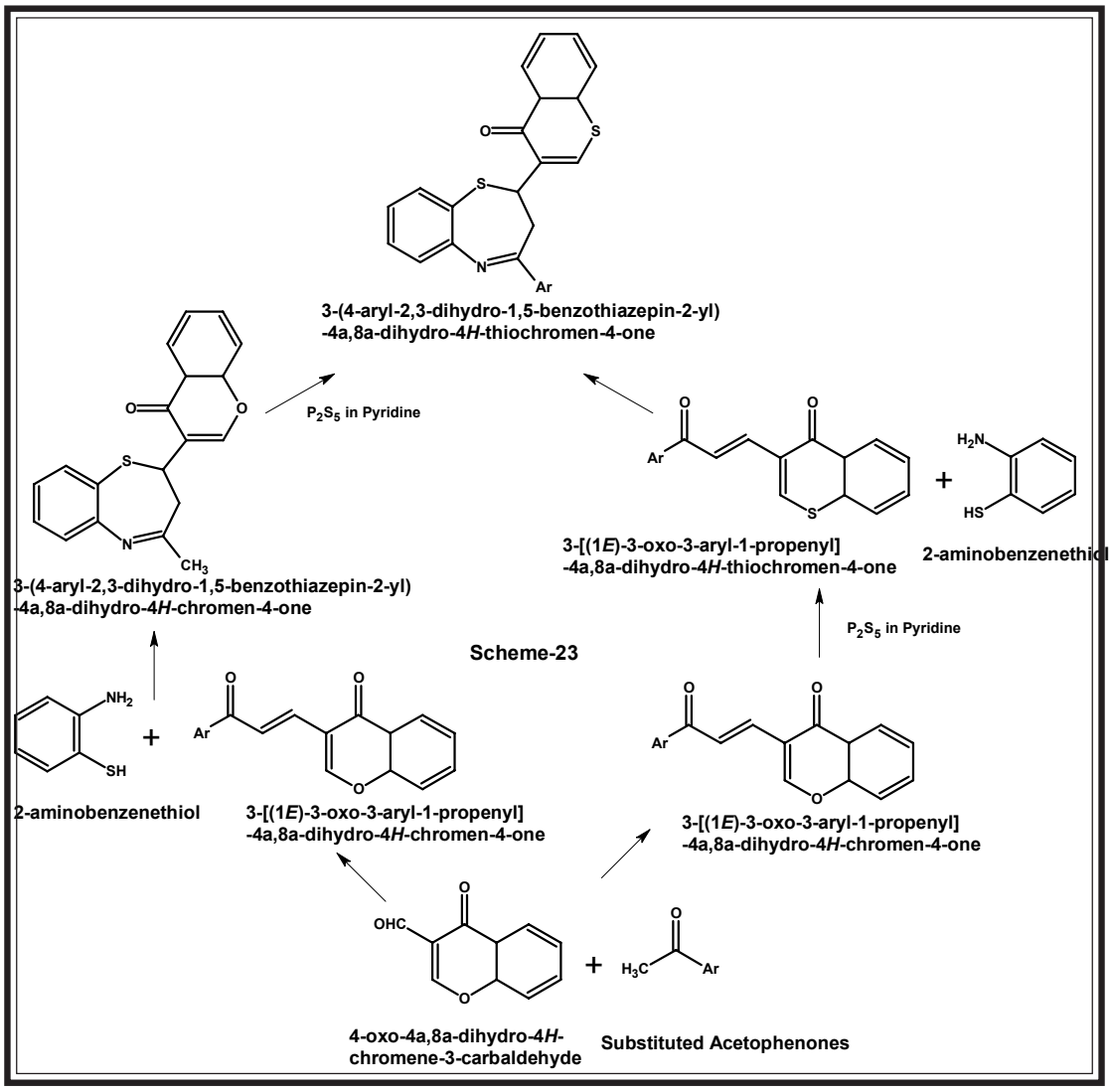
Scheme-22

2-aryl-4-(4-hydroxy-6-methyl-2-pyrone -3-yl)-2,3-dihydro-1,5-benzothiazepines

Chen X. et al[242] have suggested that Nitrodisulfides on treatment with SmI_2 in anhydrous THF at room temperature lead to reactive intermediates, which are 'living' double-anions and react smoothly with á,â-unsaturated ketones to afford 2,3-dihydro-1,5-benzothiazepines in good yields under mild and neutral conditions.

Nikalje M.A.G. and co-workers[243] have described that 3-Formyl chromones 1 were synthesized by using Vilsmeier-Haack reaction and were converted to respective 2-propane-1-ones by Claisen condensation. The cyclocondensation of the2-propane-1-ones and 2-aminobenzenethiol gave 2-chromonyl-4-substituted phenyl-2,3-dihydro-1,5-benzothiazepines. The 1,5-benzothiazepines and P_2S_5 when refluxed in pyridine were found to yield thiochromonyl-1,5-benzothiazepines. These compounds were also obtained from 3-formyl chromones by their conversion to 3-formyl thiochromones with P_2S_5 in pyridine. The 3-formyl thiochromones when condensed with aromatic aldehydes in presence of piperidine yielded á,â-unsaturated ketones. The cyclocondensation of this á,â-unsaturated ketones and 2-aminobenzenethiol gave identical products, 2-thiochromonyl-1,5-benzothiazepines (Scheme-23).

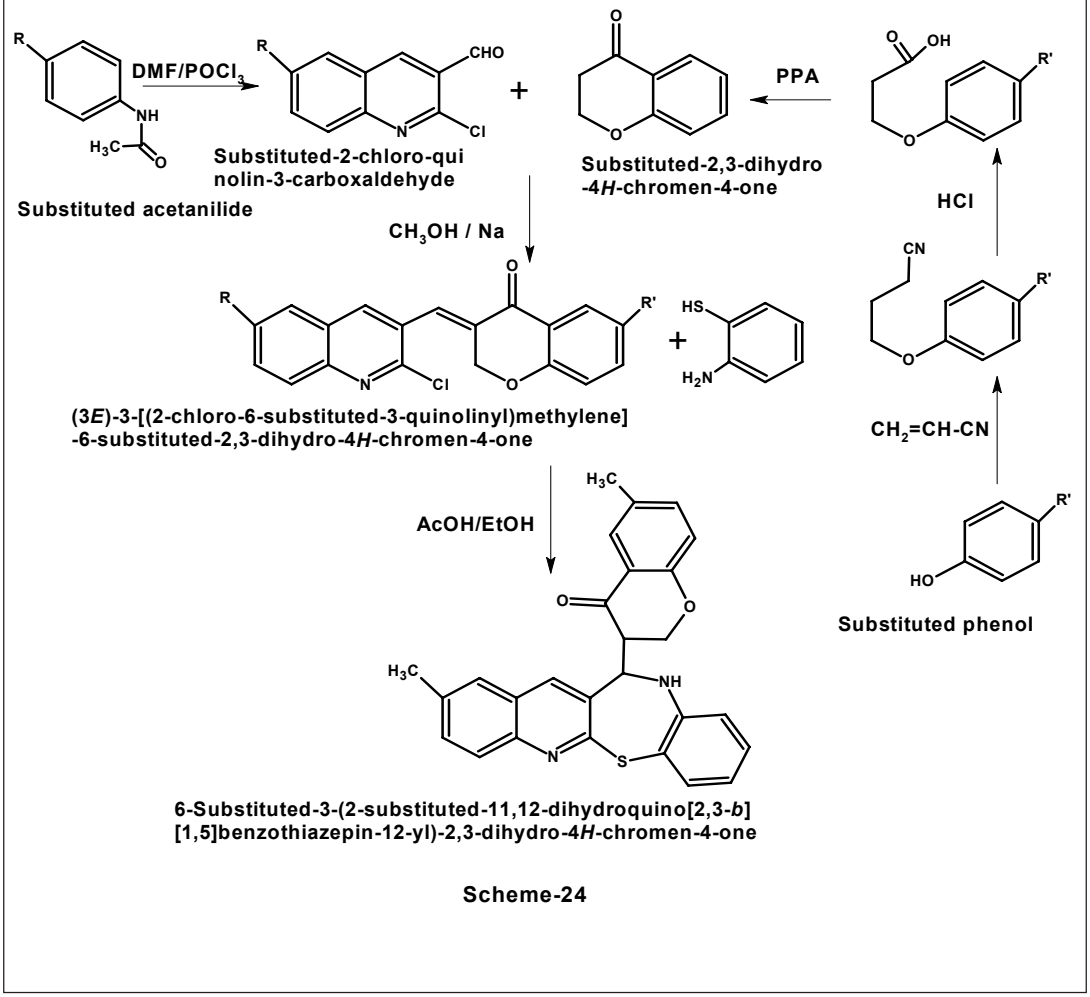
242.X.Chen, W.Zhong, Y. Zhang, *J.Chem.Res. - Part S*, **8**, 386(2000)
243.M.Nikalje, R.Ingle, V.Bhingolikar, R.Mane, *Indian J. Heterocycl Chem.*,**13**(1), 37(2003)



Liu F.M. and coworkers[244] have illustrated that 1,5-Benzothiazepines containing 2-phenyl-1,2,3-triazole which have potentially useful pharmacological properties were synthesized from á,â- unsaturated ketones and 2-aminothiophenol, and then underwent 1,3-dipolar cycloaddition reaction with aryl nitrile oxides "in situ" and a series of 1,2,4-oxadiazolo[4,5-d][1,5] benzothiazepine derivatives containing 2-phenyl-1,2,3-triazole were obtained. The experimental results show that if the substituent R_1 or R_2 at phenyl of 1,5-benzothiazepine is electron-donated, it will facilitate the 1,3-dipolar cycloaddition reaction. The structures of the products were confirmed by IR, ^{1}H NMR, MS and elementary analyses and their spectrum characters were also discussed.

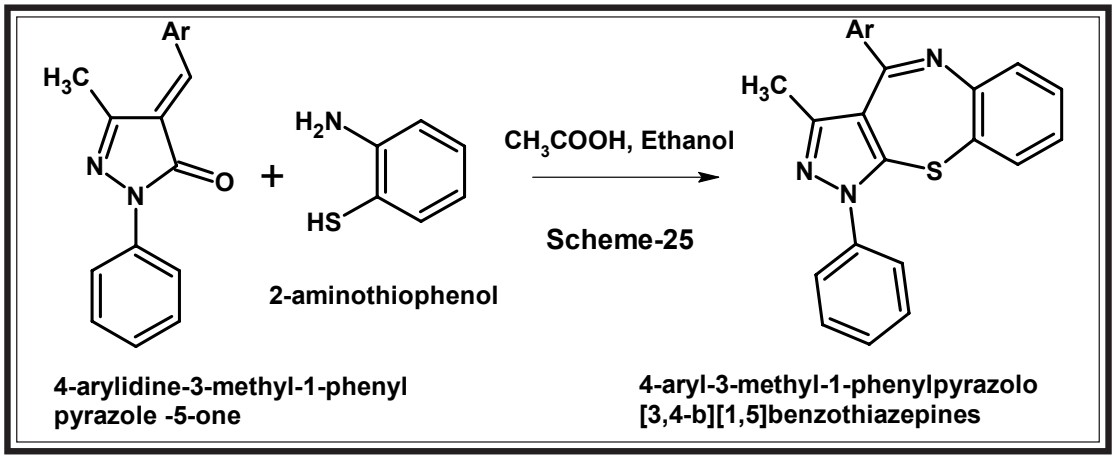
244.F.Liu, B.Wang, Y. Li, *Chemical Journal of Chinese Universities*, **23**(11), 2101(2002)
245.V.Krishnan, P.Dubey, S.Srinivas Rao, P.Prasada Reddy, *Indian Journal of Heterocyclic Chemistry*,**13**(1), 5(2003)

Krishnan et al[245] used substituted acetanilides for treatment with DMF-$POCl_3$ complex (Vilsmeier reagent) to obtain 2-chloro-quinoline-3-carboxaldehydes which on treatment with chromanones in ethanol in the presence of sodium methoxide gave chalcones. The later on treatment with o-aminothiophenol in the presence of catalytic quantity of acetic acid gave benzothiazepine system flanked by quinoline and chromanone moieties (Scheme-24).



Scheme-24

Abd El Latif F.M.[246] have synthesized 4-aryl-3-methyl-1-phenylpyrazolo[3,4-b][1,5]benzothiazepine derivatives via regioselective nucleophilic addition. 4-Arylidene-3-methyl-1-phenylpyrazole-5-one reacted with 2-aminobenzene thiol in ethanol-acetic acid solution to produce a mixture of 4-aryl-3-methyl-1-phenylpyrazolo[3,4-b][1,5]benzothiazepines, 2-arylbenzothiazoles and 3-methyl-1-phenylpyrazole-5-one (Scheme-25).



Ding Z. et al[247] have narrated an expedient synthesis of novel [1,2,4]triazolo[3,2- d][1,5]benzothiazepine derivatives via cycloaddition of the allene-like cations derived from thiochroman-4- one arylhydrazones, to the triple bond of nitriles and ensuing ring expansion annulation. The structure of one product has been unambiguously determined by X-ray diffraction.

❖Glycidic ester condensation with 2-amino thiophenol.

This perticular route of synthesis implimented selectively for the preparation of 2-substituted-2,3-dihydro-3-hydroxy-1,5-benzothiazepine-4(5H)ones. Some of their optically active derivatives are important antihypertensive agents. Depending on the reaction conditions, both 2,3-cis and 2,3-trans diastereomers can be prepared selectively.

The general route of synthesis included condensation of 2-nitrophenol with phenylglycidic esters afforded nitrocarboxylic acid esters which were reduced to their corresponding amino carboxylic acid esters. Ring closure of the later gave 2-aryl-2,3-dihydro-3hydroxy-1,5-benzothiazepine-4(5H)ones[1] (Scheme-26).

H.Inoue et al[61] have synthesized cis-2-aryl-2,3-dihydro-3-hydroxy-1,5-

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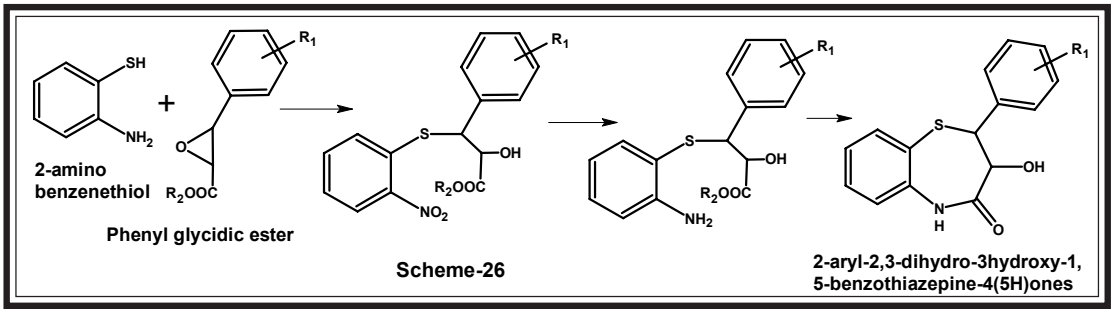
246.F.Abd El Latif, E.El Rady, *Phosphorus, Sulfur and Silicon and the Related Elements*, **179**(2), 215(2004)
247.X.Liu,Q.Wang, Y.Liu, Y.Fan, Z.Ding, *Synthesis*, **3**, 435(2000)
248.T.Hashiyama,H.Inoue M.Konda M.Takeda, *J.Chem.Soc.Perkin Trans*,**1**,1725(1984)
249.T.Hashiyama,H.Inoue, M.Takeda , *J.Chem.Soc.Perkin Trans*,**1**,421(1985)
250.T.Hashiyama, H.Inoue, *Chem.Abstr.*,**98**,125653v (1983)
251.H.Kugita, H. Inoue, M.Ikezaki, M.Konda, S.Takeo, *Chem.Pharm.Bull.*, **18**,2284(1970)
252.S.Yamada, K.Morimatsu, R. Yoshioka, Y.Ozaki, H.Seko, *Tetrahedron Asymmetry,* **9**(10),1713(1998)
253.G.Singh, N.Kumar, A.Yadav, A.Mishra, *Heteroatom Chemistry,* **13**(7), 620(2002)



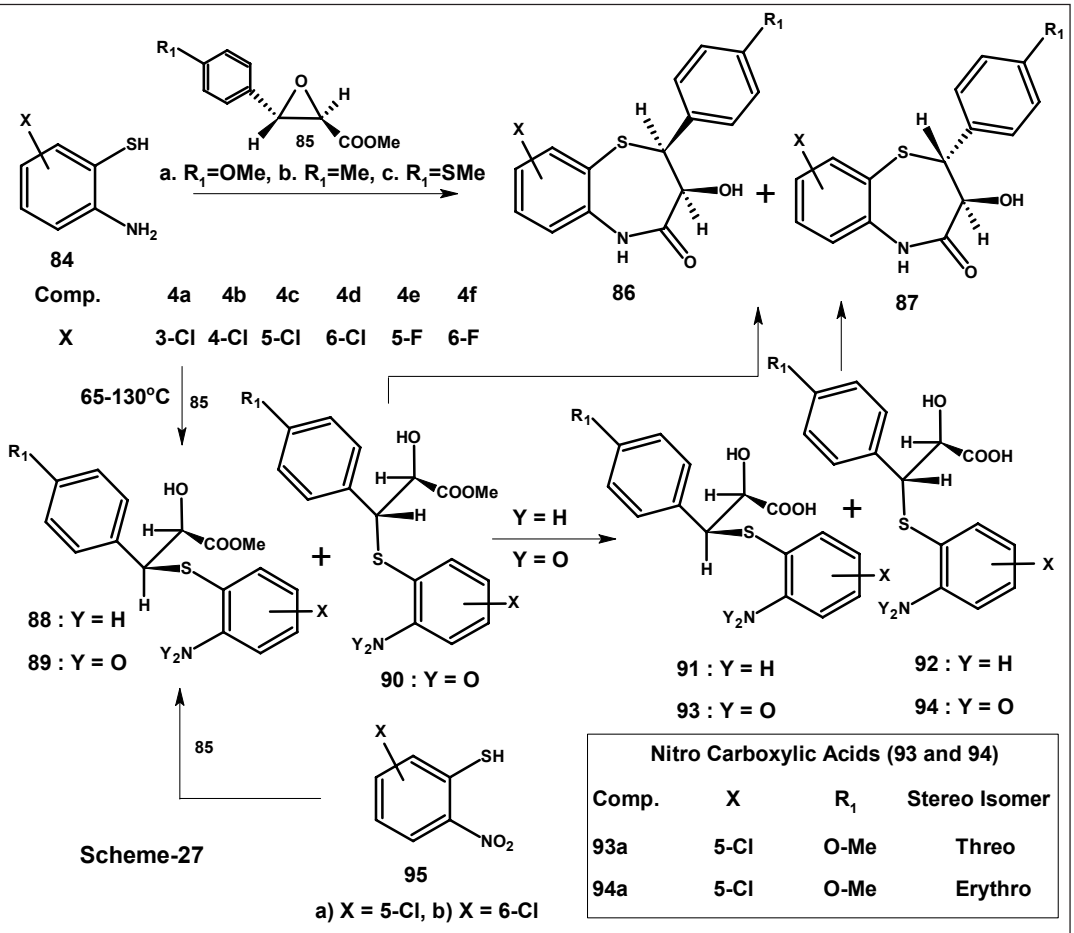
Scheme-26

benzothiazepine-4(5H)-one(86) as shown in scheme -27. Fusion of the halogen substituted 2-aminothiophenol (84) with the trans -3-arylglycidic ester(85) at about 160°C gave the cis lactam(86). This reaction involved cis opening of the oxirane ring of (85) by the thiol group(84) followed by intramolecular cyclization to give the cis lactam (86) predominantly along with minor quantity of trnas isomer (87). Although the yield was rather poor, this simplest method was mainly employed for the preliminary synthesis of (86). The stereochemistry of cis and trans lactams(86 & 87) was deduced from the vicinal coupling constant between the methine protons at C_2 and C_3 (about 6Hz and 11Hz for cis and trans isomers respectively)

The reaction of 2-aminothio-3-chlorothiophenol(84a) with glycidic ester (85a) gave intermediate aminoester (88e) predominantly together with the lactams(86a & 87a). More practically the cis lactam 86 was prepared via the amino ester(88). Heating of 2-amino-5-chlorothiophenol(84c) wth glycidic esters 85a and 85b in a nonpolar solvents at lower temperature(65-130°C) gave the threo aminoester 88a and 88b in moderate yield. Alkaline hydrolysis of 88 gave the amino acid 91.

Lewis acids such as halides or carboxylates of tin or zinc, catalytically effect ready and highly stereoselective cis opening of 3-arylglycidic esters with thiophenols[248-250]. In the presence of catalytic amount of zinc acetate, 5-or 6-chloro-2-nitrophenols(95a or 95b) smoothly reacted with 3-(4-methoxyphenyl)glycidic ester 85a at room temperature, giving the threo nitro esters 89a and 89b in good yield. In the presence of sodium bi carbonate , the erythro nitro ester 90a was obtained as a sole product by trans opening of 85a[251]. The nitro esters 89 & 90 were converted to the amino acids 91 & 92 through the nitro acids 93 & 94 or the amino ester 88.

Cyclodehydration of the amino acids 91 & 92 in boiling xylene gave the lactams 86 & 87 in good yield, respectively. Treatment of the amino acid 91 with dicyclohexylcarbodiimide(DCC) and 1-hydroxybenzotriazole (HOBt) also effected cyclization to give the lactam (86). Treatment of the amino ester (88) with methylsulfinyl carbanion in dimethyl sulfoxide (DMSO) easily gave the lactam 86 at room temperature.



Scheme-27

Nitro Carboxylic Acids (93 and 94)			
Comp.	X	R_1	Stereo Isomer
93a	5-Cl	O-Me	Threo
94a	5-Cl	O-Me	Erythro

Comp.	X	R_1
86a	6-Cl	O-Me
86b	8-Cl	O-Me
86c	8-Cl	S-Me
86d	8-Cl	Me
86e	9-Cl	O-Me
86f	8-F	O-Me
86g	8-F	Me
86h	9-F	O-Me
86i	7,8-Cl_2	O-Me
86j	8,9-Cl_2	O-Me
86k	7-Cl	O-Me

Comp.	X	R_1
87a	6-Cl	O-Me
87b	8-Cl	O-Me
87h	9-F	O-Me
87j	8,9-Cl_2	O-Me

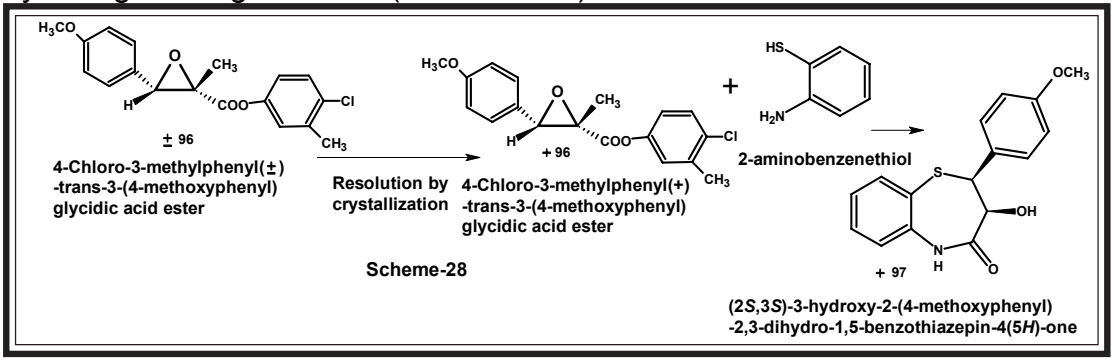
Threo Amino Esters (7)		
Comp.	X	R_1
88a	5-Cl	O-Me
88b	5-Cl	Me
88c	6-F	O-Me
88d	6-F	O-Me
88e	3-Cl	O-Me

Nitro Esters (8 and 9)			
Comp.	X	R_1	Stereo Isomer
89a	5-Cl	O-Me	Threo
89b	6-Cl	O-Me	Threo
90a	5-Cl	O-Me	Erythro

Amino Carboxylic Acids (10 and 11)			
Comp.	X	R_1	Stereo Isomer
91a	5-Cl	O-Me	Threo
91b	5-Cl	Me	Threo
91c	6-Cl	O-Me	Threo
92a	5-Cl	O-Me	Erythro

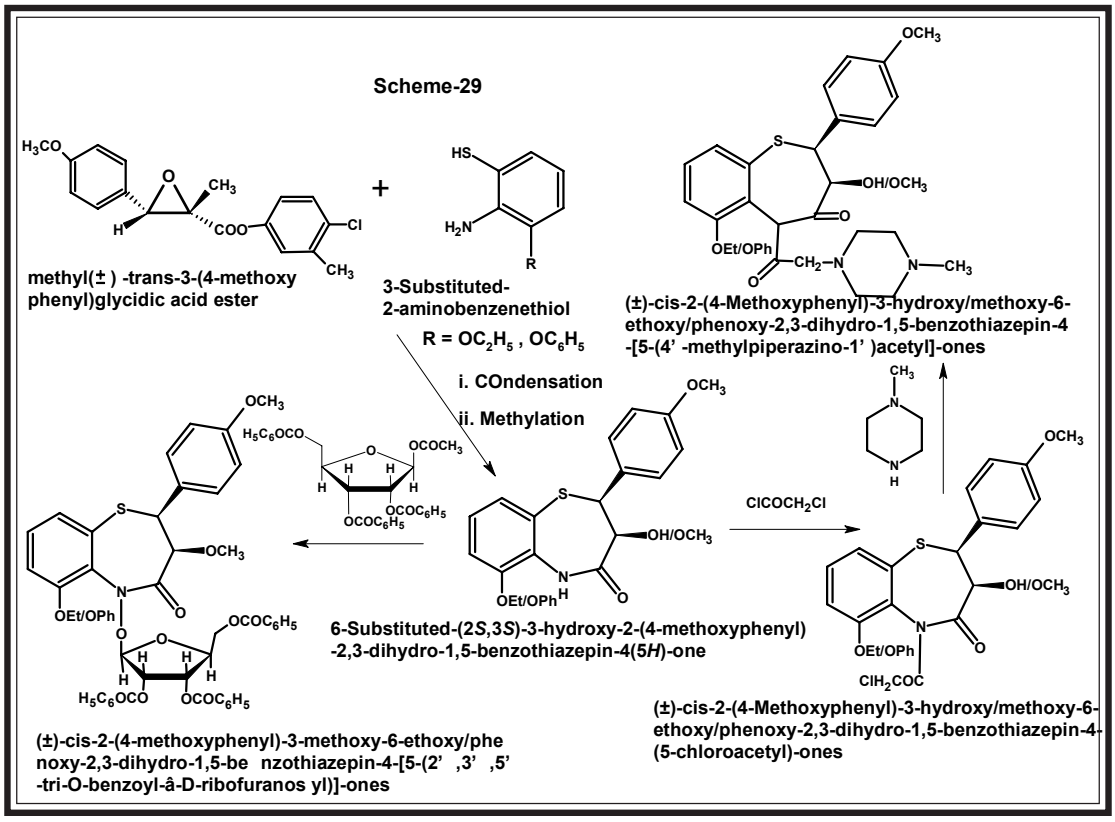
For the synthesis of optically active isomers of (86b) optical resolution of the intermediate amino acid (91a) was effected with methyl L- or D-(4-hydroxyphenyl)glycinate. More practically the nitro acid 93a could be resolved into its enantiomers via diastereoisomeric salts of L-lysine.

Yamada S.-I. et al[252] suggested that among the various esters of 3-(4-methoxyphenyl)glycidic acid, 4-chloro- 3-methylphenyl ester (±)-96 was found to exist as a conglomerate, and could be alternately resolved into (+)- and (-)-96 of >98% ee by a preferential crystallization procedure. Furthermore, a 1,5-benzothiazepine derivative, (+)-97, a significant intermediate of diltiazem, was prepared in one pot in 76% overall yield through the treatment of enantiomerically pure (-)-96 with 2-aminothiophenol followed by a ring closing reaction (Scheme-28).



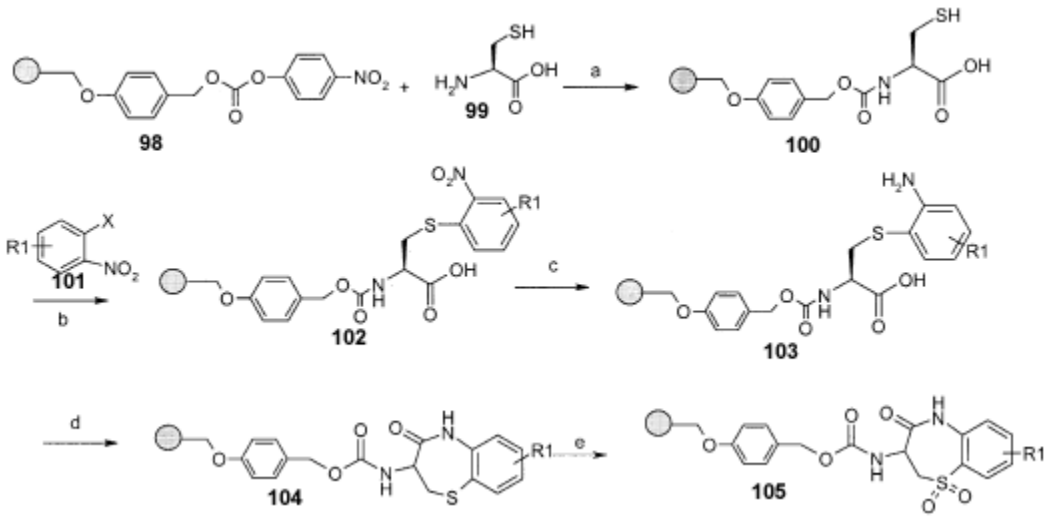
Scheme-28

G.Singh et al[253] have synthesized (±)-cis-2-(4-Methoxyphenyl)-3-hydroxy/methoxy-6-ethoxy/phenoxy-2,3-dihydro-1,5-benzothiazepin-4-[5H/5-chloroacetyl/5-(4' -methylpiperazino-1')acetyl]-ones by the condensation of 2-amino-3-ethoxy/phenoxybenzenethiol with methyl-(±)-trans-3-(4-methoxyphenyl)glycidate in xylene. Ribofuranosides, viz. (±)-cis-2-(4-methoxyphenyl)-3-methoxy-6-ethoxy/phenoxy-2,3-dihydro-1,5-be nzothiazepin-4-[5-(2' ,3' ,5' -tri-O-benzoyl-â-D-ribofuranos yl)]-ones, have been synthesized by the treatment of 3-methoxy derivatives of 1,5-benzothiazepines with a derivative, sugar viz. â-D-ribofuranose-1-acetate-2,3,5-tribenzoate, in toluene in vacuo. The structures of all the synthesized ribofuranosides and their precursors have been characterized on the basis of elemental analyses and IR, ^{1}H NMR, and ^{13}C NMR spectral data. These compounds were screened for their antimicrobial activity (Scheme-29).



❖Solid phase reactions, microwave assisted &/or one pot synthesis and Green chemistry approach &/or solvent free synthesis.

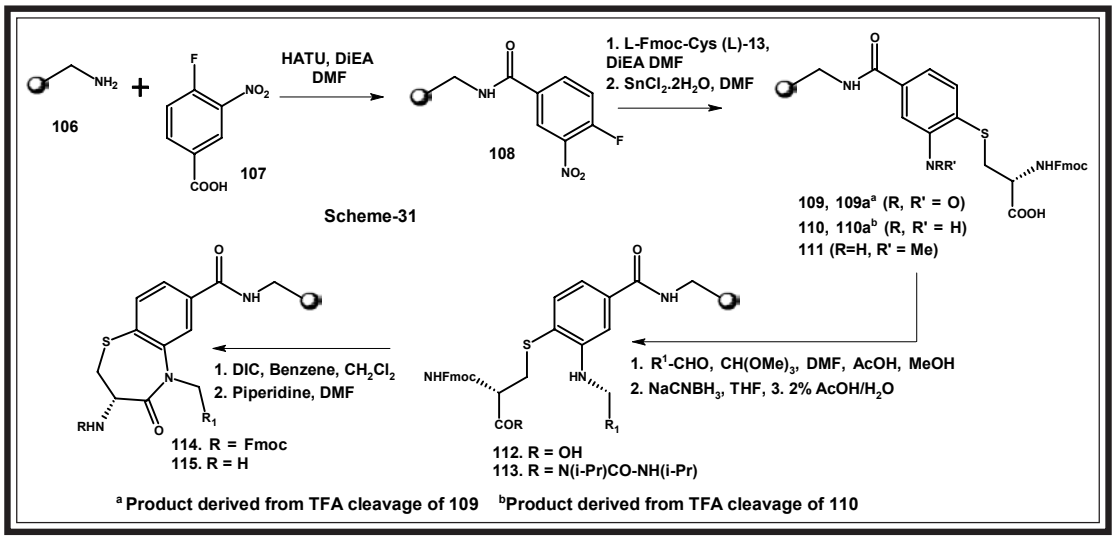
Developing solution or solid-phase synthetic routes which can provide a large range of medicinally relevant compounds in high purity has remained a challenge which has been successfully met in several instances[254-255]. George C. Morton et al[256] have synthesized 3-amino-1,5-benzothiazepine-4-one derivatives using 3-amino group as a point of attachement to the Wang resin and exploited wide variety of commercially available o-halo nitro benzene derivatives and facile substitution of the benzothiazepine amide nitrogen. As per shown in scheme 30, cysteine (99) was reacted with the nitrophenyl carbonate derivative of Wang resin 100 by first using bis-(trimethylsilyl)-acetamide(BSA) to dissolve the amino acid (Scheme 30). The thiol 100 was then reacted with a variety of halo-nitrobenzene derivatives 101. Formation of up to 25% of cystine by dimerization of the resin-bound cysteine was observed during this step. This could be suppressed by using a strictly inert atmosphere and 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU) as base. Alternatively, cysteine could be cleanly reduced with tributyl phosphine and further reacted with the



Scheme-30: (a) (1) BSA, DMF, argon, rt; (2) 10% AcOH in DMF; (b) X=Cl, Br or F, DBU, DMF, argon, rt; (c) SnCl22H2O, DMF, rt; (d) EDC, NMP, rt; (e) *m*-CPBA, DCM, rt

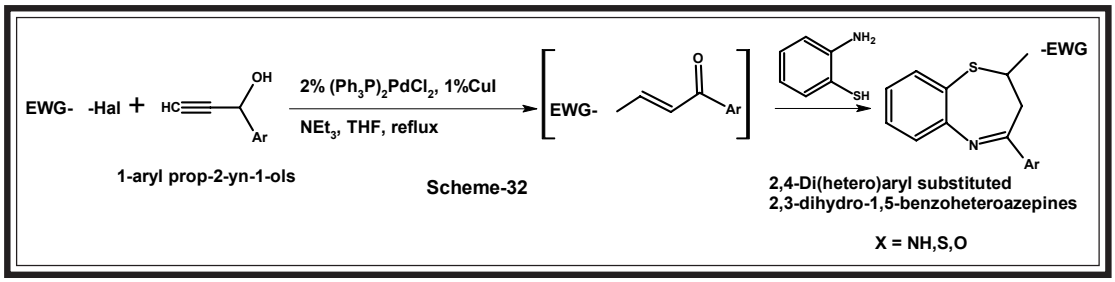
halo-nitrobenzene derivative. Reduction of the nitro group with tin-dichloride dihydrate and cyclization with ethylene dischloride afforded the benzothiazepine derivative 104. Further diversity could be obtained by oxidation of the sulfide 104 to the sulfone 105 with *m*-chloro per benzoic acid.

Schwarz M.K. et al[257] suggested a solid-phase route affording novel 3,5-disubstituted 1,5- benzothiazepin-4(5H)-ones in optically pure form. S(N)Ar reaction of polymer-bound 4-fluoro-3-nitrobenzoic acid, 108, with L-Fmoc- cysteine, L-13, under basic conditions, followed by tin(II) chloride mediated nitro group reduction, furnished the primary aniline 110. Reductive alkylation of 110 to the corresponding secondary anilines 112 was shown to be feasible for a wide range of aldehydes, using an optimized solvent system composed of trimethylorthoformate, dimethylformamide, methanol, and acetic acid, with sodiumcyanoborohydride as the reducing agent. Subsequent cyclization of the secondary anilines 112 using DIC in apolar solvents furnished the corresponding N(5)-alkylated 1,5-benzothiazepin-4- ones 114. Following Fmoc removal from 114, the primary amino group was finally reacted with carboxylic acids, isocyanates, sulfonyl chlorides, or aldehydes to afford the respective amides, ureas, sulfonamides, or secondary amines. Performing the synthesis with the D-form of Fmoc-cysteine, D-13, resulted in the corresponding antipodal products, with no detectable scrambling at C-3.(Scheme-31)



Scheme-31

Roland Braun et al[258] have synthesized 2,4-Di(hetero)aryl substituted 2,3-dihydro 1,5-benzoheteroazepines (hetero) NH, O, S) in a one-pot process initiated by a coupling-isomerization sequence of an electron poor (hetero)aryl halide and a terminal propargyl alcohol subsequently followed by a cyclocondensation with 2-amino, 2-hydroxy, or 2-mercapto anilines. The investigation based on the principle that palladium/copper catalyzed crosscoupling reactions of electron poor halogen substituted ð-systems and 1-aryl prop-2-yn-1-ols do not furnish the expected propargyl alcohols but rather the isomeric enone components[259]. Mechanistically, this isomerization, occurring after the cross-coupling reaction, is purely base catalyzed and opens a new access to electron deficient propenones (Scheme-32).



Scheme-32

Mitsuo Kodomari et al[260] have described solvent free synthesis of 1,5-benzothiazepines on inorganic supports, from chalcones and o-aminophenol. The results of the reaction of o-aminothiophenol with chalcone in the presence of various solid supports were shown in Table 1.1. From these results, Silica was found to be an effective support for the synthesis of 1,5-benzothiazepines, under identical experimental conditions.

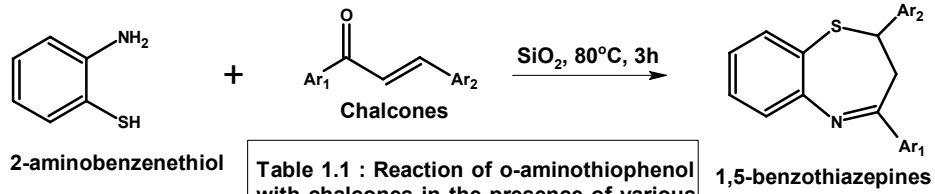
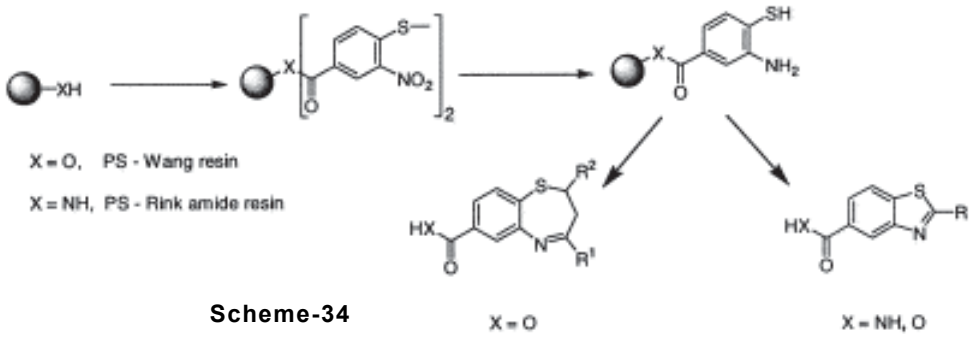


Table 1.1 : Reaction of o-aminothiophenol with chalcones in the presence of various supports

Support	%Yield of benzothiazepine
Silica Jel	87
Acidic alumina	28
Basic alumina	2
Neutral alumina	21
Molecular sieves(5 °A)	30
Keiselguhr	5
None	22

Scheme-33

Cheng Leng Lee et al[261] demonstrated solid phase combinatorial synthesis of 2,3-dihydro-1,5-benzothiazepines from bis-2(2-nitro-4-carboxy phenyl)disulphide loaded on Wang resin. The nitro group was reduced to its amine with concomitant cleavage of the disulphide bond using tin(II)chloride, which followed by nucleophillic attack on α,â-unsaturated ketone and tri fluoro acetic acid cleavage from resin (Scheme-34).



Scheme-34

254.M.Bilodeau, A.Cunningham, *J. Org. Chem.*,**63**, 2800(1998)
255.B.Dressma, L.Spangle, S.Kaldor, *Tetrahedron Lett.*,**37**, 937(1996)
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259.T.Mu¨ller, M.Ansorge, D.Aktah, *Angew. Chem., Int. Ed.*,**39**, 1253(2000).
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WORK DONE AT OUR LABORATORY

The literature survey of the work done on benzofused seven membered heterocycles suggest that 1,5-benzothiazepine derivatives have shown diversed pharmacolocal profile including antihypertensive/Ca^{+2} channel antagonist, coronary vasodilators, peripheral or mitochondrial benzodiazepine receptor antagonist, anticonvulsants, antiischemics, antiarrhythmics, anticancer agents, anti HIV agents(non nucleotide reverse transcriptase inhibitors), platelet aggregation inhibitors, antimicrobial agents and angiotensine converting enzyme inhibitors etc.

In addition to this, 4-hydroxy coumarin and its derivatives are well known for their anticoagulant and antimicrobial activities.3-substituted-4-hydroxy coumarins generated considerable interest in designing HIV protease inhibitors with high therapeutic index. Among these, most interestingly finding suggested that 4-hydroxy coumarin derivatives substituted with bulky group occupied at 3-position exhibited a wide range of diversed biological activity profile.

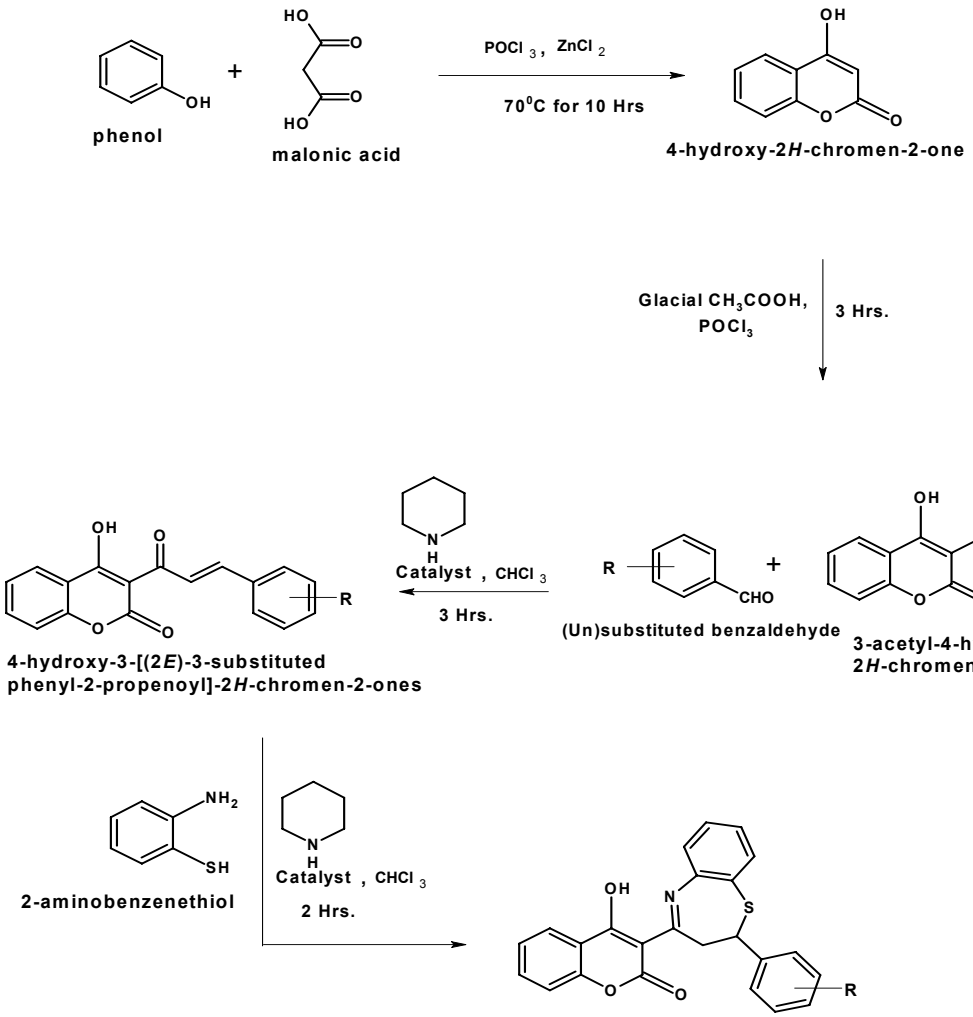
With this innovative idea, in the current chapter we decided to synthesize 2-arylsubstituted-2,3-dihydro-1,5-benzothiazepine derivatives possesing 4-hydroxy coumarin substitution at 4-position of 1,5-benzothiazepine system, which were not studied so far in the literature. The chemistry developed to synthesize such derivatives included Pechmann condensation to afford (un)substituted-4-hydroxy coumarin system followed by acetylation with selective Lewis acids and chalcone formation by Knoevenagel reaction with different substituted aromatic aldehydes. Finally regioselective nucleophilic attack followed by Micheal additionby reaction of á,â- unsaturated ketones with o-aminothiophenol yielded target compounds.

The reaction scheme designed to synthesize target compound consist of four steps. The first step deals with the synthesis of (un)substituted 4-hydroxy coumarins by Pechmann condensation between (un)substituted phenols and malonic acid using anhydrous zinc chloride and phosphorous oxy chloride as condensing agents, which are further acetylated in second step with glacial acetic acid and $POCl_3$ to yield 3-acetyl-4-hydroxy coumarins, due to comparative acidic nature of methyl group of 3-acetyl substitution, on treatment with different aromatic aldehydes it undergoes Knoevenagel condansation in basic condition to give corresponding chalcones. There were 21 chalcones synthesized using corresponding aromatic aldehydes in third step. Finally these chalcones on treatment with 2-aminothiophenol under basic condition afforded 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones.

All the synthesized compounds were characterized by IR,H^1NMR and Mass spectral data and C,H,N elemental analysis.

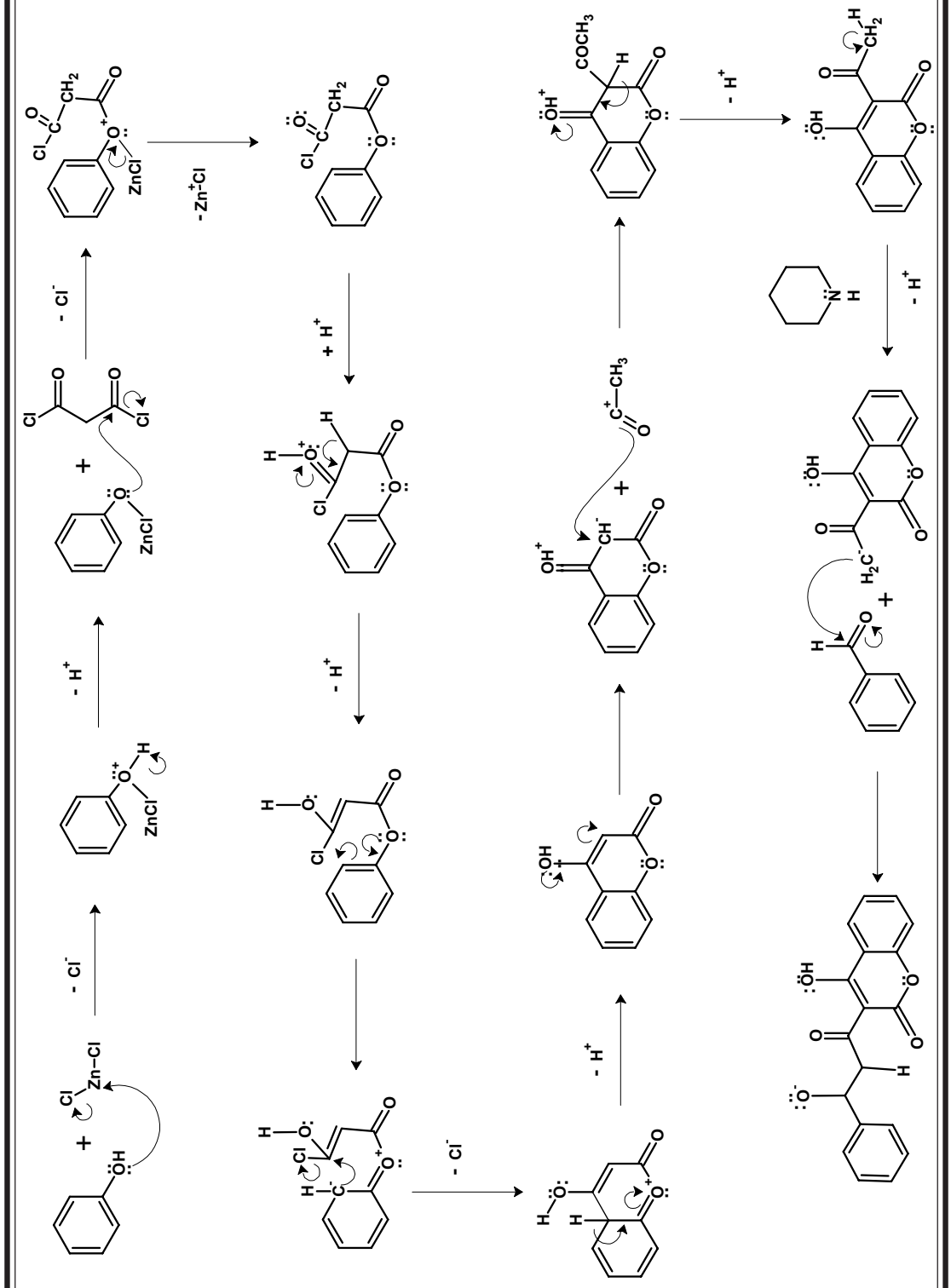
Finally the synthesized compounds were screened for different biological activities.

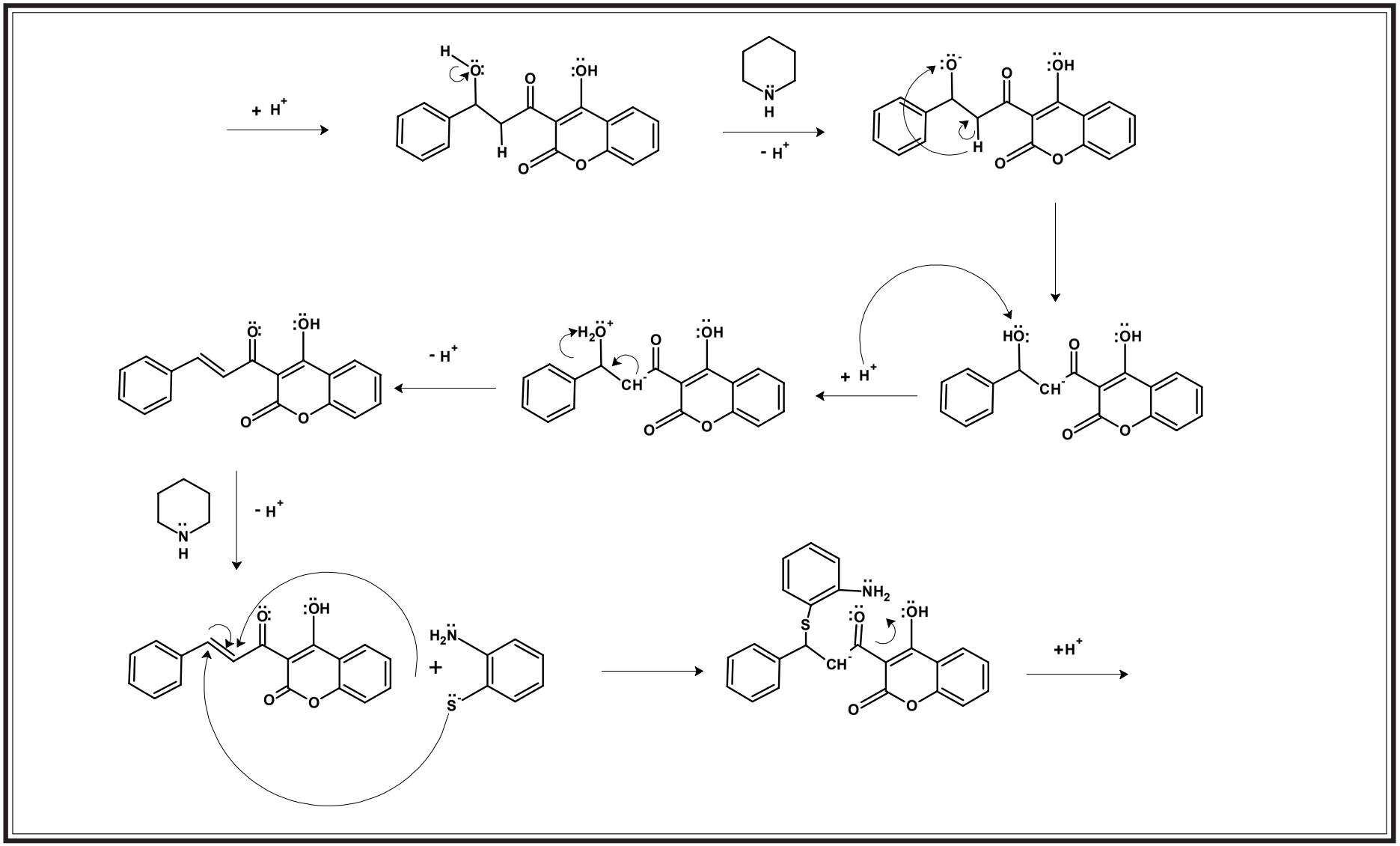
Reaction Scheme



R = Electron donating or Electron withdrawing groups

Reaction Mechanism:-

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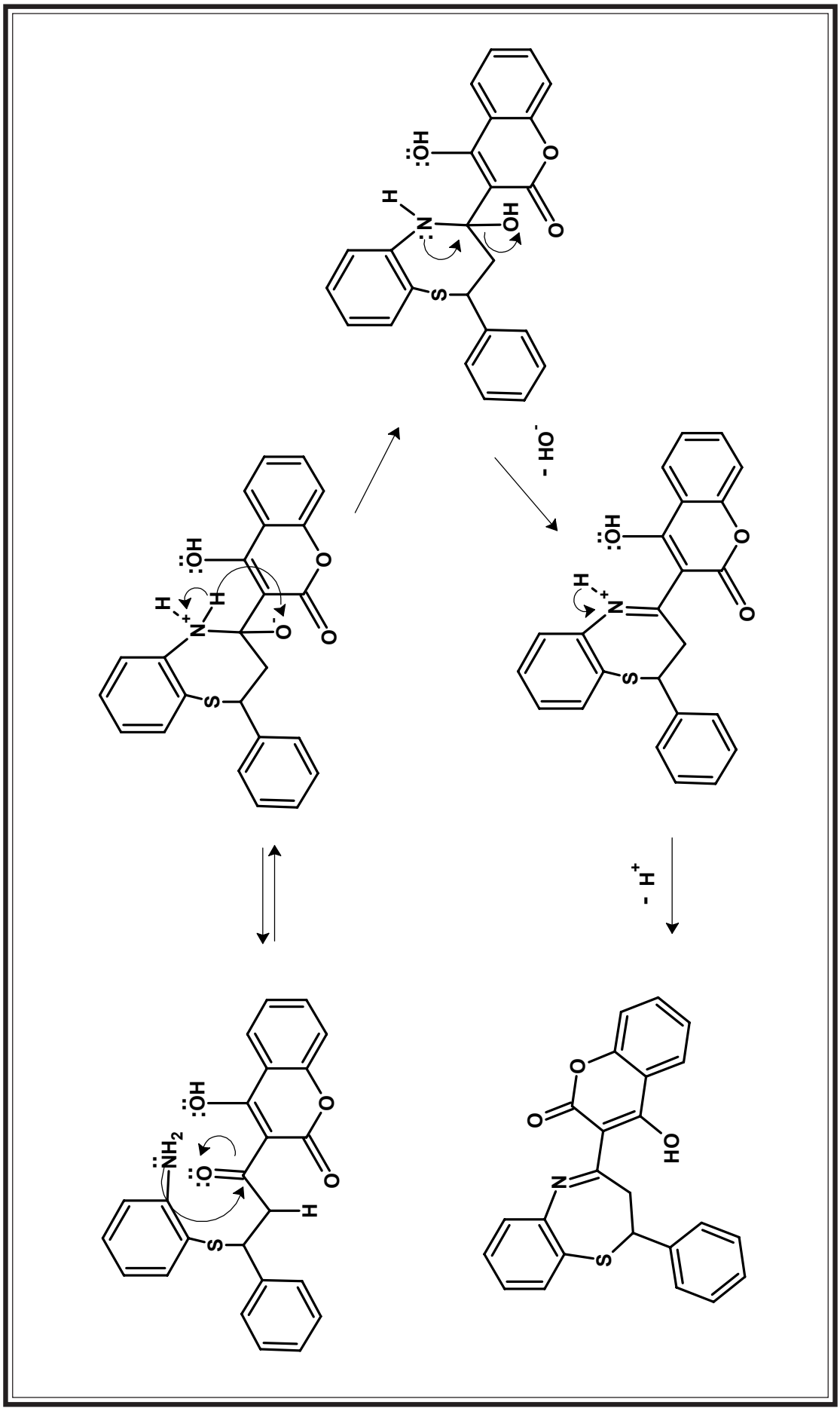
+ H+
- H+
- H+
+H+



Experimental Protocols

All the starting materials, Phenol, Malonic acid, Zinc chloride, $POCl_3$, Gla. AcOH, substituted aro matic aldehydes and o-Aminothiphenol were obtained from Spectrochem Ltd., Sisco Research Lab., s.d.fine chem. Ltd., Ranbaxy Lab., and other commercial sources. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature using standard Zeal's thermometer and are uncorrected. The commencement of the reaction and purity of the synthesized compounds were ensured using thin layer chromatography (TLC) silica gel-G, used as stationary phase, the TLC plates were purchased from Merck India Ltd. ethyl acetate: hexane was used as the mobile phase. However other solvent systems like acetone :benzene and $CHCl_3$:CH_3OH were also employed but the best results observed with Ethyl acetate:Hexane system.

Experimental

Synthesis of 4-Hydroxy-2*H*-chromen-2-one:

In a 250ml Round Bottomed Flask, phenol(0.1mole) , malonic acid(0.11mole), anhyd. zinc chloride(36gms) and phosphorous oxychloride(40ml) were mixed together and heated on a waterbath at 65-70°C till the reaction mass become viscous. The reaction mass poured into crushed ice. The crude product was filtered and thoroughly washed with water till became acid free. The crude product was treated with saturated solution of sodium carbonate and filtered. The filterate was acidified with 30%HCl solution to give 4-hydroxy-2*H*-chromen-2-one, which was filtered and dried and recrystallized from methanol. Yield 73%, m.p. 212-213°C(Reported m.p. 210°C[262]). Purity was check by TLC using ethyl acetate:hexane(4:6) as mobile phase.

Synthesis of 3-Acetyl-4-hydroxy-2*H*-chromen-2-one :

4-hydroxy-2*H*-chromen-2-one (0.06mole,) was dissolved in glacial acetic acid(50ml) then $POCl_3$(40ml) was added and the reaction mixture was refluxed in a waterbath for 2 hrs. Pour the reaction mass into crushed ice. Filter the solid and wash thoroghly with water till become acid free. Recrystallized from methanol, Yield was 87%, m.p. 122°C(Reported m.p. 121°C[262]). Purity was checked by TLC using ethyl acetate: hexane(3:7) as mobile phase.

Synthesis of 4-Hydroxy-3-[(2*E*)-3-substituted phenyl-2-propenoyl]-2*H*-chromen-2-ones (General Method):

A mixture of (un)substituted benzaldehyde (0.01mole) and 3-acetyl-4-hydroxy-2*H*-chromen-2-one (0.01mole) with catalytic amount of caustic lye were refluxed in ethanol, which on cooling give corresponding chalcone in good yields which were purified by recrystallization from methanol. Purity of each compound was checked by TLC using ethyl acetate:hexane(5:5) as mobile phase.

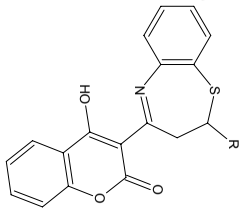
Synthesis of 4-Hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones (General method):

A mixture of appropriate 4-hydroxy-3-[(2*E*)-3-substituted phenyl-2-propenoyl]-2*H*-chromen-2-one (0.01mole) and o-Aminothiophenol (0.01mole) were refluxed in chloroform using piperidine as catalyst. The commencement and completion of the reaction was governed by TLC. At the end of reaction solid product obtained was filtered, washed with little amount of solvent. The crude product was recrystallized from chloroform. Purity was checked by TLC using ethyl acetate:hexane (5:5) as mobile phase.

The elemental analysis of all the compounds performed by **PERKIN ELMER 470R** are in total agreement with the theoritical values.

The physical constants and elemental analysis of all compounds were shown in **Table. 2.1.**

Table 2.1: Physical constants of 4-Hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
1	KUT-1	Phenyl	$C_{24}H_{17}NO_3S$	399.4	226-228	72.16 72.13	4.29 4.28	3.51 3.50
2	KUT-2	2-NO_2 Phenyl	$C_{24}H_{16}N_2O_5S$	444.4	229-231	64.86 64.84	3.63 3.62	6.30 6.31
3	KUT-3	3-NO_2 Phenyl	$C_{24}H_{16}N_2O_5S$	444.4	233-235	64.86 64.84	3.63 3.62	6.30 6.31
4	KUT-4	2-OCH_3 Phenyl	$C_{25}H_{19}NO_4S$	429.5	240-243	69.91 69.87	4.46 4.47	3.26 3.25
5	KUT-5	3-OCH_3 Phenyl	$C_{25}H_{19}NO_4S$	429.5	221-223	69.91 69.87	4.46 4.47	3.26 3.25
6	KUT-6	4-OCH_3 Phenyl	$C_{25}H_{19}NO_4S$	429.5	193-195	69.91 69.87	4.46 4.47	3.26 3.25

Note:- The underline values denote the calculated percentage of composition

Table 2.1: Physical constants of 4-Hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
7	KUT-7	3-Br Phenyl	$C_{24}H_{16}BrNO_3S$	478.3	201-203	60.26 60.24	3.37 3.36	2.93 2.92
8	KUT-8	2-Cl Phenyl	$C_{24}H_{16}ClNO_3S$	433.9	206-209	66.43 66.45	3.72 3.73	3.23 3.22
9	KUT-9	3-Cl Phenyl	$C_{24}H_{16}ClNO_3S$	433.9	185-187	66.43 66.45	3.72 3.73	3.23 3.22
10	KUT-10	4-Cl Phenyl	$C_{24}H_{16}ClNO_3S$	433.9	224-227	66.43 66.45	3.72 3.73	3.23 3.22
11	KUT-11	2-ethenyl Phenyl (Cinnamoyl)	$C_{26}H_{19}NO_3S$	425.5	229-232	73.39 73.36	4.50 4.48	3.29 3.27
12	KUT-12	4-F Phenyl	$C_{24}H_{16}FNO_3S$	417.4	215-217	69.05 69.08	3.86 3.85	3.36 3.37
13	KUT-13	4-N,N DiCH_3 Phenyl	$C_{26}H_{22}N_2O_3S$	442.5	227-230	70.57 70.55	5.01 5.02	6.33 6.35
14	KUT-14	4-OH Phenyl	$C_{24}H_{17}NO_4S$	415.4	256-259	69.38 69.35	4.12 4.14	3.37 3.39

Note:- The underline values denote the calculated percentage of composition

Table 2.1: Physical constants of 4-Hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point 0C	Elemental Analysis (C,H,N%)		
						C	H	N
15	KUT-15	4-NO2 Phenyl	$C_{24}H_{16}N_2O_5S$	444.4	189-192	64.86 64.84	3.63 3.62	6.30 6.31
16	KUT-16	4-SCH3 Phenyl	$C_{25}H_{19}NO_3S_2$	445.5	207-209	67.39 67.36	4.30 4.28	3.14 3.15
17	KUT-17	3,4-DiOCH3 Phenyl	$C_{26}H_{21}NO_5S$	459.5	208-211	67.96 67.94	4.61 4.63	3.05 3.03
18	KUT-18	3,4,5-TriOCH3 Phenyl	$C_{27}H_{23}NO_6S$	489.5	200-202	66.24 66.25	4.74 4.72	2.86 2.88
19	KUT-20	3-OH Phenyl	$C_{24}H_{17}NO_4S$	415.4	221-224	69.38 69.35	4.12 4.14	3.37 3.39
20	KUT-21	3-Indolyl	$C_{26}H_{18}N_2O_3S$	438.5	195-196	71.22 71.2	4.14 4.15	6.39 6.37
21	KUT-22	9-Anthracyl	$C_{32}H_{21}NO_3S$	499.6	148-151	76.93 76.97	4.24 4.22	2.80 2.79
22	KUT-24	2-OH Phenyl	$C_{24}H_{17}NO_4S$	415.4	236-238	69.38 69.35	4.12 4.14	3.37 3.39

Note:- The underline values denote the calculated percentage of composition

SPECTRAL CHARACTERIZATION

The constitution of newly synthesized compounds were supported by FT - IR, ^{1}H NMR and EI-Mass spectral study.

IR Spectral Study

Instrument: SHIMADZU FT-IR 8400 Spectrophotometer

Sample technique: KBr pellet

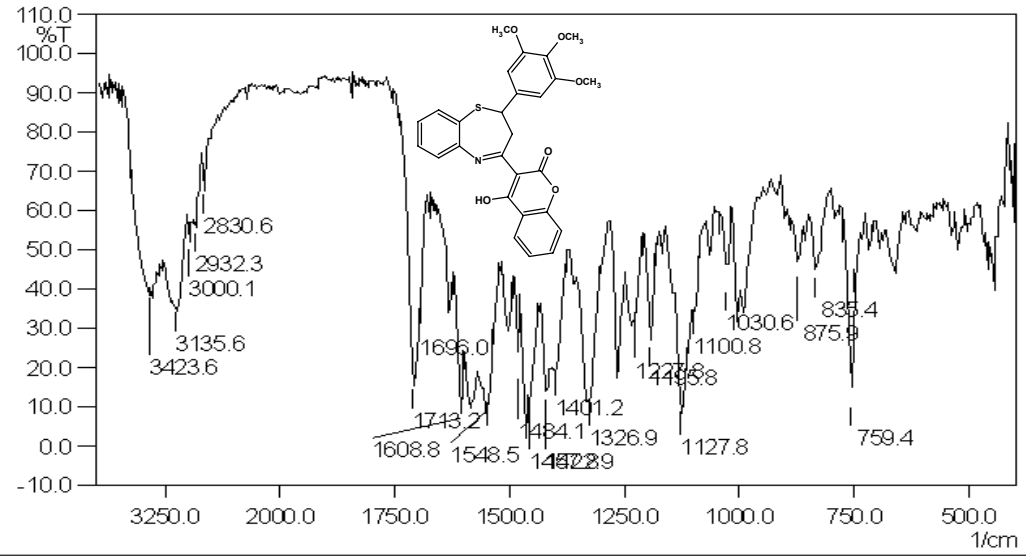
Frequency range: 400-4000 cm^{-1}

The 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones (KUT Series), indicates the presence of very specific functional groups like hydroxy group of coumarin skeleton, carbonyl group of the lactone system, C=Nstr, C-H str.(methylene and methyne group) and C-S str. vibration of thiazepine ring, aromatic ring skeleton etc., which absorb IR radiations of specific frequency and show sharp, medium or weak intense signals and hence supports in identifiction and confirmation of the molecule.

The IR spectrums of 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one shows common frequences as 3460-3300cm^{-1} for hydroxy group, 3150-3110cm^{-1} for aromatic C-H str.,1690-1760cm^{-1} for C=O str. of lactone group, 1630-1605cm^{-1} for C=Nstr. merge with aromatic ring where as aromatic region was observed extended upto 1480cm^{-1}. C-H inplane deformation of methylene group was observed in range between 1470-1450cm^{-1},while that of methyne group was observed bet ween 1400-1320cm^{-1}. Thioether linkage of thiazepine ring shows C-S str. range between 700-555 cm^{-1}. Mono,di & tri phenyl substitutions observed between 875-740cm^{-1}. While characteristic vibration corresponds to substitution at phenyl residue was clearly observed in each case.

A comparative chart of IR spectral data of all compounds is shown in **Table-2.2.**

IR Spectrum of 4-Hydroxy-3-(2-(3,4,5-trimethoxy)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-18)



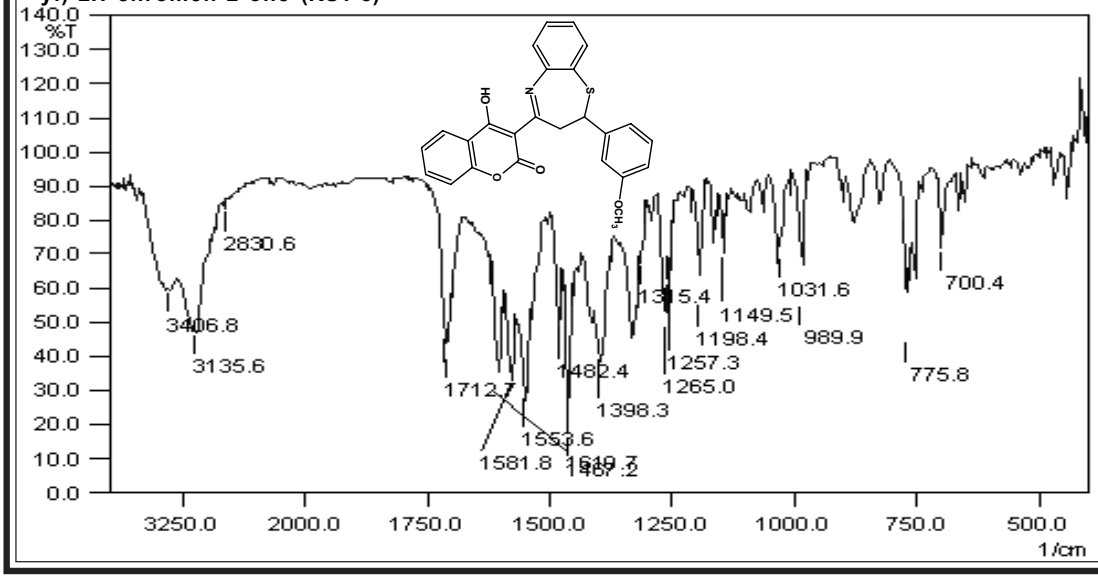
KUT-6

Functional Group	Vibration Mode	Frequency (cm^{-1})
-OH	O-H str.	3423
-CH Aromatic	-C-H str.	3135
-C=O	C=O str.	1713
-C=N	-C=N	1608
Aromatic Skeleton	-C=C- str.	1588,1512,1461
	1:3:4:5 tetra substitution	875
Methylene	-C-H str.	1452
Methyne	-C-H str.	1326
Thioether	C-S-C str.	666
$-OCH_3$	-C-H str. -C-O-C- str.	2932,2830 1227, 1195

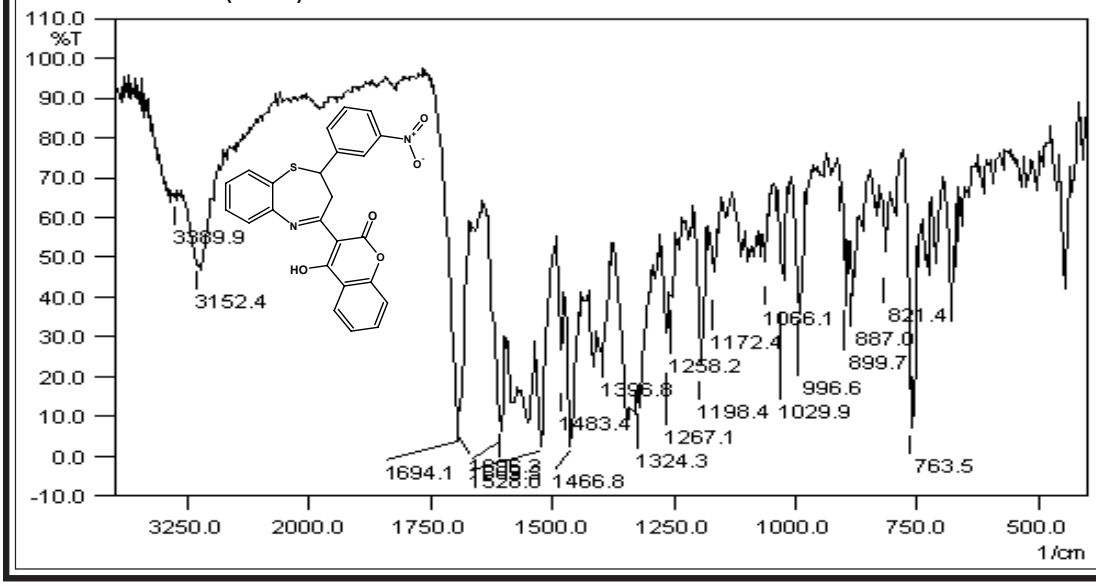
KUT-5

Functional Group	Vibration Mode	Frequency (cm^{-1})
-OH	O-H str.	3406
-CH Aromatic	-C-H str.	3135
-C=O	C=O str.	1712
-C=N	-C=N	1610
Aromatic Skeleton	-C=C- str.	1581,1553,1482
	1:3 di substitution	775
Methylene	-C-H str.	1467
Methyne	-C-H str.	1398
Thioether	C-S-C str.	700
$-OCH_3$	-C-H str., -C-O-C- str.	2915, 1198

IR Spectrum of 4-Hydroxy-3-(2-(3-methoxy)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-5)



IR Spectrum of 4-Hydroxy-3-(2-(3,nitro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-3)



KUT-3

Functional Group	Vibration Mode	Frequency (cm^{-1})
-OH	O-H str.	3389
-CH Aromatic	-C-H str.	3152
-C=O	C=O str.	1699
-C=N	-C=N	1608
Aromatic Skeleton	-C=C- str.	1584,1557,1527
	1:4 di substitution	821
Methylene	-C-H str.	1483
Methyne	-C-H str.	1396
Thioether	C-S-C str.	680
$-NO_2$	N-O str.	1355

KUT-1

Functional Group	Vibration Mode	Frequency (cm^{-1})
-OH	O-H str.	3389
-CH Aromatic	-C-H str.	3135
-C=O	C=O str.	1711
-C=N	-C=N	1609
Aromatic Skeleton	-C=C- str.	1581,1553,1481
	mono substitution	755
Methylene	-C-H str.	1467
Methyne	-C-H str.	1326
Thioether	C-S-C str.	653

IR Spectrum of 4-Hydroxy-3-(2phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-1)

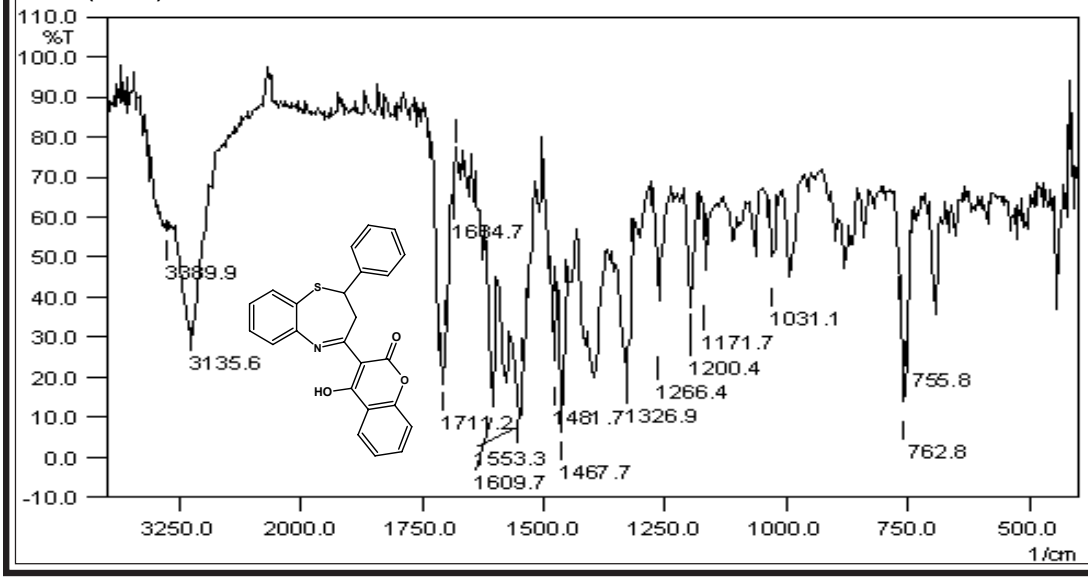
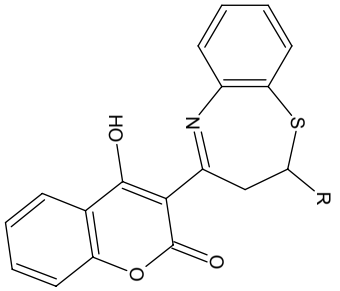
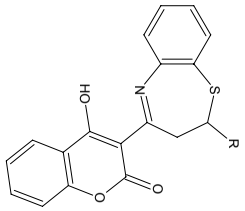


Table 2.2 : IR Spectrual data of 4-Hydroxy-3-(2-sub.phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones



Code	Substitution (R)	-OH Str.	Ar-H Str.	$>CH_2$ Str.	-CH str.	>CO Str.	>C=N Str. / Aromatic Region (>C=C< str)			$-CH_2$ deformation	-CH deformation	Phenyl Substitution	Substitution	C-S-C Str.
KUT-2	2-NO_2 phenyl	3372	3118	2926	2882	1692	1612	1598	1515	1465	1348	747 (1:2 di sub.)	1321 (-N=O str.)	656
KUT-4	2-OCH_3 phenyl	3406	3135	2932	2847	1724	1610	1582	1492	1465	1398	776 (1:2 di sub.)	1267 (C-O-C str)	686
KUT-6	4-OCH_3 phenyl	3423	3152	2949	2830	1760	1609	1588	1509	1455	1386	823 (1:4 di sub)	1200 (C-O-C str)	667
KUT-7	3-Br phenyl	3406	3152	2932	2847	1703	1610	1584	1484	1468	1398	760 (1:3 di sub)	722 (C-Br str)	693
KUT-8	2-Cl phenyl	3406	3135	2915	2817	1709	1632	1611	1551	1463	1323	754 (1:2 di sub.)	720 (C-Cl str)	690
KUT-9	3-Cl phenyl	3389	3135	2920	2835	1702	1615	1553	1483	1456	1398	875 (1:3 di sub)	730 (C-Cl str)	690
KUT-10	4-Cl phenyl	3406	3135	2931	2832	1702	1610	1578	1483	1470	1322	818 (1:4 di sub)	761 (C-Cl str)	672
KUT-11	$CH=CHC_6H_5$	3406	3135	2925	2850	1712	1610	1578	1483	1466	1398	763 (1:2 di sub)	1257 (trans >CH=CH< i.p.def.) 992 (trans >CH=CH< o.o.p def)	691

Table 2.2 : IR Spectrual data of 4-Hydroxy-3-(2-sub.phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones



Code	Substitution (R)	-OH Str.	Ar-H Str.	$>CH_2$ Str.	-CH str.	>CO Str.	>C=N Str. / Aromatic Region (>C=C< str)			$-CH_2$ deformation	-CH deformat^n	Phenyl Substitution	Substitution	C-S-C Str.
KUT-12	4-F phenyl	3406	3152	2916	2858	1700	1611	1581	1507	1466	1339	831 (1:4 di sub.)	998 (C-F str)	690
KUT-14	4-OH phenyl	3355	3152	2916	2846	1674	1618	1551	1511	1484	1319	763 (1:4 di sub.)	1319 (C-OH str.)	694
KUT-15	4-NO_2 phenyl	3406	3135	2922	2863	1691	1608	1596	1513	1464	1394	827 (1:4 di sub)	1334 (-N=O str.)	696
KUT-16	4-SCH_3 phenyl	3406	3135	2885	2860	1706	1610	1581	1480	1467	1394	859 (1:4 di sub.)	-	642
KUT-17	3,4-diOCH_3 phenyl	3406	3135	2953	2853	1709	1607	1544	1506	1468	1379	812 (1:4 di sub)	1241, 1263 (C-O-C str.)	659
KUT-20	3-OH phenyl	3423	3186	2930	2828	1709	1611	1588	1483	1463	1326	875(1:3:4:5 tetra sub.)	1266,1201 (C-OH str.)	700
KUT-21	3-Indolyl	3305	3135	2932	2830	1694	1609	1581	1483	1465	1335	871 (1:3 di sub)	1263(C-N str)	646
KUT-22	9-Anthracyl	3423	3135	2949	2881	1694	1606	1538	1489	1461	1336	740 (1:3 di sub.)	-	555
KUT-24	2-OH phenyl	3457	3152	2932	2881	1689	1608	1583	1486	1463	1380	755 (1:2 di sub)	1203,1325 (C-OH str.)	669

^{1}H NMR Spectral Study

^{1}H NMR Spectrum of the 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-ones show signals relevant to the number of protons and their electronic environment known as chemical shift. Chemical shift may move to either upfield (Shielding) or down field(deshielding), according to the electronic environment of the corresponding proton. In addition to this each ^{1}H NMR signal further splitted into number of subpeaks according to the number of neighbouring protons present in the skeleton of molecule. The splitting of the NMR signal further provides signification about the degree of interaction between neighbouring protons by means of spin-spin coupling constant J.

4-Hydroxy-3-(2-(4-thiomethyl)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-16)

^{1}H NMR Spectrum of KUT-16 shows a singlet for thiomethyl protons , at chemical shift value δ 2.469 ppm. A triplet relevant to methyne proton of the thiazepine ring, appears at δ 2.741 with J value of 12 & 12Hz, which reveals the presence of one methylene neighbouring group. A dounlet due to two protons of methylene group, appears at δ 4.320 with J value of 10Hz, which reveals the presence of one adjacent methyne group in the same thiazepine ring. Proton $H_{(5)}$& $H_{(D)}$ of benzenoid part of coumarin residue and 1,5-benzothiazepine system become most deshielded, hence each appears as doublet at chemical shift value of δ 8.118 & 7.771 with J value of 6.6Hz and 6.6Hz respectively, which reveals that because of only one proton involve in the splitting of their signals, $H_{(5)}$ & $H_{(d)}$ become nearest protons from junction of fusion, Protons $H_{(8)}$ & $H_{(A)}$ are also protons of another ends of the benzenoid part of the coumarin ring and 1,5-benzothiazepine, evidence by their appear ance as two doublets at merge chemical shift value 7.558δ ppm with J value of 8.7 & 7.2Hz respectively.

Protons $H_{(B)}$, $H_{(C)}$, $H_{(6)}$, $H_{(7)}$, $H_{(d)}$ and $H_{(e)}$ experience almost same electronic environmnet resulting to give merge signal as multiplet at chemical shift range between δ 7.397-7.198. The hydroxy proton resonates at δ 13.623.

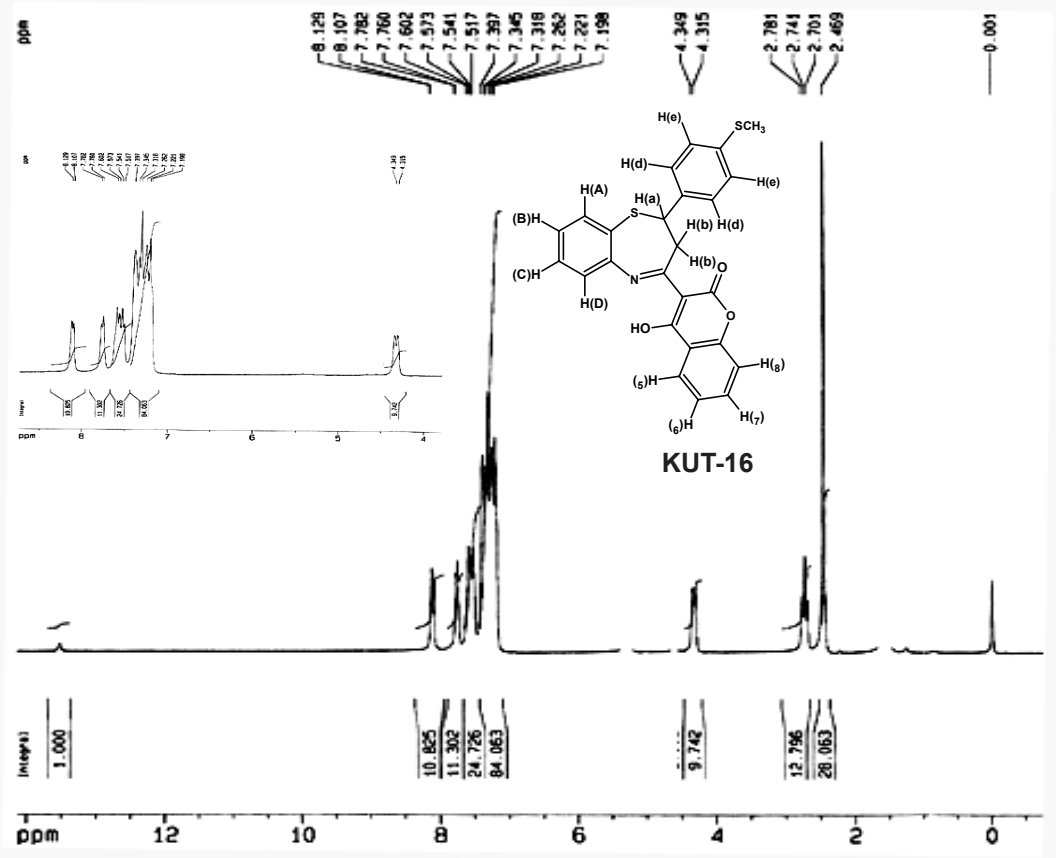
4-Hydroxy-3-(2-(4-nitro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-15)

In ^{1}H NMR Spectrum of KUT-15 a triplet due to methyne proton, appears at δ 2.801 with J value of 11.4 & 12.6Hz, where asa doublet relevant to two methylene protons appears at δ 3.339 with J value of 13.2Hz, which reveals that methyne & methylene are neighbouring protons in a same thiazepine ring. Proton $H_{(5)}$, $H_{(A)}$ & $H_{(D)}$ of benzenoid part of coumarin residue and 1,5-benzothiazepine system become most deshielded, experience almost same electronic environmnet resulting to give multiplet at chemical shift range between δ 8.212-8.099. Protons $H_{(e)}$ of phenyl residue appears as doublet at δ 7.453 with J value of 8.4Hz. Proton $H_{(7)}$ of benzenoid part of coumarin ring resonate at 7.632δ ppm as triplet with Jvalue of 7.8 & 7.5Hz. Protons $H_{(B)}$, $H_{(C)}$, $H_{(A)}$, $H_{(d)}$ & $H_{(6)}$ experience almost same electronic environment and their resonance resulting as multiplet at chemical shift range between δ 7.402-7.261 ppm. A signal for the hydroxy proton appears at δ 13.628.

4-Hydroxy-3-(2-(4-chloro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-10)

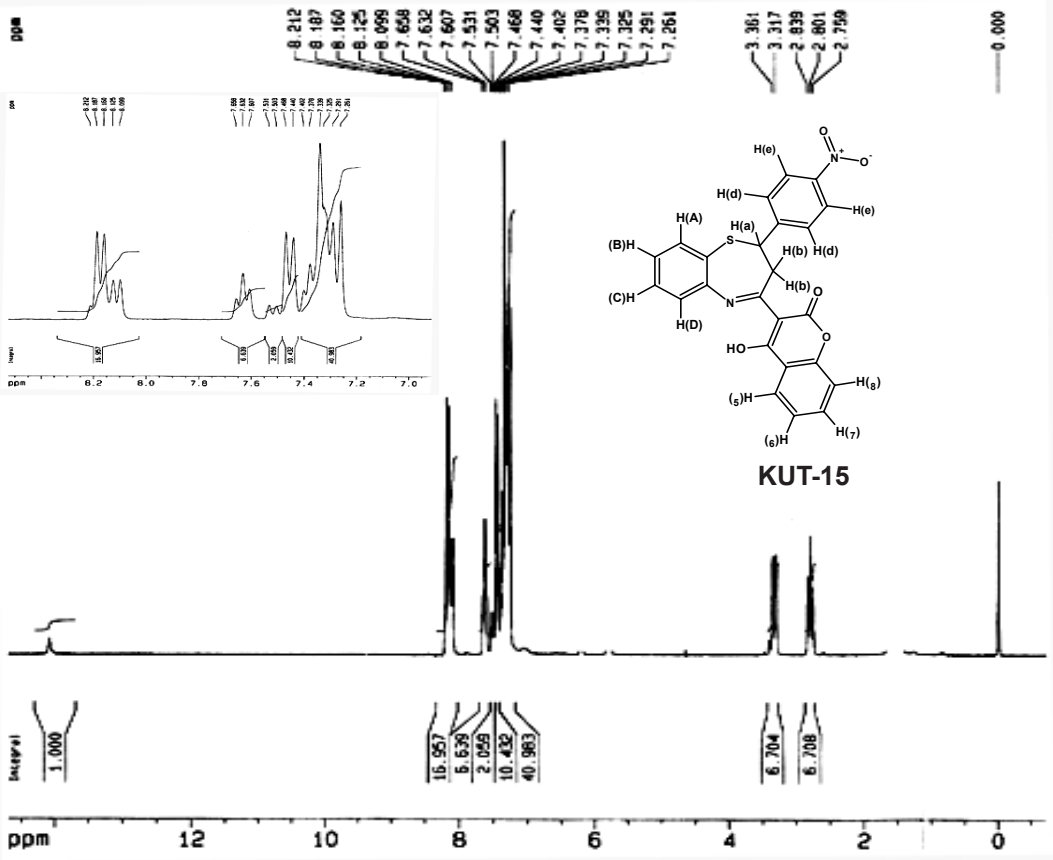
^{1}H NMR Spectrum of KUT-10 shows a triplet for methyneproton at δ2.725 ppm with J value of 12.3 & 12.3Hz, where as a doublet due to two protons of methylene group appears at δ 4.330 with J value of 11.1Hz, which reveals the presence of a methyne & a methylene group as neighbour to each other . Proton $H_{(5)}$ & $H_{(D)}$ of benzenoid part of coumarin residue and 1,5-benzothiazepine system become most deshielded, hence each appears as doublet at chemical shift value of 8.125 & 7.776δ ppm with J value of 7.8Hz and 7.5Hz respectively. Protons $H_{(8)}$ & $H_{(7)}$ as well as protons $H_{(d)}$ & $H_{(e)}$ felt almost same electronic environment and resonated to give multiplets at range between δ 7.638-7.532 and δ 7.412-7.357 respectively. Protons $H_{(A)}$, $H_{(B)}$, $H_{(C)}$ and $H_{(6)}$ experience almost same electronic environment resulting to give merge signal as multiplet at chemical shift value range between δ 7.309-7.260 ppm. Where as a the hydroxy proton resonates at 12.625δ ppm

^{1}H NMR Spectrum of 4-Hydroxy-3-(2-(4-thiomethyl)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-16)



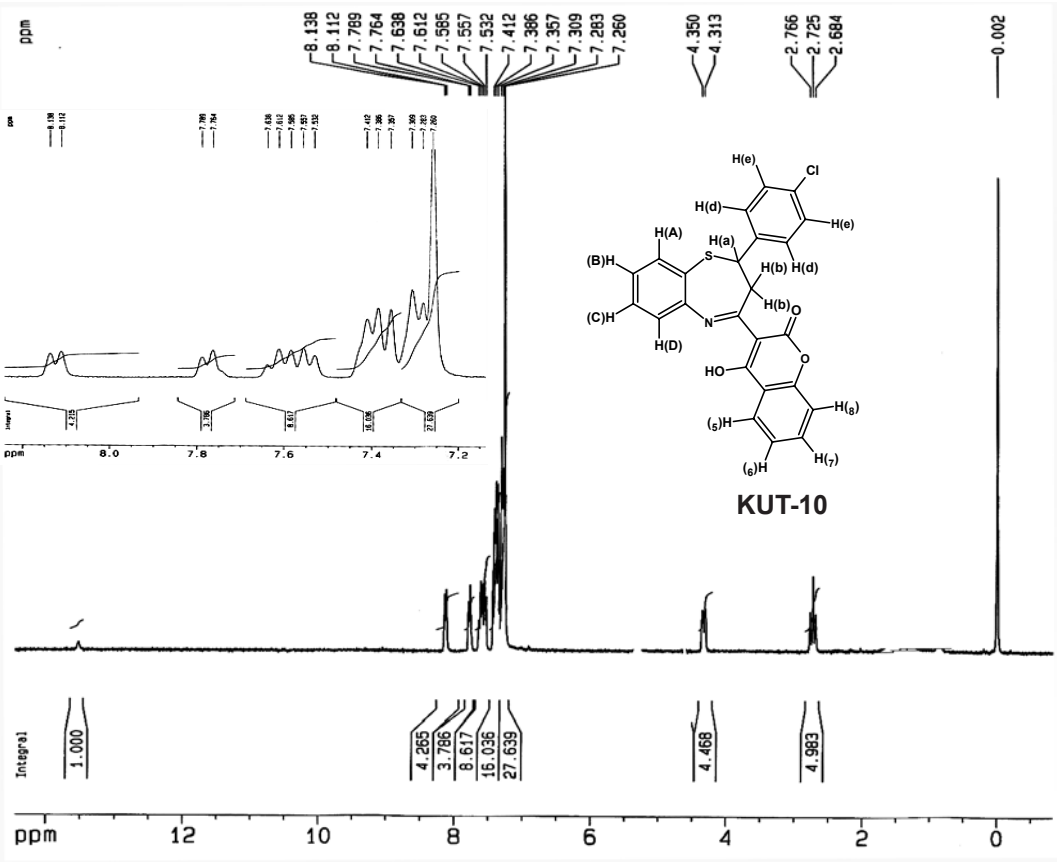
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value (Hz)
- $CH_{(a)}$	2.741	1	t	12.0 & 12.0
- $CH_{2(b)}$	4.320	2	s	10
-SCH_3	2.469	3	s	-
Ar-$H_{(D)}$	7.771	1	d	6.6
Ar-$H_{(A,8)}$	7.558	2	2d	8.7 & 7.2
Ar-$H_{(b,c,d,e,6,7)}$	7.397-7.198	8	m	-
Ar-$H_{(5)}$	8.118	1	d	6.6
O-H	13.623	1	s	-

^{1}H NMR Spectrum of 4-Hydroxy-3-(2-(4-nitro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-15)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{(a)}$	2.801	1	t	11.4 & 12.6
- $CH_{2(b)}$	3.339	2	d	11.2
Ar-$H_{(5,8,D)}$	8.212-8.099	3	m	-
Ar-$H_{(e)}$	7.454	2	d	8.4
Ar-$H_{(7)}$	7.632	1	t	7.8 & 7.5
Ar-$H_{(B,C,A,d,6)}$	7.402-7.261	6	m	-
O-H	13.628	1	s	-

^{1}H NMR Spectrum of 4-Hydroxy-3-(2-(4-chloro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-10)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{(a)}$	2.725	1	t	12.3 & 12.3
- $CH_{2(b)}$	4.330	2	d	11.1
Ar-$H_{(D)}$	7.776	1	d	7.5
Ar-$H_{(7,8)}$	7.638-7.532	2	m	-
Ar-$H_{(d,e)}$	7.412-7.357	4	m	-
Ar-$H_{(A,B,C,8)}$	7.309-7.260	4	m	-
Ar-$H_{(5)}$	8.125	1	d	7.8
O-H	12.625	1	s	-

EI MASS Spectral Study

As EI-Mass spectrum of the 4-Hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2H-chromen-2-ones exhibited special features like molecular ion peak and fragmentation pattern relavent to the nature of the molecule, it become one of the most important tool for characterization of the the structure of molecule.

4-Hydroxy-3-(2-(4-thiomethyl)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-16)

Molecular Ion peak at m/z 445, a peak due to loss of methyl and hydroxy group from molecular ion was observed at m/z 412, the peak at m/z 308 reveals loss of hydroxy and thiomethyl phenyl group from the molecular ion, where as base peaks observed at m/z 137 and 150, other ions observed are m/z 295, m/z 175, m/z 122 etc.

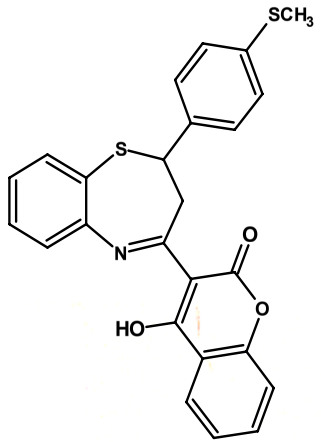
4-Hydroxy-3-(2-(4-nitro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-15)

Molecular Ion peak at m/z 444, a peak due to loss of 4- hydroxycoumarin group from molecular ion was observed at m/z 280, where as base peak observed at m/z 308, other ions observed are m/z 252, m/z 188, m/z 160, m/z 121 etc.

4-Hydroxy-3-(2-(4-Fluoro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-12)

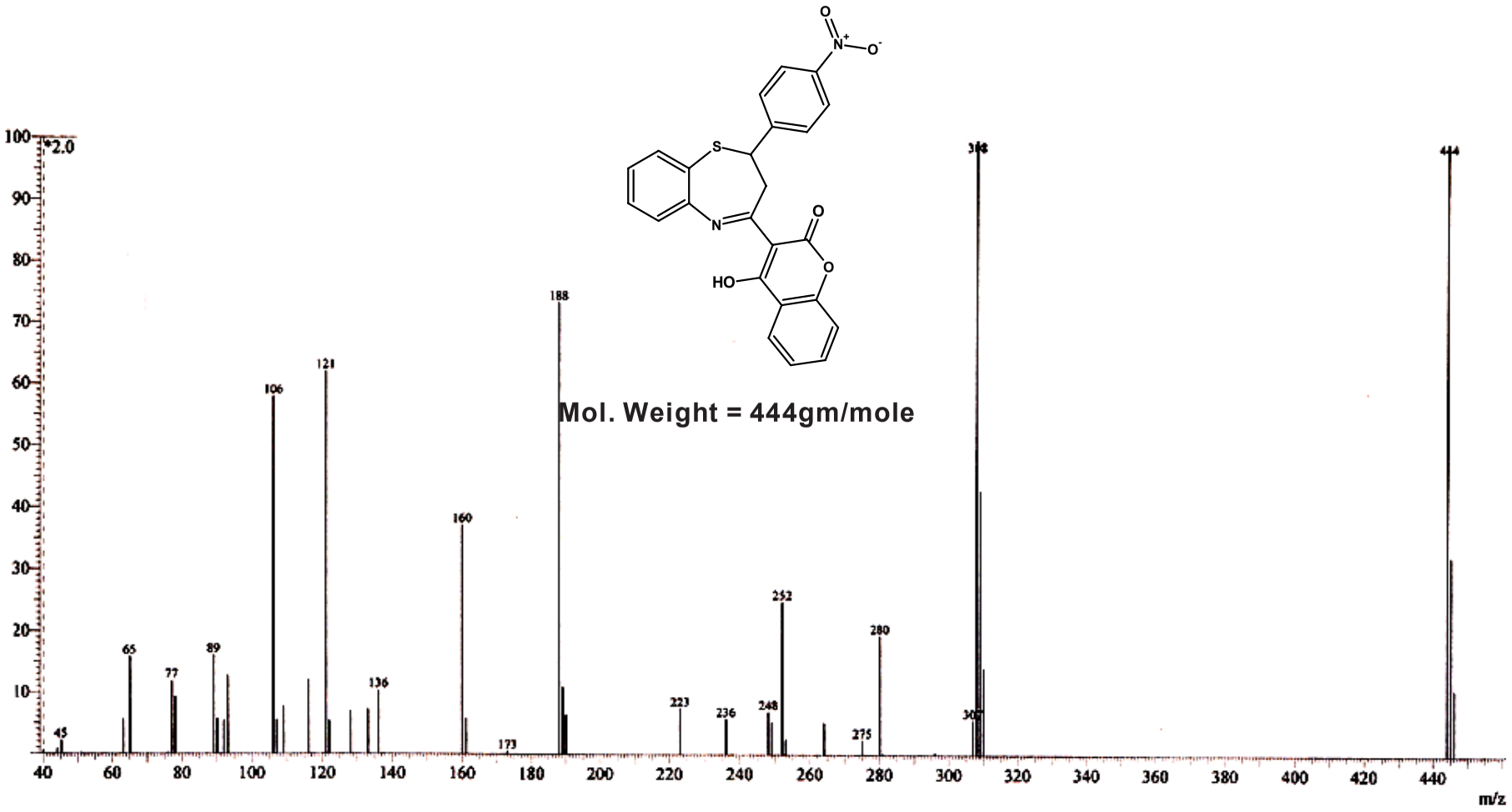
Molecular Ion peak along with typical characterestic ionization patern of (m+1), (m+2), (m-1) peaks of fluoro substitution observed at m/z 417, a peak due to loss of 4- hydroxycoumarin group from molecular ion was observed at m/z 280, where as base peak observed at m/z 308, other ions observed are m/z 295, m/z 256, m/z 160, m/z 175 etc.

EI MASS Spectrum of 4-Hydroxy-3-(2-(4-thiomethyl)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-16)

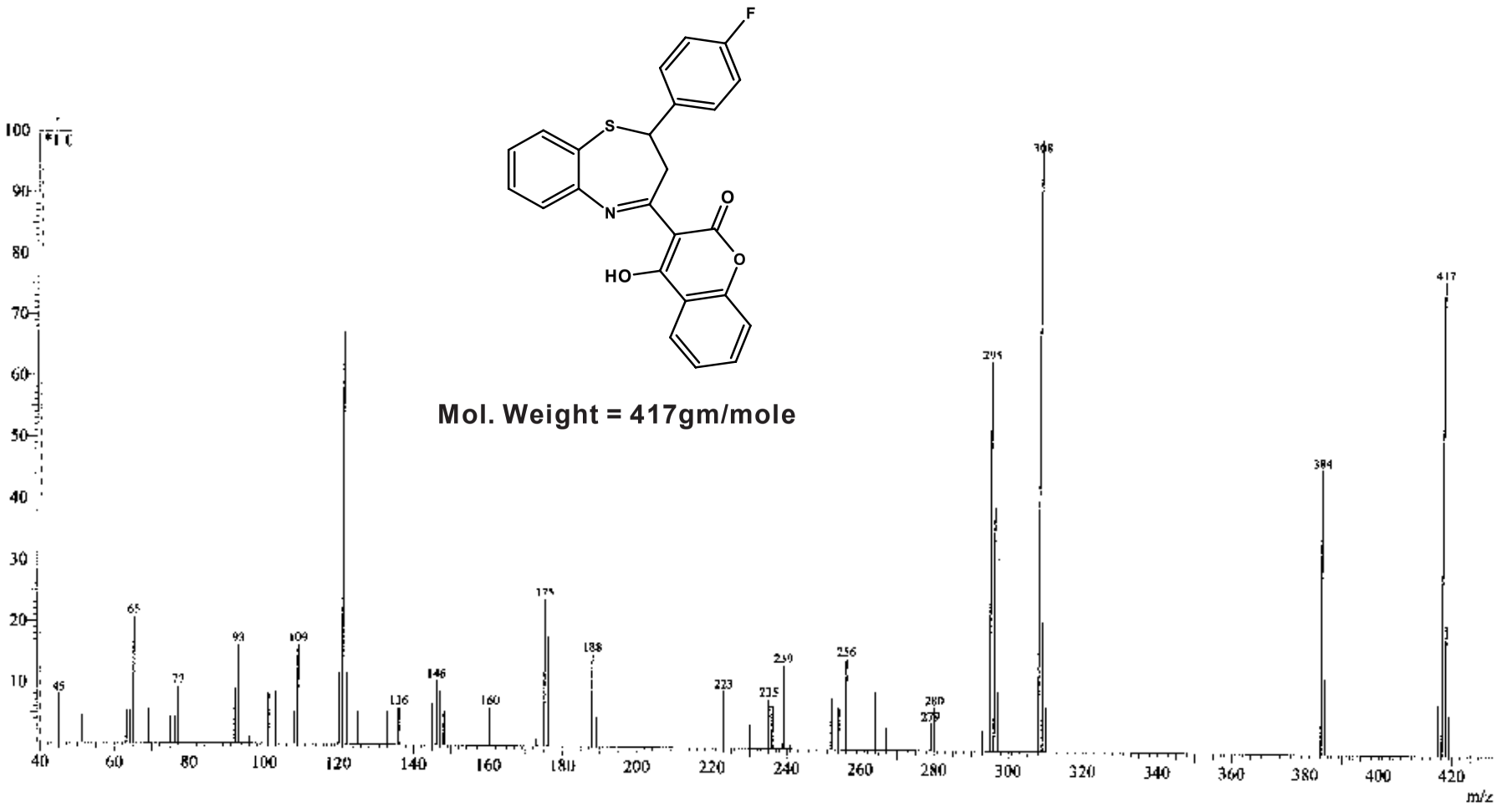


Mol. Weight = 445gm/mole

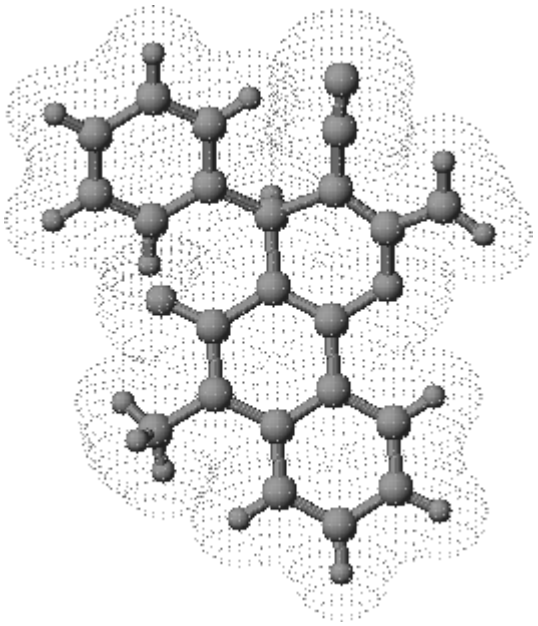
EI MASS Spectrum of 4-Hydroxy-3-(2-(4-nitro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-15)



EI MASS Spectrum of 4-Hydroxy-3-(2-(4-fluoro)phenyl-2,3-dihydro-1,5-benzothiazepin-4-yl)-2*H*-chromen-2-one (KUT-12)



CHAPTER-3



Synthesis and Characterization of Some 2-Amino -6-alkyl/alkylaryl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c] quinoline -3-carbonitriles

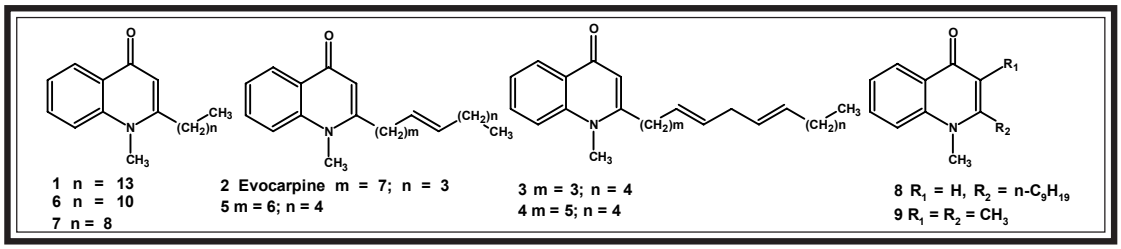
Introduction

4-hydroxy-2-quinolones are important compounds, not only because this moiety is present in a number of natural products but quite a few derivatives exhibit variety of interesting pharmacological properties[1]. While pyranofused heterocycles are biologically important as antibacterial[2], antihistamines[3], antimicrobials[4], enzyme substrates[5] and alkaloids[6]. Pyranoquinolinones shows good pharmacological activities[1,7] such as, antibacterial, antimicrobial, anti-HIV and antiviral and antitumor. The derivatives of fused 2-quinolones are useful as cardiovascular agents[7]. Quinolone alkaloids are known to possess antimicrobial activity and marked cytotoxicity against animal and plant tumors[7.1]. A novel class of 4-hydroxyquinolin-2(1H)-ones has recently been described[7.2] as selective glycinesite NMDA antagonists with potent *in vivo* activity after oral administration. However, depending on their structural types, quinolone derivatives exhibit different activities [7.3]. Furo[2,3-c]quinolin-4(5H)-one and 2H-pyrano[3,2-c]quinolin-5(6H)-one derivatives are abundantly distributed in nature [7.4].

❖Quinolone alkaloids

1-methyl tetradecylquinolin-4(1H)-one (1), was isolated from the fruits of *Evodia rutaecarpa* together with evocarpine (2) and related alkadienes (3) and (4) after activity-guided fractionation based on inhibition of diacylglycerol acyltransferase (DGAT)[8].

These are the first quinolone alkaloids to have been tested as DGAT inhibitors; the moderate activity of the purified compounds (IC_{50} 69.5, 23.8, 20.1 and 13.5 ì M, respectively) indicates possible utility in the design of hypolipidaemic and antiobesity agents. Evocarpine and the positional isomer (5) showed strong antibacterial activity against *Helicobacter pylori* both *in vitro* and *in vivo* by inhibiting respiration, the results reinforcing previous indications of this class of alkaloids as novel therapeutical agents for the treatment of ulcers[9]. Another *Evodia* alkaloid, 1-methyl-2-undecylquinolin-4(1*H*)-one (6), has proved to be an irreversible and selective inhibitor of type B monoamine oxidase (*K*i 0.01 ì M), suggesting potential value in the treatment of neurological disorders such as Parkinson's andAlzheimer's diseases and Huntington's chorea[10]. The related alkaloids (7–9) from the genus *Boronia*, all with saturated side chains, showed moderate to significant ability to inhibit the growth of several pathogenic bacteria[11].



The herb rue (*Ruta graveolens*), known since antiquity for its medicinal properties, continues to yield new alkaloids. Bioassaydirected isolation of antifungal compounds from a leaf extract produced the previously unknown alkaloid (10), and the related compounds graveoline (11) and rutavarin (12) in which methylene “spacers” of different lengths separate the quinolone and aromatic moieties, as well as 1-methyl-2-nonylquinolin-4(1*H*)-one[12]. All of the purified alkaloids inhibited the growth of seven different fungi to a greater or lesser extent; *Phomopsis* species were especially sensitive, while the new alkaloid (10) was highly active against *Botrytis cinerea* even at a low concentration (30 ì M). In a separate investigation of the biological activity of *R. graveolens* metabolites, graveoline (11) was found to be cytotoxic towards the HeLa cancer cell line (ED50 3.35 ì g ml^{-1}), and its

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7.1 C.Neville, M.Grundon, V.Ramchandran, G.Reisch, J.Reisch, *J Chem Soc Perkin Trans.*,**1**, 2261 (1991)
7.2 a) R.Carling, P.Leeson, K.Moore, J.Smith, C.Moyes, I.Mower, S.Thomas, T.Chan, R.Baker, A.Foster, S.Grimwood, J.Kemp, G.Marshall, M.Tricklebank, K.Saywell, *J.Med Chem* **36**, 3386(1993)
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c)R.Carling, P.Leeson, K.Moore, J.Smith, C. Moyes, I.Mawer, S.Thomas, T.Chan, R. Baker, A.Foster, S.Grimwood, J.Kemp, G.Marshall, M.Tricklebank, K.Seywell, *J Med Chem.*, **36**, 3397(1993)
7.3 J.Kugalowski, R.Baker, N.Curtis, P.Leeson, I.Stansfield, A.Foster, S.Grimwood, R. Hill, J.Kemp, G.Marshall, K.Saywell, M.Tricklebank, *J Med Chem*, **37**,1402(1994)
7.4a) R.Brown, J.Hobbs, G.Hughes, E.Ritchie, *Aust J Chem,* **7**, 348(1954)
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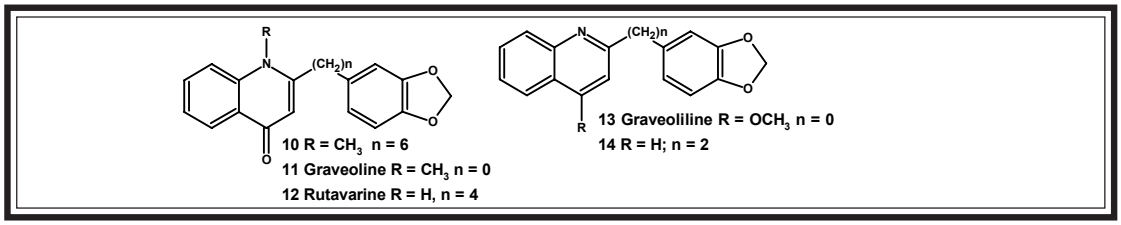
c) D.Lavie, N.Danieli, R. Weitman, E.Glotter, *Tetrahedron*, **24**, 3011(1968)
d) D.Dreyer, A.Lee, *Phytochemistry*, **11**, 763 (1972)
e)D.Taylor, J.Warner, *Phytochemistry*, **12**, 1359 (1973)
f) J.Reisch, J.Korosi, K.Szendred, I.Novak, E.Minker, *Phytochemistry*, **14**, 1678(1975)
g) L.Jurd, M.Bensen, *J Chem Soc Chem Commun* 92(1983)

isomer graveolinine (13) was an effective inhibitor of platelet aggregation induced by arachidonic acid and collagen at low concentration (5 ìg ml^{-1})[13]. A related alkaloid from *Galipea longi.ora*, compound (14), inhibited the growth of cells infected with human T-lymphotropic virus type 1 (HLTV-1) at a concentration of 10 ì M, but less effectively than a number of synthetic quinolines, among them the simple compound 2-vinylquinoline[14].



It has been known for some time that simple quinoline alkaloids from *Galipea* and related rutaceous genera show substantial trypanocidal, antileishmanial and antimalarial activity. Some of these findings have now been reviewed by Fournet and Munoz[15]. One of the simplest *Galipea* alkaloids,2-propylquinoline, has been proposed as a new oral treatment for visceral leishmaniasis after it was found to have decreased intestinal P-glycoprotein activity in mice infected with *Leishmania donovani*[16].

The unique structural feature in helietidine (15), a new alkaloid isolated from the Brazilian medicinal plant *Helietta longifoliata*[17], is the pyrano[3,2-*g*]quinoline ring system, hitherto unprecedented among the hemiterpenoid quinoline alkaloids; indeed, even 6-prenylquinolines, likely precursors for this tricyclic ring system, are unknown as natural products. The structure was presented as a quinolin-2-ol, but the quinolin-2-one tautomer is undoubtedly more plausible.

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9.K.Tominaga, K.Higuchi, N. Hamasaki, M.Hamaguchi, T.Takashima, T.Tanigawa, T.watanabe, Y.Fujiwara, Y.Tezuka, T.Nagaoka, S.Kadota, E.Ishii, K.Kobayashi, T.Arakawa, *J.Antimicrob.Chemother.*, **50**, 547(2002)
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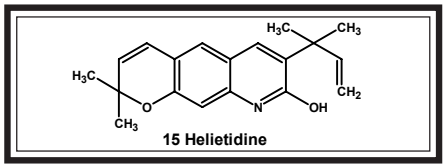
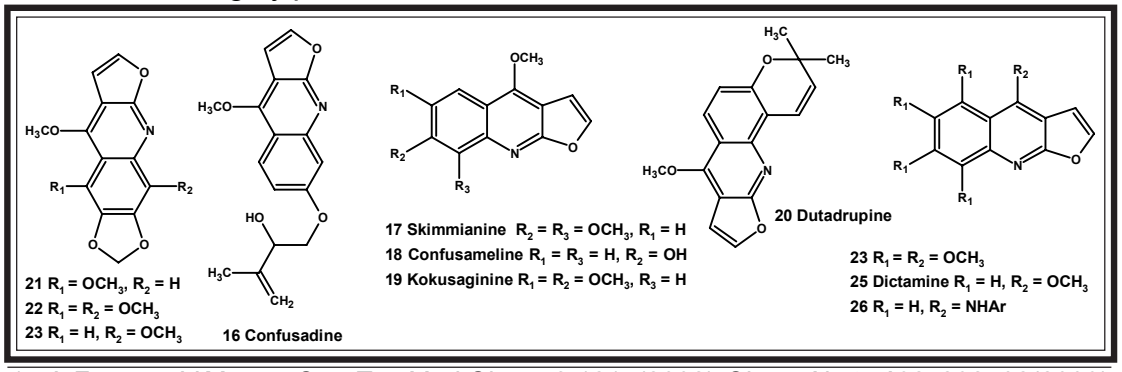


Among the *Melicope* metabolites, confusadine (16) was found to be a noteworthy inhibitor of platelet aggregation triggered by various inducers[18]. The new alkaloids were generally poorly cytotoxic towards a range of human cancer cell lines, and substantially worse than the previously isolated *Melicope* alkaloids confusameline (18) and dutadrupine (20) (ED_{50} 0.03–2.3 ì g ml^{-1})[19]. New furo[2,3-*b*]quinoline alkaloids with more conventional structures isolated from the wood of the Madagascan plant *Vepris punctata* include 5-methoxymaculine (21), 5,8-dimethoxymaculine (22) and 4,5,6,7,8-pentamethoxyfuroquinoline (23)[20]. The somewhat rare 6,7-methylenedioxy substituent seen in the first two compounds is also present in flindersiamine (24) and maculine, alkaloids previously isolated from this plant species and confirmed in the present study. All the isolated compounds were weakly cytotoxic towards the A2780 human ovarian cancer cell line. Among a range of furoquinoline alkaloids isolated from *Ruta graveolens*, skimmianine (17) and dictamnine (25) were significant inhibitors of platelet aggregation, while dictamnine(25) and kokusaginine (19) were cytotoxic towards HeLa cancer cell lines[21]. Dictamnine has itself been used as a starting material for the synthesis of 4-anilinofuro[2,3-*b*]quinolines (26) (ArNH in place of OMe), several of which were highly potent inhibitors of a number of human cancer cell lines.[22,23].



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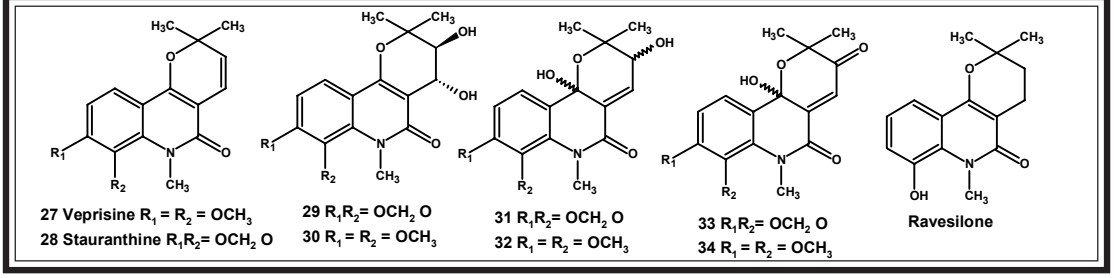
A cytotoxic fraction fromthe stem bark extract of *Stauranthus perforatus*, was analysed by the comparatively rare technique of HPLC–NMR, HPLC–MS measurements, showed the new compounds to be analogues of veprisine (27) (7,8-dimethoxy-*N*-methyl.indersine), which had previously been found in this species and which was again detected in this study. One of the new alkaloids was (28), the 7,8-methylenedioxy equivalent of veprisine, to which the name stauranthine was given. The remaining alkaloids were oxidised analogues of both veprisine and stauranthine, and included the *trans*-3,4-dihydroxy-3,4-dihydro derivatives (29) and (30), the 3,6-dihydroxy-3,6-dihydro derivatives(31) and (32), and the 6-hydroxy-3-keto analogues (33) and (34). The 3,4-dihydroxypyran component in (29) and (30) has previously been found in other rutaceous quinoline and acridone alkaloids, but the involvement of the hemiterpenoid moiety in the remaining alkaloids in hemiketal formation with a quinolin-4-one is unprecedented. It is likely that *trans*-3,4- dihydroxy-3,4-dihydroveprisine (30) is identical to the alkaloid araliopsinine, reported from *Araliopsis tabouensis* by Ngadjui *et al.* in 1988[24]. Interestingly, the alkaloids of the stauranthine series were all more stable than those of the veprisine series, perhaps reflecting the greater tendency towards oxidation of the vicinal dimethoxy grouping as compared with the methylenedioxy substituent. A pyrano[3,2-c]quinolone alkaloid ravesoline was isolated from the leaves of *Ravenia spectabilis*[24a].



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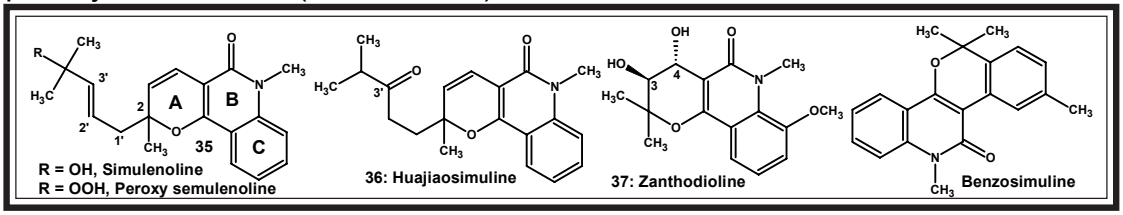
Pyranoquinoline alkaloids simulenoline (35, R=OH)[25,26] and huajiaosimuline (36)[27] were isolated from root barks of *Zanthoxylum simulans*, a shrub found in Taiwan and mainland China. While simulenoline (35, R=OH) was the most recently isolated of the two alkaloids, a third pyranoquinoline alkaloid zanthodioline (37) from the same species was just recently disclosed in the literature[25]. These novel monoterpenoid pyranoquinolines are potent inhibitors of platelet aggregation. For example, at a concentration of 100 ì g/mL, simulenoline (35, R=OH) demonstrates a nearly complete suppression of platelet aggregation induced *in vitro* by collagen, arachidonic acid, and PAF in general[25]. While simulenoline (35, R=OH) and zanthodioline (37) are not cytotoxic, huajiaosimuline(36) is toxic toward several human cultured cell lines, especially the estrogen receptor-positive breast cancer cells, ZR-75-1[27]. Structurally, these alkaloids contain three fused six-membered rings with a unique terpenoid side chain at C-2. The BC-ring is essentially the quinolone nucleus, and the A-ring is a 2*H*-pyran. Where as three pyranoquinoline alkaloids isolated were from stem bark of Formasan *Zanthoxylum simulans* Hance(Rutaceae), namely simulenoline(35, R=OH), peroxysimulenoline(35, R=OOH) and benzosimuline[25]



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(*b*) M.Grundon, *Tetrahedron*, **34**, 143(1978)
(*c*) S.Barr, C.Neville, M.Grundon, D.Boyd, J.Malone, T.Evans, *J. Chem. Soc.*, *Perkin Trans. 1*, 445(1995);
(*d*) B.Ngadjui, J.Ayafor, A.Bilon, B.Sondengam, J.Conolly, D.Rycroft, *Tetrahedron*, **48**, 8711(1992).
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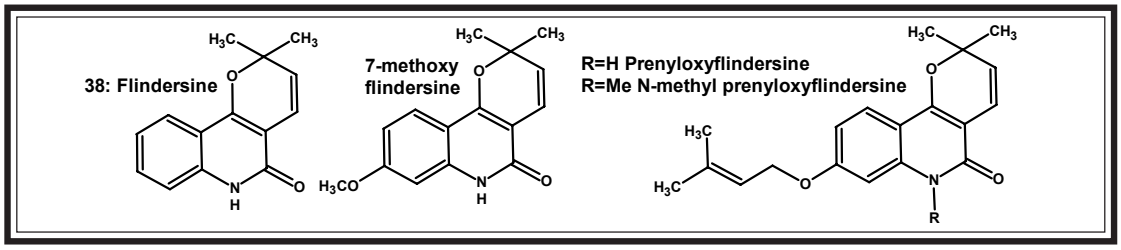
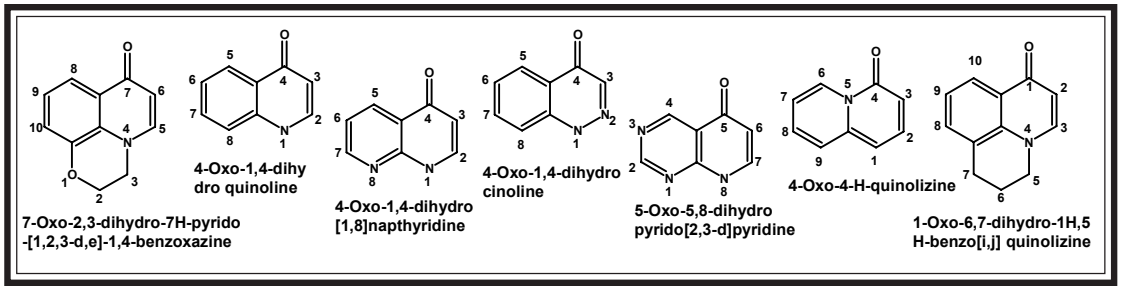
(*b*) F. Piozzi, P. Venturella, A. Bellino, *Gazz. Chim. Ital.*,**99**, 711(1969);
(*c*) A. Groot, B.Jansen, *Tetrahedron Lett.*,3407(1975);
(*d*) R. Bowman, M.Grundon, K. James, *J. Chem.Soc.*, *Perkin Trans. 1*, 1055(1973);

Pyrano[3,2-*c*]quinolin-5-one derivatives constitute a large group of naturally occurring alkaloids represented by, *e.g.*, flindersine 38[26]. These pyrano[3,2-*c*]quinolin-5-one derivatives and their synthetic analogues are of current research interest not only because they have a wide range of biological activities and therefore have potential medical and other applications[27b,28], but also because they are often used as synthetic precursors for the preparation of other natural products such as dimeric quinoline alkaloids[29] and other polycyclic heterocycles[31]. Several syntheses of flindersine and benzene-ring-substituted flindersine derivatives have been reported[32]. A suite of pyrano[3,2-*c*]quinolinones isolated from the leaf extract of *Vepris bilocularis*, a forest tree of south India,includes the known compound haplamine(6-methoxy flindersine) and three new flindersine derivatives , namely 7-methoxyflindersine, 7-prenyloxyflindersine and *N*methyl-7-prenyloxyflindersine[32a].



❖Antibiotic activity profile of Quinolone derivatives

The quinolone anti-infective agents are of wholly synthetic origin and are not modeled knowingly after any natural antibiotic. Several ring systems are or have been involved. Those of greatest prominence and their numbering systems are illustrated in figure below[33].



(*e*) M. Ramesh, P.Mohan, P.Shanmugam, *Tetrahedron*, **40**, 4041(1984)
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(*h*) W.Campbell, B.Devidovitz and, G.Jackson, *Photochemistry*, **29**, 1303(1990).
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The first antimicrobial quinolone discovered called nalidixic acid demonstrated Gram negative antibacterial activity , remains in the market today, so-called first generation quinolones. Despite its convenient oral activity, bactericidal action, and ease of synthesis, its limited antimicrobial spectrum (primarily activity against *Escherichia coli*) and poor pharmacokinetic characteristics limit its use primarily to treatment of sensitive community acquired urinary tract infections.

Norfloxacin, the first of the second-generation family of quinolones[34]. This agent had dramatically enhanced and broader spectrum anti Gram-negative activity and possessed significant anti Gram-positive activity as well. The potency of norfloxacin was in the same range as that of many fermentation-derived antibiotics, and its comparative structural simplicity and synthetic accessibility lead to a very significant effort to find even more improved analogues. Norfloxacin and its *N*-methyl analogue pefloxacin ultimately failed to find major use outside of the genitourinary tract because of poor active blood levels and limited potency against Gram-positives.

Shortly thereafter, ciprofloxacin[35-37] and ofloxacin, [37,38] as well as its optically active form levofloxacin[39], were introduced. The second-generation agents have significant broad-spectrum antimicrobial activity including important Gram-positive pathogens. This is coupled with gratifying safety and pharmacokinetic characteristics.

A wide variety of clinical indications have been approved for quinolones including many infections commonly encountered in community practice including upper and lower respiratory, gastrointestinal and gynecologic infections, sexually transmitted diseases, prostatitis, and some skin,bone, and soft tissue infections[40].

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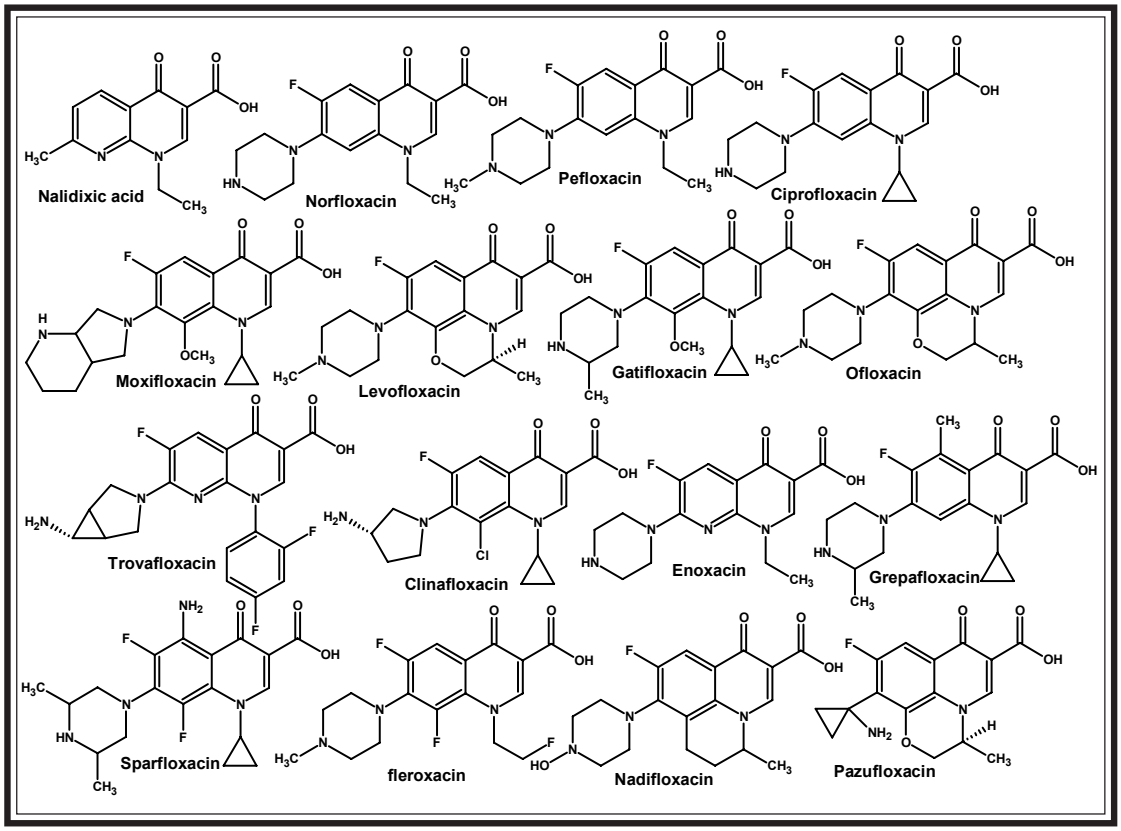
Recently introduced members of the fluoroquinolone family belong to the third generation. These include gatifloxacin[41] and moxifloxacin[42] which possess further enhanced activity against Gram-positive infections, and anti-anaerobic coverage is now present although at present only trovafloxacin[43] is approved for this indication. Among the agents still in preclinical study, clinifloxacin[44] is the most promising anti-anaerobic agent. When first introduced, there was no idea of the molecular mode of action of these agents. Indeed, the availability of nalidixic acid was instrumental in assisting the discovery of the targeted bacterial type II topoisomerases[45]. Those of importance to the quinolones are bacterial topoisomerase II,[46] also known as DNA gyrase, and bacterial topoisomerase IV[47,48]. These enzymes are vital for dictating the proper topology of DNA important for protein biosynthesis, DNA replication and repair, and DNA decatenation. Fluoroquinolones form a ternary complex consisting of drug, DNA, and enzyme that interferes with DNA transcription, replication, and repair and promotes its cleavage, leading to rapid bacterial cell death. They are without apparent significant action on individual molecules of DNA or topoisomerases alone, but the interaction of DNA with enzyme creates a binding pocket for the quinolones.

Generally anti Gram-negative activity is more closely associated with DNA gyrase inhibition and anti Gram-positive activity is more closely associated with bacterial topoisomerase IV inhibition[49]. With a number of quinolones activity is attributed to interference with the function of both of these enzymes. For example, a survey of the ability of a collection of quinolones to inhibit the catalytic action of topoisomerases showed that the ratio of DNA gyrase to topoisomerase IV action for *E. coli* was between about 15 and 27, whereas for *Staphylococcus aureus* the ratio was reversed, with topoisomerase IV inhibition over DNA gyrase inhibition being from about 1.7 to 21[50]. In a later study of 15 quinolones, they were divided into three groups on the basis of their relative ability to inhibit *S. aureus* strains with

a resistance mutant toward one or the other enzyme. With group I (norfloxacin, enoxacin, fleroxacin, ciprofloxacin, lomefloxacin, trovafloxacin, grepafloxacin, ofloxacin, and levofloxacin) topoisomerase IV was the more sensitive target. With group 2 (sparfloxacin and nadifloxacin) DNA gyrase was the more sensitive target. With group 3 (gatifloxacin, pazufloxacin, moxifloxacin, and clinafloxcin) both were equivalently sensitive. The later were termed the dual targeting quinolones[48].



Human topoisomerase II is generally not inhibited by these agents at the doses normally employed since it is often at least 100-1000times less sensitive to them[51]. Despite significant homologies with the bacterial enzyme, creative analoguing is able to distinguish clearly between them, and safe agents are readily produced.

Absorption of quinolones following oral administration is usually good, mostly 50% or better, and, in some cases, in excess of 95%[52].They are well distributed in the body; however, efflux pumps protect the central nervous system to some extent[53]. Comparatively little metabolism takes place with them, and excretion is mostly in active form in the urine.

Overall, the quinolones possess antimicrobial spectra and potency attractive for clinical use. In particular, they are bactericidal in achievable oral doses and possess significant postantibiotic effects. These features are especially useful for treating infections of immune-suppressed patients. Microorganisms regarded as highly susceptible to quinolones have minimum inhibitory concentration values ranging from 0.01 to 0.2 ì g/ml. Examples include *E. coli*, *Klebsiella pneumoniae*, *Enterobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Hemophilus influenzae*, *Neisseria*, and *Legionella*. Less susceptible but still sensitive microorganisms lie in the range of 0.25-2 ì g/ml. These organisms include those that become resistant more easily. Especially notable are *Pseudomonas aeruginosa* and *S. aureus* (with particular emphasis on MRSA). Organisms that are regarded as insensitive have MIC values of 2 ì g/ml or higher. Examples include *Nocardia*, *Treponemia*, and anaerobes.

A comparison of the susceptibilities of a wide number of pathogenic bacteria with representatives of each generation of quinolones is presented in Table-1

microorganism	Nor	Cipro	Enox	Oflox	Levo	Gati	Lome	Moxi	Spar	Trov Alatro
				Gram-Positives						
S. aureus	+	+	+	+	+	+	+	+	+	+
MRSA							+			
Staphylococcus epidermidis	+	+	+	+	+	+	+	+	+	+
MRSE						+	+	+		
Staphylococcus hemolyticus		+								
Streptococcus pyogenes		+		+	+	+		+	+	+
Streptococcus viridans					+				+	+
Enterococcus faecalis	+	+			+					+
				Gram-Negatives and Special Microorganisms						
E. coli	+	+	+	+	+	+	+	+	+	+
Chlamydia trachomatis				+						
Chlamydia pneumoniae				+	+	+	+	+	+	+
Enterobacter sp.	+	+	+	+	+	+	+	+	+	
Gardnerella vaginalis				+						+
H. influenzae		+		+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+
Legionella pneumoniae		+		+	+	+	+	+	+	+
Mycoplasma hominis				+						+
Mycoplasma pneumoniae				+	+	+	+	+	+	+
Neisseria gonorrhoeae	+	+	+	+		+				
Proteus mirabilis	+	+	+	+	+	+	+	+	+	+
Proteus vulgaris	+	+	+	+	+	+	+		+	+
Providencia rettgeri	+	+		+	+		+			
Providencia stuartii	+	+	+	+	+					
P. aeruginosa	+	+	+	+	+		+			+
Salmonella typhi		+								
Seratia marcescens	+	+	+	+	+		+			
Shigella sp.		+		+	+	+		+	+	+
Ureaplasma urealyticum	+			+						+
Vibrio chloerae		+								
				Anaerobes						
Bacteroides fragilis										+
Clostridium perfringes	+			+		+				+

[a] "+" means that the culture is normally regarded as sensitive to ordinary concentrations.

Table 1. Antimicrobial Sensitivities of Selected Quinolones[a]

Resume of Structure-Activity Relationships of Quinolones

Figure-1 contains a pictorial summary of findings with reference to the parts of the molecule that are presumed to be in contact with the enzyme, with DNA, and with other quinolone moieties (thus forming the ternary complex). At the left is a cartoon viewed from the top. In this view, north and south represent DNA-binding sites wherein the keto and carboxyl groups hydrogen bond to single-stranded segments made available to them by enzymic action. West and east represent binding sites to DNA gyrase. In the interior of the complex, the quinolone molecules are associated with each other through hydrophobic contacts. Virtually every portion of the quinolone molecule is employed in one or more of these interactions. The requirement for the enzyme to open a saturable drug-binding pocket is implicit. The important role of having a basic moiety attached to C-7 is also apparent. The need for the groupsattached to N-1 and C-8 to be small and hydrophobic is also rationalized. The two molecules are aligned “head to tail” with respect to each other by electrostatic interactions involving the carboxyl and the amino substituents. A second pair of interacting quinolone molecules can also be aligned in a vertical stack by the ð-ð interactions and the same electrostatic interactions.

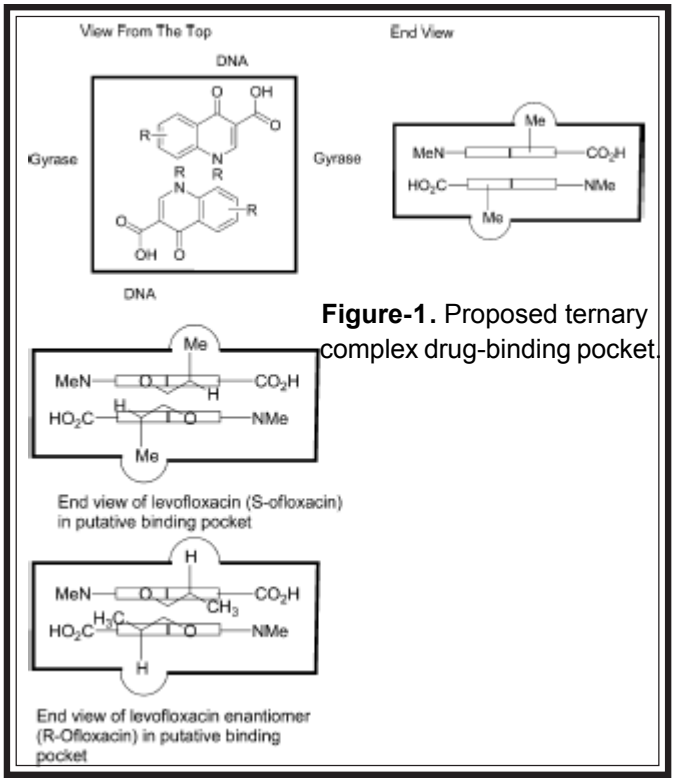


Figure-1. Proposed ternary complex drug-binding pocket.

An end view with the N-1, C-8 edge closest to the viewer is shown on the right side of the figure-1. The significance of the chiral preference conveyed by the methyl group of flumequine and levofloxacin and their analogues is rationalized by invoking the presence of a lipophilic drug-binding site above and below the pocket. The methyl portion of the *N*-ethyl moieties is illustrated as fitting into this pocket and providing a favorable interaction.

Pharmacology

❖Voltage-Gated Potassium Channel Blockers (Kv1.3)

The *Shaker*-related K^+ channel Kv1.3 is involved in the control of membrane potential, production of lymphokines, and proliferation of human T-lymphocytes[54,55]. Inhibition of Kv1.3 channels causes depolarization which reduces the electrochemical driving force for the increase in intracellular Ca^{2+} concentration required for lymphocyte activation: stimulation of gene transcription, production of lymphokines(e.g. interkeukin 2, IL-2), DNA synthesis, and finally cell division[54-56]. The Kv1.3 channel has a limited distribution in the periphery, being found mainly in B-cells, macrophages, platelets, osteoclasts, and fibroblasts, besides T-lymphocytes. In addition, the K^+ channel in human T-cells is a homomultimer of Kv1.3 subunits in contrast to the heterotetrameric form of channels in brain, where Kv1.3 is combined with at least Kv1.1 and Kv1.2. Thus, a selective Kv1.3 blocker may have ion channel selectivity against other tissues[56].

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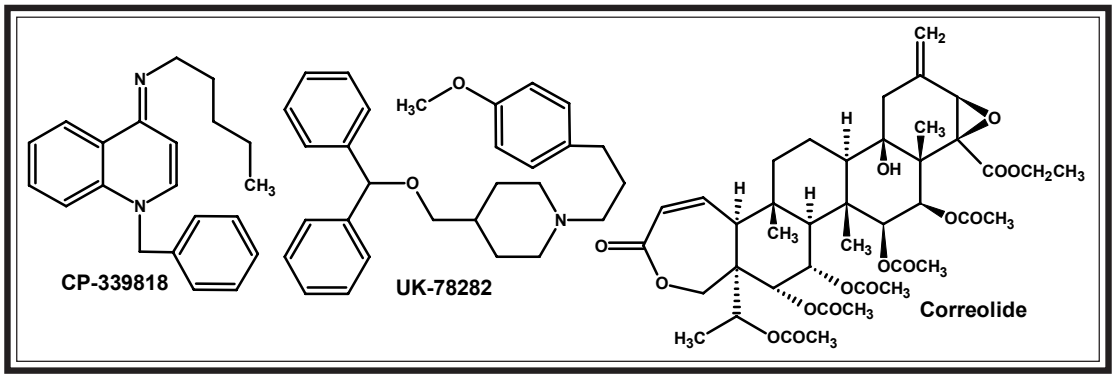
Using peptidyl inhibitors, such as margatoxin (MgTX), several groups have demonstrated that blockade of Kv1.3 channels suppresses activation and proliferation of human T-cells[57]. MgTX has been shown to suppressdelayed-type hypersensitivity and allogenic-antibody responses in miniswine[58], providing *in vivo* evidence that Kv1.3 is a novel pharmacological target for immunosuppressive therapy[56,59]. Potent, specific Kv1.3 inhibitors have the potential to be novel immunosuppressive agents with utility in transplantation, autoimmune disease, and inflammation therapy[56]. Efforts to identify a small, nonpeptidyl inhibitor of Kv1.3 channels have resulted in the discovery of submicromolar Kv1.3 blockers such as the dihydroquinoline CP-339818[60], the piperidine UK-78282[61] and correolide, a triterpene isolated from the root and bark of a Costa Rican tree, *Spachea correae* [62-64]. However, both CP-339,- 818 (half-blocking concentration, IC_{50}, for Kv1.3 channels, 0.23 ì M)[60] and UK-78282 (IC_{50}(Kv1.3)) 0.28 ì M)[61] block Kv1.3 and Kv1.4, a *Shake*r-related K^+ channel expressed in the heart and brain, with similar potency,[60,61] thus lacking selectivity. The most promising compound seems to be correolide, which has a 4-14-fold selectivity for Kv1.3 over other *Shaker*-related Kv channels[64].



Inga Butenschon, et. al[65] have revealed that the voltage-gated potassium channel Kv1.3 constitutes an attractive target for immunosuppression because of its role in T-lymphocyte activation and its functionally restricted expression to lymphocytes. Blockade of Kv1.3 channels by margatoxin has previously been shown to prevent T-cell activation and attenuate immune responses *in vivo*. Several furo and pyranoquinoline derivatives were synthesized and screened

for their blocking activities of Kv1.3 channels, stably expressed in mice-fibroblasts L929. In addition the activities of the compounds on Kv currents of the neuroblastoma cell line N1E-115 were determined.

The screening results suggested that Angular dihydropyranoquinolinones were in general more potent than their linear isomers (Table 2): angular dihydropyranoquinolinones bearing hydroxy groups (39a,b) displayed weak blocking activities, whereas angular dihydropyranoquinolinones lacking the hydroxy group (39c-e) were found to block both Kv channels of N1E-115 and Kv1.3 currents and N-alkylation increased the blocking activity of the angular dihydropyranoquinolinones on Kv currents of both cell lines (Table 2).The substituent in position 9 of the angular dihydropyranoquinolinones was important for their selectivity: Compounds (39c,d) without substitution in position 9 were found to be more potent blockers of Kv channels of N1E-115, whereas a methoxy group (39e) increased the blocking activity of Kv1.3 currents.

The second remarkable result is the higher potency of furoquinoline derivatives with an aromatic furan ring compared to dihydrofuroquinolinones. The linear furoquinolines kokusaginine (43a, R_1=R_2=OCH_3, R_3 =H), skimmianine(43b, R_1=H, R_2=R_3=OCH_3,), and dictamnine (43c, R_1=R_2=R_3 =H) as well as the linear dehydrated furoquinolinones (42a, R_1=H) and (42b, R_1=OCH_3) were in general more potent than dihydrofuroquinoline derivatives. Of these linear furoquinolines kokusaginine (43a, R_1=R_2=OCH_3, R_3 =H) seems to be the most promising compound, blocking Kv1.3 currents in the low-micromolar range and with higher potency than the N1E-115 K+ current. Again the angular isomers (40b,c) displayed higher affinities with the exception of (40a), the sodium salt of a sulfonic acid, being ineffective, thus corroborating that hydrophilic substituents diminish potency (Table 2). Finally, the pyranoquinolinone (41a) showed a slightly higher blocking activity than the corresponding hydrogenated derivative (39e). The dehydrated pyranoquinolinone veprisine (41b) bearing two methoxy groups in positions 7 and 8 was less effective than (41a). Moreover, in contrast to

veprisine (41b), compound (41a) as well as (40b,c) displayed higher blocking activities of Kv1.3 currents compared to Kv channels of N1E-115 (Table 2). Na+ currents were largely uneffected by all compounds tested. Thus, the effective quinolines are selective blockers of Kv channels. The compounds (39e), (41a), and especially (40c) were found to be the most potent and moreover selective blockers of Kv1.3 channels, showing a much higher potency than the furoquinoline kokusaginine. For these effective compounds we determined the half-blocking concentrations for N1E-115 and Kv1.3. The IC_{50} values are listed in Table 3.

By concluding these results, the following pharmacophoric model for the Kv channel blocking activity of the quinolines can be deduced. **First, an angular structured furo- or pyranoquinolinone seemed to be required for optimal interaction with a hydrophobic pocket of limited size at the receptor** site of Kv1.3 channels as well as Kv channels of N1E-115 cells. Moreover, it is striking that the **most potent compounds (40c and 41a) on Kv currents have a double bond in the furan or pyran ring in common, thus pointing toward ð-ð interaction with the receptor site.** Hydrophilic substituents such as hydroxy or sulfonic groups in this region diminish potency almost completely. For a favored binding to the receptor site of Kv1.3 the methoxy group in position 8 of the angular furoquinolinones 40c or in position 9 of the angular pyranoquinolinones 39e and 41a is of crucial importance. Compound 40c reveals 8-fold selectivity (Table-3) for Kv1.3 over Kv channels of N1E-115 cells, where as (40b) lacking a substituent in the quinoline ring is much less selective. Compound (39e) reveals 2-fold selectivity (Table-3) for Kv1.3, and 6d lacking the methoxy group is even more potent for Kv currents of N1E-115 cells just like the 7,8-dimethoxy-substituted angular pyranoquinolinone veprisine (41b). Thus, the substitution pattern in the quinoline ring is critical for Kv1.3 selectivity.

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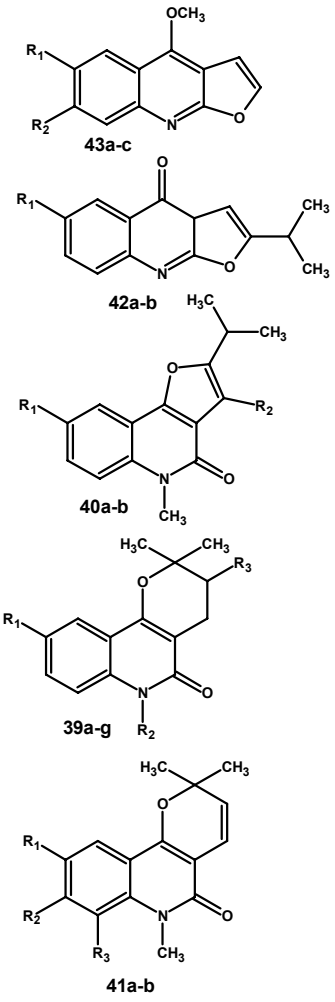

Table 2. Compound Structures of Angular Furo- and Pyranoquinolinones and Block of Peak K^+ Currents of N1E-115 and Kv1.3[a]

compd	R_1	R_2	R_3	B_K (%) N1E-115	B_{Na} (%) N1E-115	B_K (%) Kv1.3
39 a	H	CH_3	OH	15	4	4 (25 μM)
39 b	OCH_3	CH_3	OH	21	2	4
39 c	H	H	H	37	3	15
39 d	H	CH_3	H	65	7	48
39 e	OCH_3	CH_3	H	39 (25 μM)	3 (25 μM)	57 (25 μM)
				52	4	83
39 f	NO_2	H	H	23	8	nd
39 g	NO_2	CH_3	H	14 (25 μM)	0 (25 μM)	7 (25 μM)
40 a	OCH_3	SO_3Na		0 (25 μM)	0 (25 μM)	nd
40 b	H	H		25 (25 μM)	0 (25 μM)	44 (25 μM)
				42	11	70
40 c	OCH_3	H		32 (25 μM)	5 (25 μM)	98 (25 μM)
				57	11	98
41 a	OCH_3	H	H	44 (25 μM)	1 (25 μM)	88 (25 μM)
				64	12	93
42 b	H	OCH_3	OCH_3	55	12	41

[a] B_K N1E-115: blockade of peak steady-state K^+ currents of neuroblastoma cells N1E-115 normalized to the corresponding K^+ currents in normal bathing medium (pulse amplitude 40 mV, pulse duration 100 ms); B_{Na} N1E-115: accompanying blockade of peak Na^+ currents (pulse amplitude 0 mV); B_K Kv1.3: blockade of peak steady-state K^+ currents of Kv1.3 channels (pulse amplitude 40 mV, pulse duration 200 ms). Compounds were tested at 50 μM unless otherwise stated; blockades are given as mean of at least three experiments; nd, not determined.

Table 3. Half-Blocking Concentrations of Selected Furo- and Pyranoquinolines for Blockade of Kv1.3 Channels and N1E-115 Kv Currents[a]

	$IC_{50} \pm$ SEM (μM)		
compd	Kv1.3	N1E-115	$S \pm$ SEM
40 c	5 ± 1	38 ± 3	7.6 ± 2.2
39 e	18 ± 1	42 ± 3	2.3 ± 0.3
41 a	10 ±1	31 ± 4	3.1 ± 0.7
43 a	49 ± 7	200 ± 30	4.1 ± 1.2

[a] IC_{50} values are the mean of at least three experiments. S: selectivity of Kv1.3 current blockade over N1E-115 Kv channel blockade given by IC_{50}(N1E-115)/IC_{50}(Kv1.3).

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❖Anticancer activity

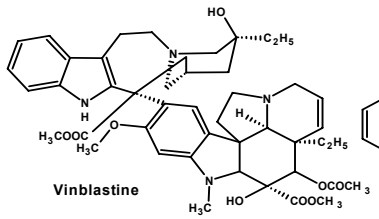
Ih-Sheng Chen, et. al[27b] have isolated pyranoquinolone alkaloids zanthosimuline (44) and huajiaosimuline (45) from the root bark of *Zanboxylum simulans*, exhibited cytotoxic activity. In addition, compound (45) showed significant antiplatelet aggregation activity and induced terminal differentiation with cultured HL-60 cells.

The cytotoxic activity of the new pyranoquinoline alkaloids, zanthosimuline (44)[27a] and huajiaosimuline (45)[27a] is summarized in Table-4. Compound (44) demonstrated a general cytotoxic response when evaluated with a variety of cultured human cancer cell lines and cultured P-388 cells using published methods of bioassay [66]. Multidrug-resistant KB-VI cells demonstrated a sensitivity that was approximately equivalent to that observed with cultured KB cells. In the presence of vinblastine, activity was enhanced about 3-fold with KB-VI cells, suggesting reversal of the drug resistance of the phenotype [67]. Conversely, oxidation of the side-chain produced a more selective profile of cytotoxic activity, as exhibited by compound (45). Of particular note, of the human tumor cell lines tested, greatest activity was observed with the estrogen receptor-positive breast cancer cells, ZR-75-1, and even more pronounced reversal of resistance to vinblastine was demonstrated with KB-VI cells.In addition, prompted by the structural similarity of the compound (44) and (45) side-chains and vitamin D metabolites, studies were performed with cultured HL-60 cells. As summarized in Table-5, both compounds were able to induce the expression ofcellular markers associated with cell differentiation, and compound (45) was more active than compound (44) in this capacity.

However, relative to the positive control compound, 1á,25-dihydroxyvitamin D_3 both compounds are regarded as weakly active.

Finally, zanthosimuline(44) *,* huajiaosimuline (45) *,* simulanoquinoline, and toddaquinoline were evaluated for antiplatelet aggregation activity [68]. The aggregation of rabbit platelets induced by arachidonic acid (100ì M), collagen (10 ì g/ml), or PAF (2 ng/ml) was inhibited by 100%, 83.9%, and

100%, respectively, in the presence of huajiaosimuline (45) at 100 ì g/ml. Compound (44), simulanoquinoline, and toddaquinoline were not active at this concentration.



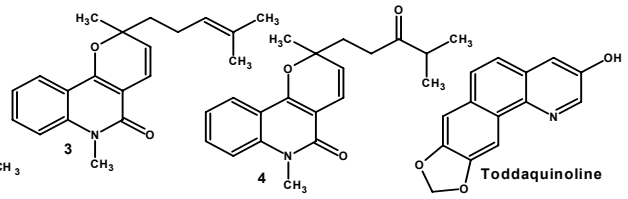


TABLE 4. Cytotoxic Activity of Compounds 44 and 45.

	Cancer cell line (ED_{50}, μM)						
Compound	BC-1	HT-1080	Lu1	Me1	Col2	KB	KB-V1 (+VLB)
44	28.8	28.8	28.8	28.4	30.4	15.2	6.5
45	>60	>60	>60	36.6	>60	46.4	4.0
	KB-V1 (−VLB)	P-388	A431	LNCaP	ZR-75-1	U373	
44	21.3	5.2	28.8	18.7	10.0	17.1	
45	24.3	9.8	>60	>60	11.1	>60	

Abbreviations: BC-1, human breast; HT-1080, human fibrosarcoma; Lu1, human lung; Mel, human melanoma; Col2, human colon; KB-V1, drug-resistant KB; VLB, vinblastine; A431, human squamous cell; LNCaP, human prostate; ZR-75-1, human breast; U373, human glioma.

TABLE 5 Induction of Cell Differentiation by Compounds 44 & 45 with Cultured HL-60 Cells.

Compound	% cell viability[1]	# of cells[2] ($X10^4$)	Conc. tested (μM)	Inhibition of [^{3}H] thymidine incorp. (%)	NBT reduction (%)	NSE activity (%)	SE activity (%)
44	9	1	64.6	Toxic	Toxic	Toxic	Toxic
	78	29	32.3	0.0±11.4	15.4±4.2	24.3±0.1	14.5±1.3
	93	51	16.2	0.0±10.2	9.0±1.0	7.4±0.3	6.5±0.3
	95	89	8.1	0.0±2.2	8.0±1.0	5.5±0.5	5.5±0.5
	98	143	4.0	0.0±0.5	3.5±0.5	4.5±0.5	5.0±0.0
45	85	16	61.5	31.6±1.8	53.1±6.0	48.9±2.9	5.0±1.0
	96	49	30.7	0.0±2.6	9.9±2.3	12.2±2.2	9.4±1.4
	98	94	15.4	0.0±9.0	8.0±2.0	5.5±0.5	8.0±0.9
	98	110	7.7	0.0±1.2	4.5±0.5	5.0±1.0	6.5±1.5
	97	106	3.8	0.0±3.8	5.5±0.5	5.0±0.0	5.5±0.5
$1\alpha,25(OH)_2D_3$	92	98	1	95.3±3.5	90.5±4.2	95.3±2.7	2.0±0.5
	95	125	0.1	92.0±3.0	80.0±1.3	90.1±0.6	2.0±0.0
	97	139	0.01	58.3±4.5	73.3±8.3	68.0±4.9	4.0±0.6
	99	145	0.001	32.3±8.0	28.5±2.9	14.0±0.9	4.6±0.5
	98	128	0.0001	6.0±0.7	8.8±5.7	2.5±0.7	5.0±0.8

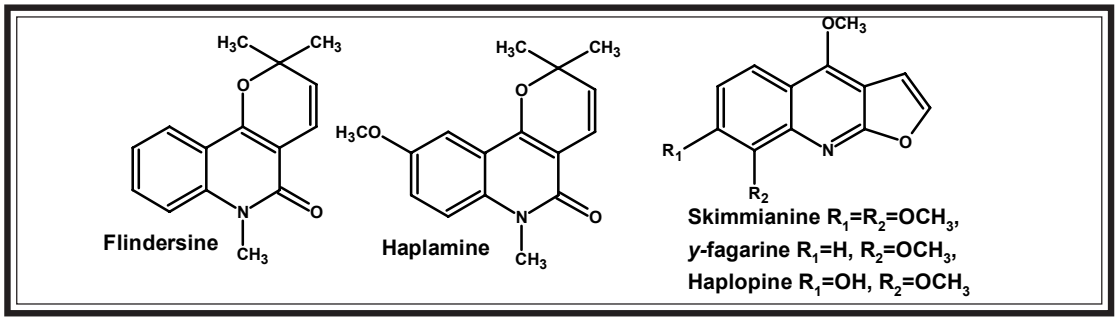
[1]The cell viability after 4 days of incubation with compounds was determined by trypan blue exclusion. [% cell viability = viable cells/(viable cells + dead cells) × 100].

[2]The starting cell number (day 0) was 20×10^4 cells/ml; the reported values are the number of cells observed after 4 days of incubation.

Olivia Jansen, et.al[69] during their systematic chemotaxonomic study of Uzbek *Haplophyllum* A. Juss. plants selected on ethnopharmacological data, have screened 14 alkaloids for their cyctotoxic properties. As a first selection for interesting compounds, each alkaloid was tested against two human cancer cell lines (HeLa and HCT-116), using WST-1 reagent.

Of the 14 alkaloids, 5 were cytotoxic when tested against the HeLa line with an IC_{50}<100ì M. These five compounds consisted of three furoquinolines: skimmianine; haplopine and γ-fagarine and two pyranoquinolones: flindersine and haplamine. Only haplamine was active against the HCT-116 line.

The cytotoxic properties of these five alkaloids were further investigated against five additional human cancer cell lines. Of these five pre-selected alkaloids, only haplamine showed significant cytotoxic activity against all the tested cell lines. This was the first report of the cytotoxic activity of haplamine. Finally, this pyranoquinolone alkaloid was tested here against 14 different cancer cell lines and against normal skin fibroblasts.



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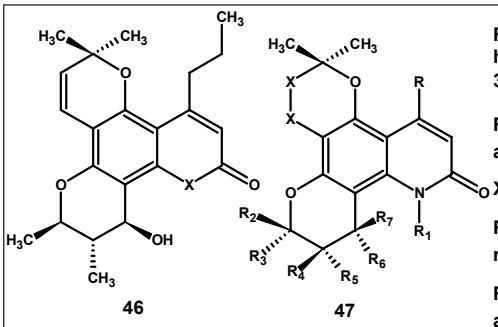
❖Anti-HIV activity

Antiviral nucleoside agents such as AZT, ddC, ddI, d_4 T and 3TC being Reverse Transcriptase (R.T.) inhibitors are approved for clinical use.Although these nucleoside based drugs can extend the life of the patient, they are associated with several side effects and are not capable of curing the disease. The urge for the promising RT inhibitors to cure AIDS, resulted in the identification of a group of coumarin derivatives isolated (Ref: M R Boyd et al, J. Med. Chem., 1992, 35, 2735-2743) from *genus Calophyllum* as HIV-1 specific non-nucleoside inhibitors, among which Calanolide A represented by (46, X=O) is the most potent and is currently undergoing clinical trials (phase III).



46

Calanolide, X = O

47

R = H, alkylC(1-10), alkenylC(1-10), alkynylC(1-10), aryl, heteroaryl, carbocyclic aryl, alkyl aryl, alicyclic aryl, 3°alkylamine, thioalkyl, hydroxy alkyl groups

R_1=H, lower dialkylamino alkyl or amino acid, hydroxyalkyl, acid amides such as aliphatic, aromatic sulphonic, trihalo acids

X-X = C-C or C=C

R_2 and R_3, R_4 and R_5 are each independently H, CH_3 there by resulting into diastereoisomers and enantiomers

R_6 and R_7 = are each independently H, OR_8, where R_8 = alkyl, alyl, aryl, amino alkyl, hydroxy alkylC(1-10), monosaccharides both furanose and pyranose, aminosugars, disaccharides, aminoacids, small peptides through lower alkyl spacer groups there by resulting into diastereoisomers and enantiomers

Calanolides, a ‘dipyrano-coumarin‘ class of compounds are active not only against the AZT-resistant strain of HIV-1, but also against virus strains resistant to some other non-nucleoside inhibitors such as TIBO pyridinone, and nevirapine. Calanolides are in the advanced stage of biological evaluation as anti-HIV agents in clinical trials (phase III).The main drawbacks of this class of compounds are (a) poor solubility of this class of compounds in the physiological medium and (b) lesser stability of the coumarin ring system in biological environment. It, thus, would be desirable to prepare the New Chemical Entities (NCEs) having calanolide skeleton but with better therapeutic index. In addition, the NCEs are desirable to overcome the problems associated with calanolides such as stability and solubility in the physiological medium.

Quinolinones are shown to be part structures of several bio-active compounds with profound bio-efficacy. Unlike the lactone bond in coumarins, the lactam bond in quinolinones is highly stable.

M.K.Gurjar, et al[70] have patented a novel 'dipyrano-quinolinone' class of compounds related to calanolide structural frame work as NCEs and envisaged to circumvent the problems associated with calanolides and have improved therapeutic indices.

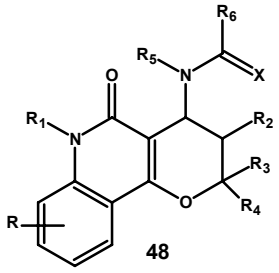
The invention deals with the synthesis of novel and new 'dipyrano-quinolinone' class of compounds, where the major differences in the structural arrangement is the replacement of coumarin ring oxygen (at position 1) of calanolide structure with nitrogen (at position 1) in the new 'quinolinone' ring system represented by (47). These quinolinone analogues of calanolides are NCEs and are envisaged as potential candidate molecules as anti-HIV agents. The rationale for the synthesis of these 'dipyrano-quinolinones' reported in this specification are as follows: (1) Replacement of oxygen (at position 1) of natural products, calanolides, with nitrogen leads to dipyrano-quinolinones as anti-HIV agents with better therapeutic index. (2) The inherent problems associated with naturally occurring calanolides such as metabolic stability and solubility in physiological medium can be circumvented with the new dipyrano-quinolinone derivatives that are reported in this patent. (3) The derivatisation of water-soluble derivatives of these new chemical entities represented in this patent are easily possible. (4) The metabolic stability is expected to enhance due to the presence of nitrogen atom in the skeleton of dipyrano-quinolinones derivative presented in this patent. (5) The structure activity relationship coupled with positive activity against calanolide resistant strain of HIV virus can be explored due to the presence of nitrogen atom in the dipyrano-quinolinone system.

The dipyrano-quinolone of the present invention screened for anti-HIV activity (reverse transcriptase inhibition), have shown potential therapeutic values

as “Candidate Molecules”. The above compound (46, X=NH) reported in this invention was shown equal potency of activity against HIV infected cell lines that observed for calanolide in the *in vitro* preliminary screening studies. The IC_{50}, EC_{50} and TI values of both calanolides and the ‘new dipyrano-quinolinone chemical entities‘ (46, X =NH) described in the present invention have equal and more protecting capacity against the HIV infection. The NCE prepared in this this invention has shown a mean therapeutic index value of 12 and indicative of superior activity for the new compound compared to the natural one.

❖As Calcium Channel Blockers

Karnail Atwal[71] have patented novel pyranyl quinolines (48) having calcium channel blocking activity.



48

X is oxygen or sulfur;
R is hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, haloalkyl, cycloalkyl, (cycloalkyl)alkyl, --NO_2, --CN, --CF_3, alkoxy or halo;
R_1 is hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, haloalkyl, cycloalkyl, (cycloalkyl)alkyl;
R_2 is hydrogen, hydroxy,-$OCOR_1$, where R_1 is not hydrogen;
R_3 and R_4 are independently selected from hydrogen, alkyl, arylalkyl or taken together with the carbon atom to which they are attached form a 5-, 6- or 7-membered ring;
R_5 is R_1 ;
R_6 is R_1, heteroaryl, amino, substituted amino or --OR_1, where R_1 ?hydrogen, or R_5 and R_6 taken together with the nitrogen atom to which they are attached for a 5-, 6- or 7-membered saturated or unsaturated ring which may contain an additional nitrogen atom or an oxygen or sulfur atom.

It was suggested that compounds (48) and their pharmaceutically acceptable salts are cardiovascular agents. They act as calcium entry blocking vasodilators and are useful as antihypertensive agents. Thus, by the administration of a composition containing one (or a combination) of the compounds of this invention, the blood pressure of a hypertensive mammalian (e.g., human) host is reduced. A single dose, or two to four divided daily doses, provided on a basis of about 0.1 to 100 milligrams per kilogram of body weight per day, preferably from about 1 to about 50 milligrams per kilogram per day, is appropriate to reduce blood pressure.

As a result of the calcium entry blocking activity of the compounds (48), and the pharmaceutically acceptable salts thereof, these compounds, in addition to being antihypertensive agents, are especially useful as anti-ischemic agents, and are also useful as anti-arrhythmic agents, anti-anginal agents, anti-fibrillatory agents, anti-asthmatic agents, as an agent to increase the ratio of HDL-cholesterol to total serum cholesterol in the blood and in limiting myocardial infarction.

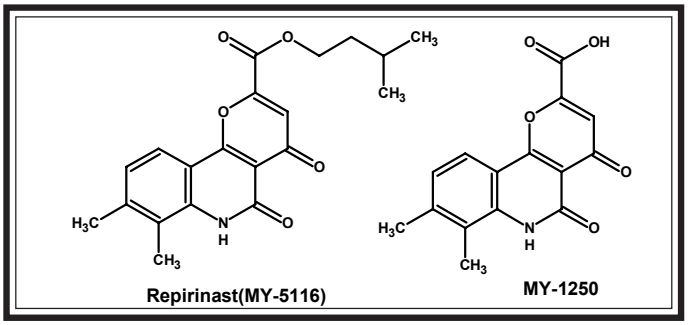
Additionally, the compounds of this invention are useful as therapy for congestive heart failure, therapy for peripheral vascular disease (e.g., Raynaud's disease), as anti-thrombotic agents, as anti-atherosclerotic agents, for treatment of cardiac hypertrophy (e.g., hypertrophic cardiomyopathy), for treatment of pulmonary hypertension, as an additive to cardioplegic solutions for cardiopulmonary bypasses and as an adjunct to thrombolytic therapy.

Compounds of this invention are also expected to be useful in the treatment of central nervous system vascular disorders, for example, as anti-stroke agents, anti-migraine agents, therapy for cerebral ischemia and therapy for subarachnoid hemorrhage, as well as in the treatment of central nervous system behavioral disorders, for example, in the treatment of psychiatric conditions including depression, mania, anxiety and schizophrenia, or for epilepsy or cognition benefit.

Further, compounds of this invention are expected to be used as anti-diarrheal agents, as therapy for dysmenorrhea, as therapy for tinnitus and other auditory and vestibulatory disorders, for the alleviation of the various forms of oedema, for reversal of adriamycin resistance, regulation of cell growth, for treatment of glaucoma, renal failure, hepatoxicity (e.g., liver cirrhosis), various endocrine hypersecretory states (e.g., diabetes, pheochromocytoma), drug-induced tardive dyskenesia, allergies, muscular dystrophy and cancer.

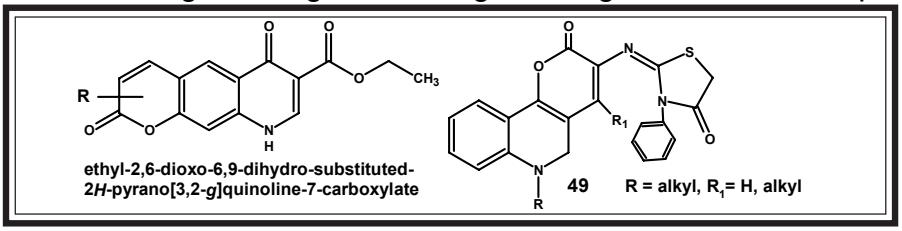
❖Antiallergic activity

Repirinast (MY-5116; isoarnyl 5,6-dihydro-7,8-diniethyl-4,5-dioxo-4H-pyrano[3,2-c]quino-line-2-carboxylate) is an anti-allergic drug of demonstrated effectiveness for treating bronchial asthma in humans. N.Yamada, et al[72] have demonstrated that MY-1250(5,6-dihydro-7,8-dimethyl-4,5-dioxo-4H-pyrano[3,2-c]quinoline-2-carboxylic acid), a major metabolite of repirinast, inhibits antigen induced histamine release from rat peritoneal mast cells. MY-1250 causes a rapid increase in cyclic adenosine monophosphate(cAMP) levels in rat peritoneal mast cells MY-1250 inhibited cyclic AMP phosphodiesterase activities from rat peritoneal cells and guinea pig lung tissue in a concentration dependent manner with IC_{50} value of 2 ì g/ml and 1.67 ì g/ml respectively. However MY-1250 showed no effect on adenylate cyclase activity from rat peritoneal cells. These results suggested that the inhibitory effect of MY-1250 on histamine release may be partly due to the inhibition of cyclic AMP phosphodiesterase activity.



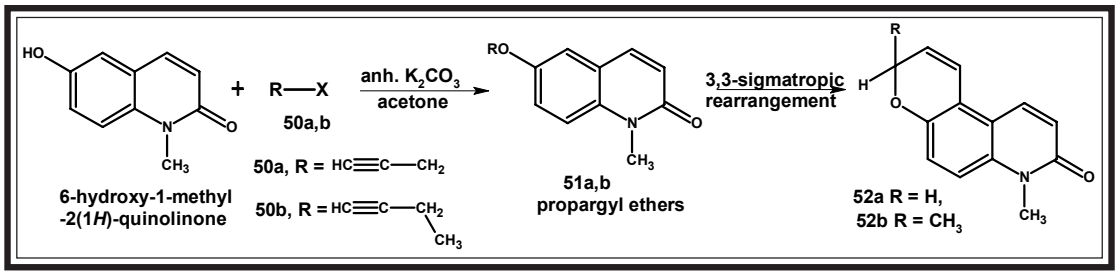
❖Antimicrobial activity

Trkovnik et al[73] have reported ethyl-2,6-dioxo-6,9-dihydro-substituted-2***H***-pyrano[3,2-g]quinoline-7-carboxylates and their antimicrobial activity against *S.aureus, P.aenrgiizosa, Streptococcus pyogens, Streptococcus faecalis, Staphiloc epiderms* etc. El-Taweel et al[74] have evaluated polysubstituted pyrano[3,2-c]quinolones(49) for their bactericidal activity against various gram negative and gram negative bacterial species.

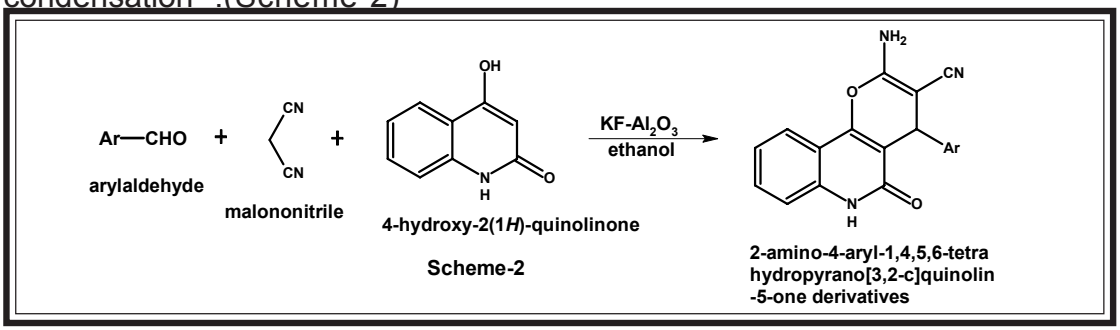


Synthetic Approaches

K.C.Majumdar et al[75] have synthesized pyrano[3,2-f]quinolin-2(7H)-ones (52a,b) by a thermal [3,3]-sigmatropic rearrangement with 60±65% yield by heating the propargyl ethers (51a,b) in refluxing N,N-dimethylaniline.(Scheme-1)



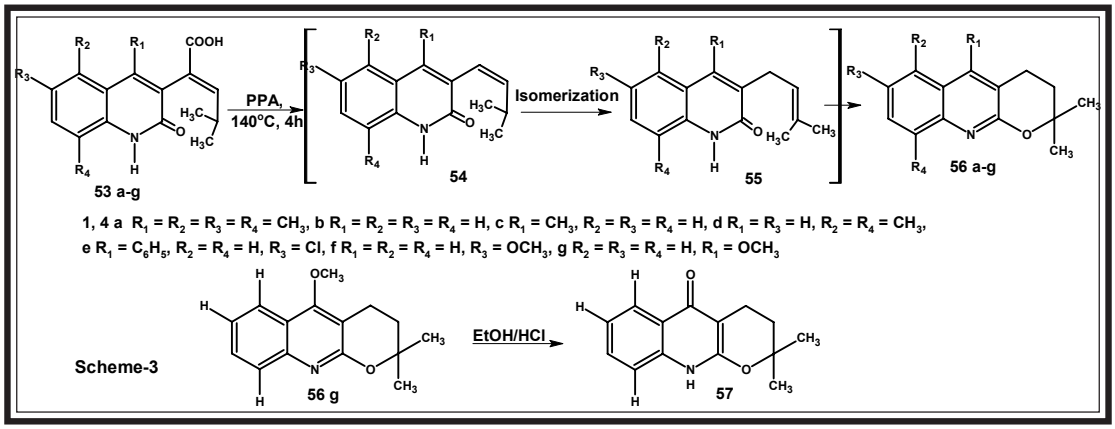
X.Wang et al[76] have described one-step synthesis of 2-amino-4-aryl-1,4,5,6-tetrahydropyrano[3,2-c]quinolin-5-one derivatives from arylaldehyde, malononitrile and 4-hydroxyquinolin-2-one by treatment with KF-Al_2O_3 as catalyst in refluxing ethyl alcohol. Prior to this investigation, the synthesis of pyrano[3,2-h]quinoline derivatives was reported[77] from arylmethylidene malononitriles and 8-hydroxyquinoline using KF-Al_2O_3 catalyst which was a versatile solid support reagent for many reactions such as Knoevenaegel condensation[78].(Scheme-2)



Scheme-2

The synthesis of the pyranoquinoline system is based on either oxidative cyclization of 4-hydroxy-3-(3'-methybut-1'-enyl)-2-quinolinones with 2,3-dichloro-5,6-dicyanoquinone(DDQ)[79] or the Prevost reaction of 3-prenyl-2-quinolones[80]. Though these methods have proven to be fairly satisfactory, the overall yield of the alkaloids was only 15-35% because the routes to obtain the precursor prenylquinolines gave low yields (21-35%)[81,82] and often were attended by undesired side reactions (such as the formation of unwanted 3-(3'-methylbut-1'-enyl)-2-quinolinones as the major product[82]). This note reports a facile novel one-pot synthesis of 3,4-dihydro-2,2-dimethyl-2*H*-pyrano[2,3-*b*]quinolines (56). khaplofoline (57) utilizing

3-(1'-carboxy-3'-methylbut-1'-enyl)-2-quinolinones[81] (53). M.Sekar and K.Rajendraprasad have suggested[85] that treatment of the carboxy derivative of the 2-quinolinone[81] (53a) with polyphosphoric acid (PPA) at 140 °C for 4 h furnished in 60% yield the desired 6-methyl-3,4-dihydro-2,2-dimethylpyrano[2,3-*b*]quinoline (56a), identical with an authentic sample[81] Extension of this method to compounds 53b-f gave the respective pyranoquinolines (56b-f). Analytical and spectroscopic data were consistent with the proposed structures (56) (Scheme-3). The formation of (56) from (53) can be assumed to proceed via the decarboxylated product (54) formed in situ under acidic condition at high temperature[84,85]. Compound (54) is isomerized to a more stable isoprenyl cation(55), which then cyclizes to product (56). A similar polyphosphoric acid reaction of 4-methoxy-2-ono-3-vinylquinolinecarboxylic acid (53g) yielded the quinoline derivative (56g). Compound (56g) was refluxed with 10% ethanolic hydrochloric acid for 30 h to give khaplofoline(57)



The condensation of â-ketoesters with phenolic compounds in the presence of sulfuric acid yields coumarin derivatives[85]. This synthesis is known as Pechmann condensation[86] and has extensive application to prepare many naturally occuring coumarins. Acidic catalyst such as aluminium

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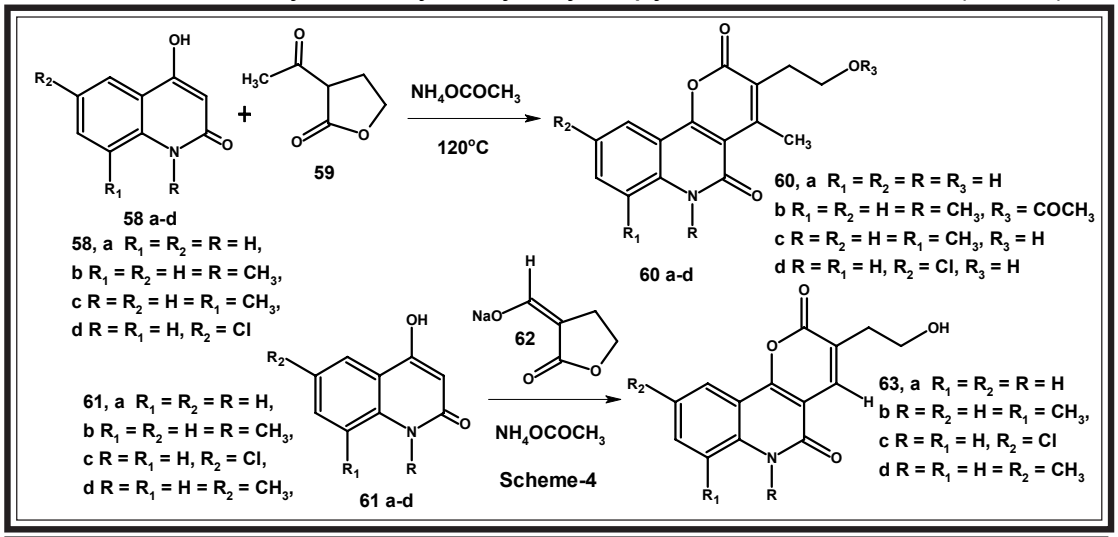
85.H. Pechmann, C. Duisberg, *Ber.*, **16**, 2119(1983)
86.S.Sethna, R.Phadke, *Org.React.*, **7**, 1(1953); Organic Name Reactions in the Merck index, S.Budavi, ed. Merck & Co., Whitehouse Station, N.J.,USA,12th Ed., ORN 67(1996)
87.C.Mayer, *Ph.D. Thesis*, K.-F. University of Graz, Austria(1971)
88.T. Kappe, C.Mayer, *Synthesis*, 524(1981)

trichloride, zinc chloride, phosphoryl oxychloride, phosphoric acid, polyphosphoric acid, trifluoro acetic acid and hydrochloric acid have been used. C.Mayer[87] and T.Kappe[88] have found that ammonium acetate can be used as a source of ammonia and that readily react with phenolic heterocyclic compounds to yield corresponding á-pyrone derivatives.

á-acetyl-*y*-butyrolactone(59), an interesting cyclic â-ketoesters can be used for the synthesis of heterocyles containing a hydroxyethyl side chain[89]. R. Toche, et al[90] have reported the pechmann reaction of 4-hydroxy-2(1H)-quinolones(58) with (59). Further more this reaction was extended to á-formyl-*y*-butyrolactone(used as its sodium salt (62)). (Scheme-4)

Thus the condensation of 4-hydroxy-2-quinolones(58a-d) with á-acetyl-*y*-butyrolactone (59)in the presence of ammonium acetate at 120°C gave pyrano[3,2-c]quinoline-2,5-diones(60a-d) in 70-75% yield, while sodium salt of á-formyl-*y*-butyrolactone(62)[91](obtained by formylation of *y*-butyrolactone) reacted smoothly with 4-hydroxy-2-quinolones(61) in the presence of ammonium acetate to yield 3-hydroxyethyl-2-pyranone derivatives (63a-d)

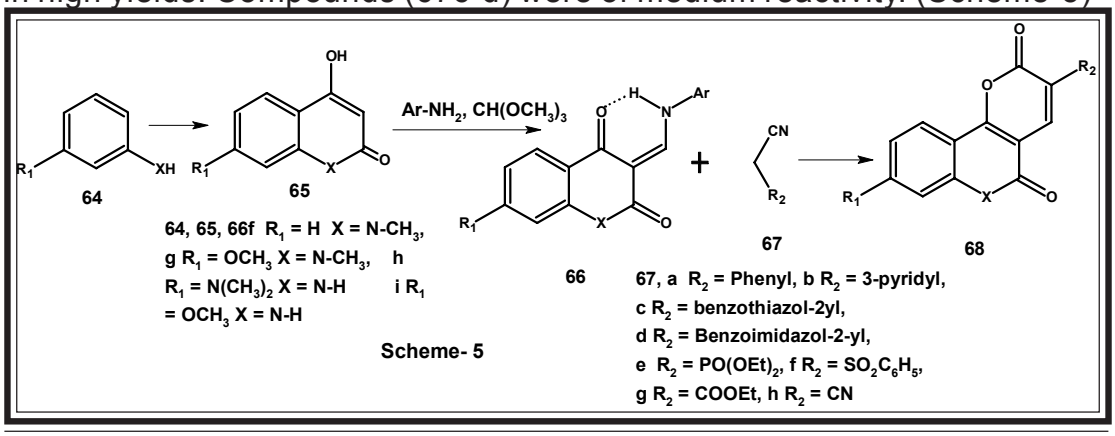


Scheme-4

89(a). E.Badawey, T.Kappe, *J. Heterocycl. Chem.*, **32**, 1003(1995)
(b).E.Badawey, *J. Heterocycl. Chem.*, **33**, 229(1996)
(c).E.Badawey, T.Kappe, *Eur. J. Med. Chem.*, **32**, 815(1997)
(d).E.Badawey, T.Kappe, *Arch. Pharma./Pharm. Med. Chem.*, **330**, 59(1997)
90.R.Toche, M.Jachak, R.Sabnis, T.Kappe, *J. Heterocycl. Chem.*, **36**, 467(1999)
91. A. Murray, N. Murray, *Synth. COmmun.*, **16**, 853(1986)
92.(a) W. Kayser, A. Reissert, *Ber.*, **25**, 1193(1892),
(b). H. Harnisch, A. Brach, *Ann. Chem.*, **740**, 164(1970)
93.E. Zeigler, K. Gelfert, *Monatsh. Chem.*, **90,** 822(1959)
94.T.Kappe, M.Chirazi, H. Stelzl, E.Zeigler, *Monatsh. Che.*, **103**, 586(1972)
95. A. Knierzinger, O. Wolfbeis, *J. Heterocycl. Chem.*, **17**, 225(1980)

2-quinolones (65g-i) were synthesized by several methods like (i) thermal cyclization of aniline with diethyl malonate at 180-200°C[92], (ii) condensation of aniline with diethylmalonate in the presence of phosphorous oxychloride[93], or (iii) condensation of anilines with bis-2,4-dichlorophenyl or bis-2,4,6-trichlorophenyl malonate[94]. A. Knierzinger and O. Wolfbeis[95] suggested that conversion of 2-quinolones(65g-i) into their 3-anilino methylene derivatives (66) was effected by heating (65) with an equimolar amount of and a 1.5 molar excess of triethoxy methane in glacial acetic acid or DMF.

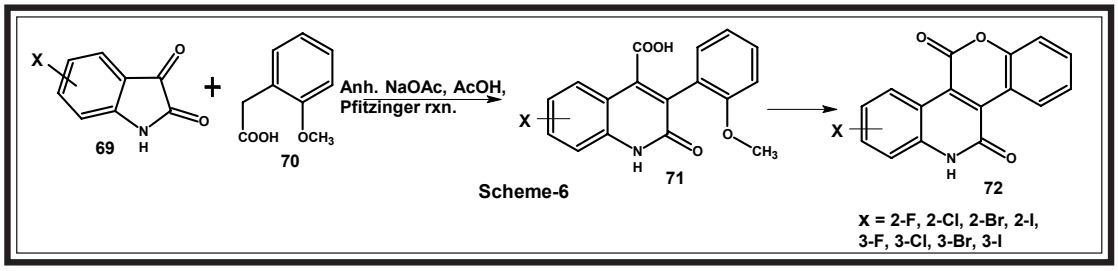
2-aminomethylene-1,3-diketones are synthetic equivalents to the less readily available and less stable 2-formaldehyde of 1,3-diketones. Thus when the enamine (66) was reacted with equimolar amounts of a nitrile(67) in the presence of strong base in DMF, aniline was replaced by nitrile anion and subsequent acidification affords the corresponding pyrones (68). The base chosen for must be strong enough to generate the anion of nitrile(67) but not so strong as to lead to reaction with enamine(66). Dry potassium hydroxide (potassium t-butoxide for less active nitriles 67a,b) was found to be a suitable reagent. Since the methylene hydrogens in (67a,b) are weekly acidic, their anions are increasingly reactive, causing side reactions which lower the yield of corresponding pyrones (68). On contrary, the pyrones formed by cyclizations with the strongly acidic (67f-h) were obtained in high yields. Compounds (67c-d) were of medium reactivity. (Scheme-5)



Scheme- 5

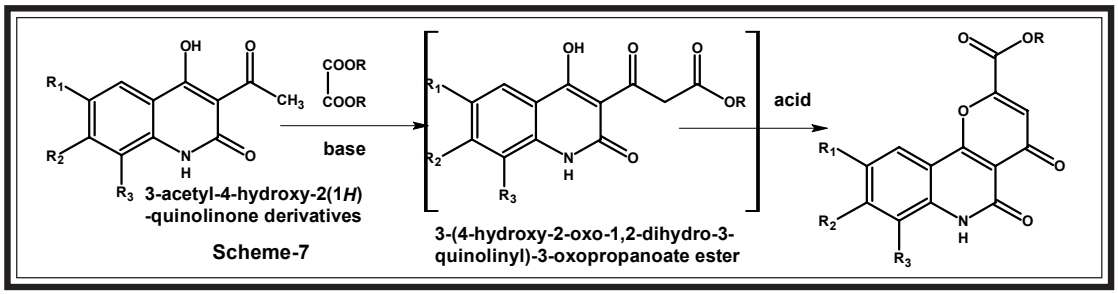
96.S. Yamaguchi, K. Tsuzuki, M. Kinoshita, Y. Oh-hira, Y. Kawase, *J. Heterocyclic. Chem.*, **26**, 281(1989)
97.Y.Morinaka, K. Takahashi,*US Patent*, **4298610**
98.J.Asherson, D.Young, *J. Chem. Soc.*, *Perkin Trans. 1*, 512(1980).
99.K.Majumdar, P. Choudhury, *Synth. Commun.*, **23**,1087(1993).

S.Yamaguchi et al[96] have synthesized eight number of 2- and 3-halo-11,12-dihydro-5H-[1]-benzopyrano[4,3-c]quinolin-5,11-dione(72) by demethyl cyclization of corresponding halo-3-(2-methoxyphenyl)-2-oxo-1,2-dihydroquinoline-4-carboxylic acids(71), which were synthesized by Pfitzinger condensation of 5- or 6-halo isatins(69) with (2-methoxyphenyl)acetic acid (70)(Scheme-6).



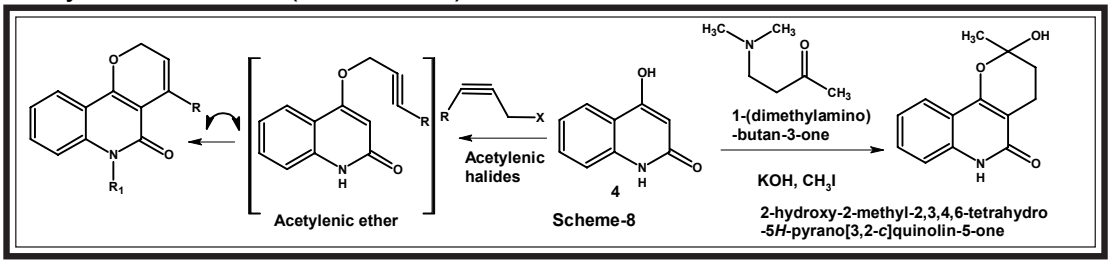
Scheme-6

Morinaka and Takahashi have patented[97] ester derivatives of pyrano[3,2-c]quinolin-2-carboxylic acid, synthesized by following route.(Scheme-7)



Scheme-7

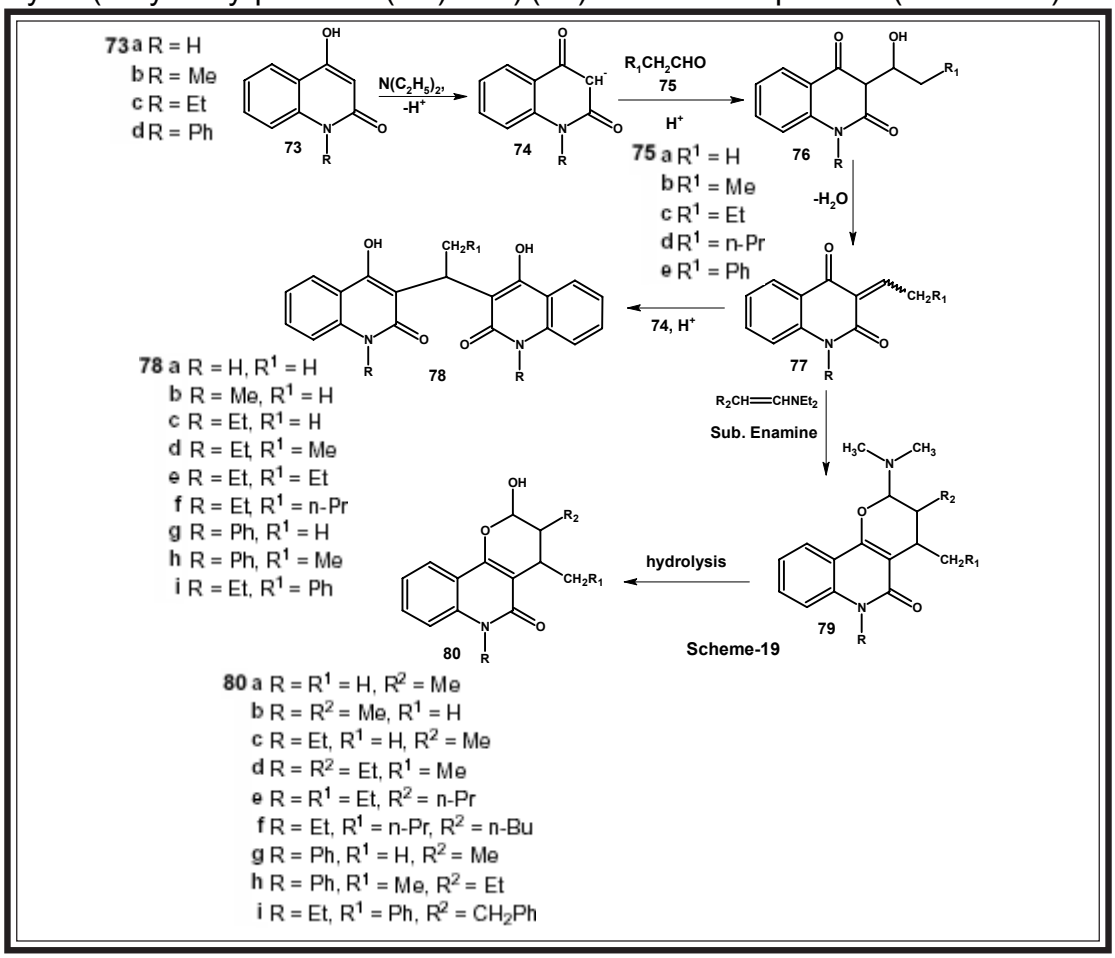
A couple of 2,3,4,6-tetrahydro-2-hydroxy-2-methylpyrano[3,2-*c*]quinolin-5-ones have been prepared by the reaction of 4-hydroxyquinolin-2(1*H*)-ones with 1-(dimethylamino)butan-3-one in the presence of potassium hydroxide and methyl iodide[98]. Some 6-alkyl-3,4-dihydropyrano[3,2-*c*]quinolin-5-ones were prepared by the reaction of 4-hydroxyquinolin-2(1*H*)-ones 4 with acetylenic halides and the subsequent intramolecular cyclization of the acetylenic ethers[99].(Scheme-8)



Scheme-8

Jai-Hai Ye et al[100] have reported convinient one pot synthesis of 2,3,4,6-Tetrahydro-2-hydroxypyrano[3,2-*c*]quinolin-5-one derivatives (80) from 4-hydroxyquinolin-2(1*H*)-ones(73) by tandem Knoevenagel condensation of (73)

with aliphatic aldehyde–Michael-type 1,4-addition of the enamine (derived from the aldehyde and diethylamine *in situ*) with the quinone methide (quinomethane) (77). This reaction sequence was achieved in one pot by direct thermal reaction of (73) with an aldehyde in the presence of diethylamine as a base in refluxing benzene, which afforded the corresponding 2,3,4,6-tetrahydropyrano-[3,2-*c*]quinolin-5-one (80) as the main product together with the 3,3'-alkane-1,1-diylbis(4-hydroxyquinolin-2(1*H*)-one) (78) as the minor product.(Scheme-9)



Scheme-19

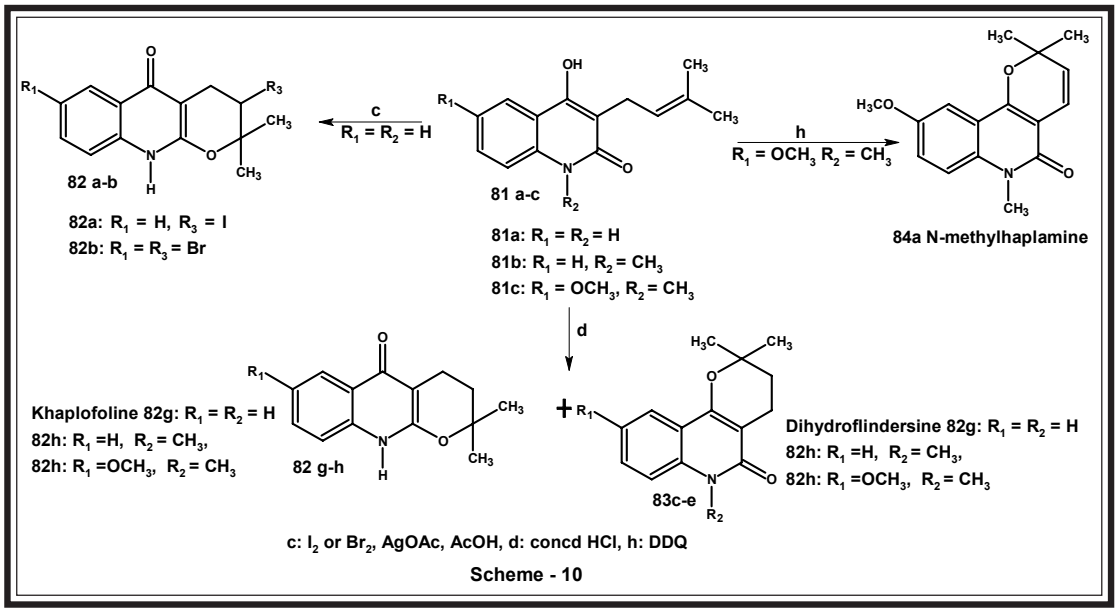
Two possible mechanisms could account for the formation of product (80) in these reactions. By analogy with reactions of 4-hydroxy-2-pyrone[101] with aliphatic aldehydes, which yield the corresponding quinone methide, base-catalyzed condensation of a quinolinone (73) with an aldehyde (75) yields the corresponding 4-hydroxy-3-(1-hydroxyethyl)quinolin-2-one (76), which is dehydrated on heating in the basic reaction medium to furnish the highly electrophilic quinone methide intermediate (77). The quinone methide (77) then undergoes competitive Michael addition at the exocyclic methylene

carbon by the preformed enamine (from DEA and the aldehyde) and the carbanion 7 derived from the deprotonation of (73). The Michael-type addition of the enamine proceeds in a 1,4-fashion and results in an intramolecular cyclization to give the 2-(diethylamino)pyrano-[3,2-*c*]quinolin-5-one (79), which on hydrolysis during the reaction at reflux temperature under the action of a trace amount of water in the reaction mixture, affords the final product (80). At the same time, addition of the carbanion 7 afforded the alkane-1,1-diylbisquinolone(78).

Alternatively, in the case of (80a–c) and (80g) from (73) and acetaldehyde, these syntheses were carried out by way of a photochemical variant by photolysis of a benzene solution of (73) as an electron acceptor and triethylamine as an electron donor, where acetaldehyde and diethylamine are generated *in situ* from triethylamine in redox processes initiated by single-electron transfer (SET) between photoexcited (73) and triethylamine[100].

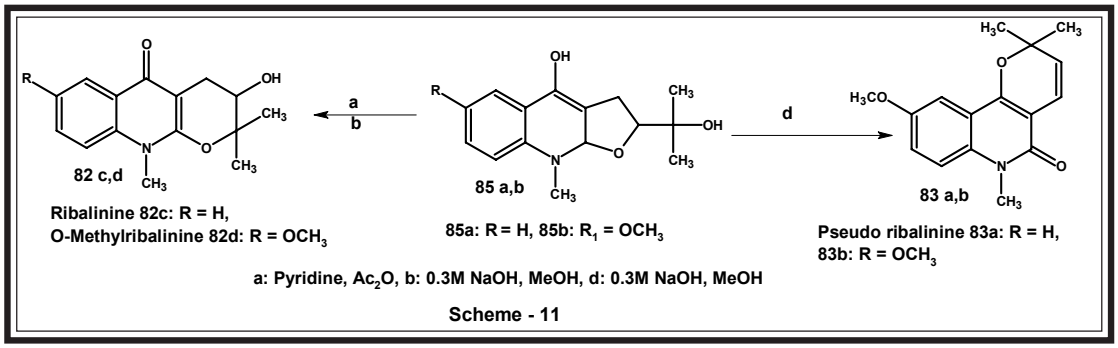
Inga Butenschon, et al[65] have synthesized Voltage-Gated potassium channel blocker pyranoquinolinones from central intermediates substituted 4-hydroxy-3-(3-methylbut-2-enyl)quinolin-2-ones[102-105], the strategy suggested that Oxidative cyclization of (81a) with iodine and silver acetate gave the linear dihydropyrano[2,3-*b*]quinolinone(82a)[106]. Use of bromine instead of iodine led to the formation of the main product the linear 3,7-dibromodihydropyranoquinolinone (82b). Mechanistically, the halogen is first added to the double bond of the prenylquinolinone, which then spontaneously undergoes intramolecular cyclization by an SN^1 reaction involving a tertiary carbonium ion. Thus, the cyclization proceeds via the electronically favored 6-*endo* process. Dihydropyranoquinolinones without substitution in position 3 are obtained via acid-catalyzed ring closure of 3-prenylquinolinones. Refluxing (81a-c) in concentrated hydrochloric acid resulted in mixtures of linear (82g-i) and angular (83c-e) dihydropyranoquinolinones, the later being the main products[109, 110]. *N*-Methylhaplamine (84a) was prepared via dehydrocyclization of (81c) with

DDQ(2,3-dichloro-5,6-dicyanobenzoquinone) according to the analogous synthesis of haplamine[111](Scheme 10).



Scheme - 10

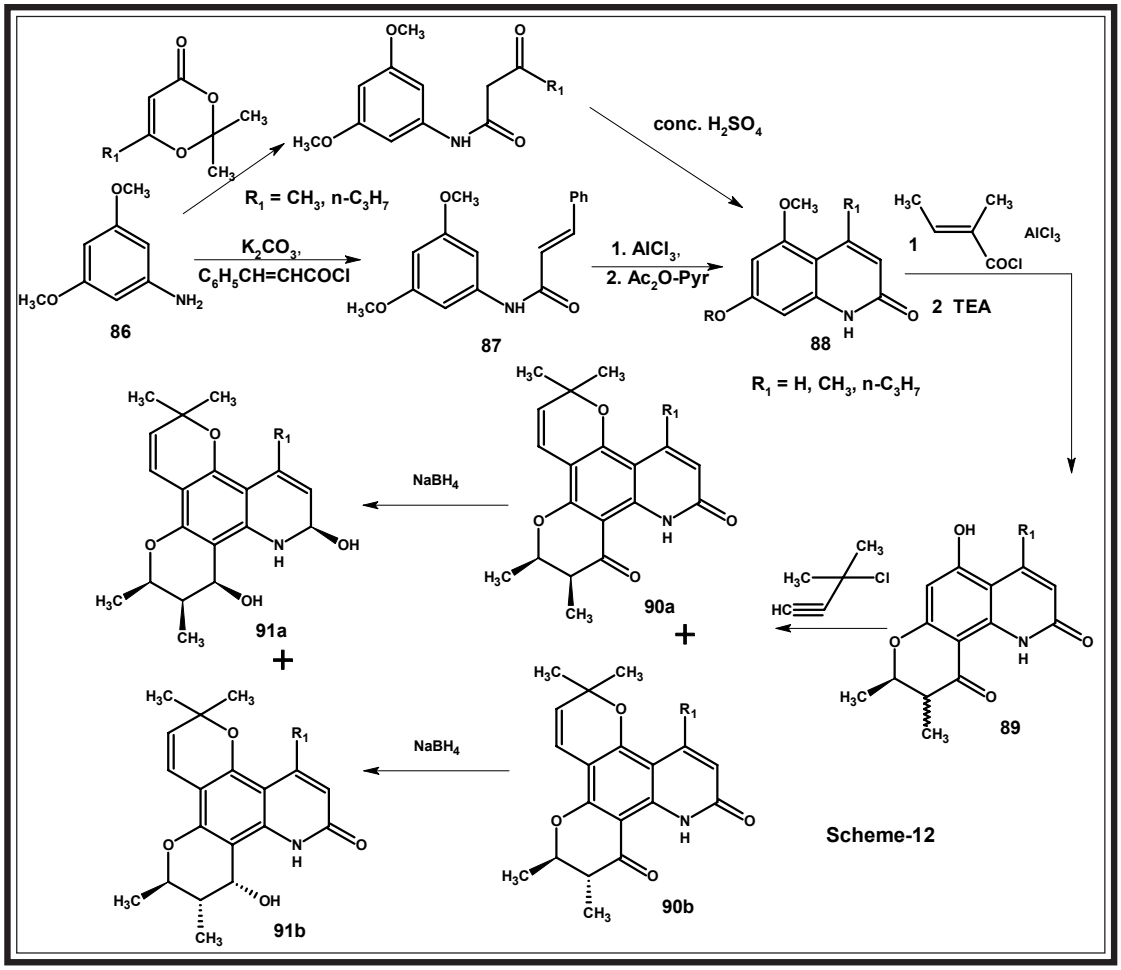
The reaction pathway with iodine and silver acetate failed in the case of the *N*-methyl-substituted adducts (81b,c). Thus, the isomeric linear dihydropyranoquinolinones (82c,d) to isoplatydesmine (85a) and *O*-methylriba-line (85b) were synthesized by rearrangement reaction of the dihydrofuroquinolinones in pyridine and acetic anhydride[107]. The corresponding angular dihydropyranoquinolinones were obtained from dihydrofuroquinolines(85a,b) via rearrangement reaction, when heated for 18 h under reflux with sodium hydroxide in methanol[108]. (Scheme-11)



Scheme - 11

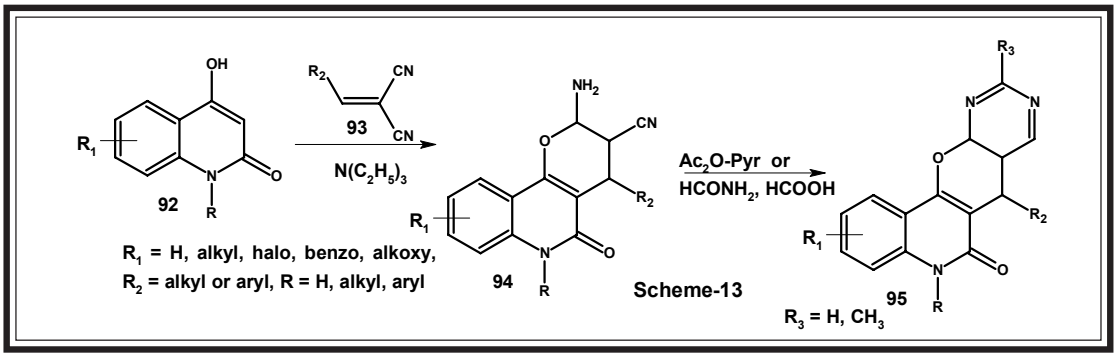
100.J. Ye, K. Ling, Y. Zhang, N. Li , J. Xu,*J. Chem. Soc., Perkin Trans. 1*, 2017(1999)
101.(a) P.March, M. Moreno-Manas, R. Pi, A.Trius, *J. Heterocycl. Chem.*,**19**,335(1982)
(b).P. March, M. Moreno-Manas, J. Casado, R. Pleixats, J. Roca, A. Trius, *J. Heterocycl. Chem.*, **21**, 85(1984)
(c).M. Moreno-Manas, E. Papell, R. Pleixats, J. Ribas , A. Virgili, *J. Heterocycl.Chem.*,**23**,413(1986)
102. D.Boulanger, B.Bailey, W.Steck, *Phytochemistry*,**12**, 2399(**1973)**.
103. E.Clarke, M.Grundon, *J. Chem.Soc.*, 438(**1964)**.
104. R.Corral, O.Orazi, I.Benages, *Tetrahedron*,**29**, 205(**1973)**.
105. J.Gaston, M.Grundon, *J. Chem. Soc. Perkin Trans.1,*,2294(**1980)**.

M. Gurjar et al[70] have synthesized dipyranoquinolinone class of compounds as a analogues of calanolide A (coumarin based natural product) and patented them as anti HIV agents by following synthetic routes(Scheme-12).

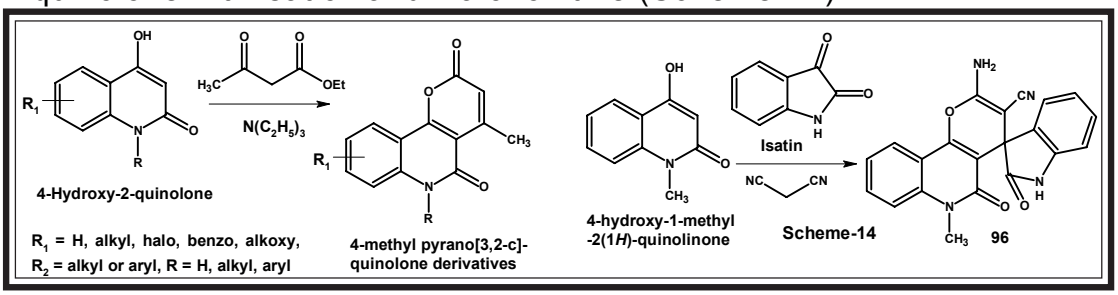


H. Abd el-Nabi[112] suggested that 4-Hydroxy-2-quinolone (92) reacts with cinnamonitrile derivatives(93) in presence of catalytic amounts of triethylamine to afford pyrano[3,2-c]quinolines (94). The reaction of (94) with reagents such as acetic anhydride/pyridine, formamide and formic acid/ formamide gave the fused heterotetracyclic system of the type of pyrimido[4',5':6,5]pyrano[3,2-c]quinoline (95).(Scheme-13)

106. M.Subramanian, P.Mohan, P.Shanmugam, K.Prasad, *Naturforsch.***47b**, 1016(**1992)**.
107.H.Rapoport, K.Holden, *J. Am. Chem.Soc.* **82**, 4395(**1960)**.
108.K.James, M.Grundon, *J. Chem. Soc. Perkin Trans. 1,* 1467(**1979)**.
109. E.Clarke, M.Grundon, *J. Chem. Soc.*, 4190(**1964)**.
110. R.Bowman, M.Grundon, *J. Chem. Soc. C*, 1084(**1966)**.
111. P.Venturella, A.Bellino, F.Piozzi, *Heterocycles* , ***3***, 367(**1975)**.
112. H. Abd el-Nabi, *Pharmazie*, **52**(1), 28(1997)
113. N. Venkateshkumar, S. Rajendran, *Heterocycl. commun.*, **10**(4-5), 289(2004)
114. V.Mulwad, M.Lohar, *Ind. J. Heterocycl. Chem.*, **12**(1), 57(2002)



N. Venkatesh Kumar and S. Rajendran[113] have described that 4-hydroxy-2-quinolone being endowed with both nucleophilic and electrophilic properties furnishes the dimeric quinoline in a base catalyzed self-condensation process. A one step synthesis, starting from 4-hydroxy-2-quinolone with ethyl acetoacetate and pyridine proceeded through the Michael addition, which was followed by cyclization, gave an angular isomer 4-methylpyrano[3,2-c]quinolin-2,5[6H]-dione(Scheme-14). V. Mulwad and M. Lohar[115] have synthesized novel spiro [3H-indole-3,7' -(9' -amino-8' -cyano-[7'H]-pyrano- [3,2-c]-quinolin)]-2,6' -[1H]-diones (96) by the reaction of N-methyl-4-hydroxy-2-quinolone with isation and malononitrile.(Scheme-14)



115.A.Shah, N.Bhatt, R.Raval, V.M.Thakor, *Curr. Sci.*, **53**, 1289(1984)
116. V.Shah, J.Bose, R.Shah, *J.Sci.Industr.Res.*, **19B**, 176(1960)
117. N.Dodia, *Ph.D.Thesis*, Saurashtra University(2000)
118. H.Acharya, *Ph.D.Thesis*, Suarashtra University(2005)
119. A.Mishra, *Ph.D.Thesis*, Suarashtra University(2005)
120. D.Manvar, *Ph.D.Thesis*, Suarashtra University(2005)
121.R.Bowman, A.Campbell, E.Tanner, *J.Chem.Soc.*, 444(1959)
122. J.Bossen, M.Rasmussen, E. Ritchie, A.Robertson, W.Taylor, *Aust.J.Chem.*, **16**, 480(1963)
123. Vogel's Textbook of Practical Organic Chemistry, Longman singapore publication Pvt.Ltd.,(1948)
124. Aldrich advancing science (2005-2006)
126.T.Kappe, R. Aigner, P.hohengaxner, W. Stadlbamer , *J.Prakt.Chem.,* **336**, 596)(1994)

Work Done at Our Laboratory

The reaction of phenols with malonic acid in the presence of an admixture of condensing agents zinc chloride and phosphorous oxychloride giving yields of 4-hydroxy coumarins[115] more over the specific condensing action of this admixture have also been observed[116].

From the begining of our ongoing research on studies of 2,4-dihydroxyquinoline derivatives and their analogues, Dodia[117] has extending this reaction with primary aromatic amines and found that with malonic acid and admixture of the condensing agents zinc chloride and phosphorous oxychloride at temperature suitably between 60°C to 100°C to give fairly good yields of 2,4-dihydroxyquinolines.

By using 2,4-dihydroxyquinolines as a central intermediate a good number of substituted fused and dimeric analogues have been prepared, which includes 3-substituted-2,4-dihydroxyquinolines, pyrano[3,2-c]quinolines, pyrimido[5',4':5,6]pyrano[3,2-c]quinolines and dimeric 2,4-dihydroxy quinoline derivatives and screened them for various biological activity. A number of 2,4-dihydroxyquinoline analogues have shown potent activity as anti-HIV, antitubercular, antimicrobial and antimalarial activity.

Acharya[118], Mishra[119] and Manvar[120] have extended the analogue making with the condensation of N-methylaniline, N-ethylaniline and N-benzyl anilines with diethylmalonates in 1:2 mole proportion to afford pyrano[3,2-c]quinolones which further reacted with cinnamonitrile derivatives to yield pyrano[2',3':4,5]pyrano[3,2-c]quinolone derivatives.

The current work encompasses with N-methyl and N-benzyl substitution of quinoline ring so as to study the changes in the biological activity. The current work presents a direct, efficient and operationally convenient approach to the synthesis of some novel Pyranoquinolone derivatives.

In the present Chapter Micheal addition and cyclocondensation reaction between N-methyl-2,4-dihydroxyquinoline and cinnamonitrile derivatives of aromatic and heteroaromatic aldehydes were studied.

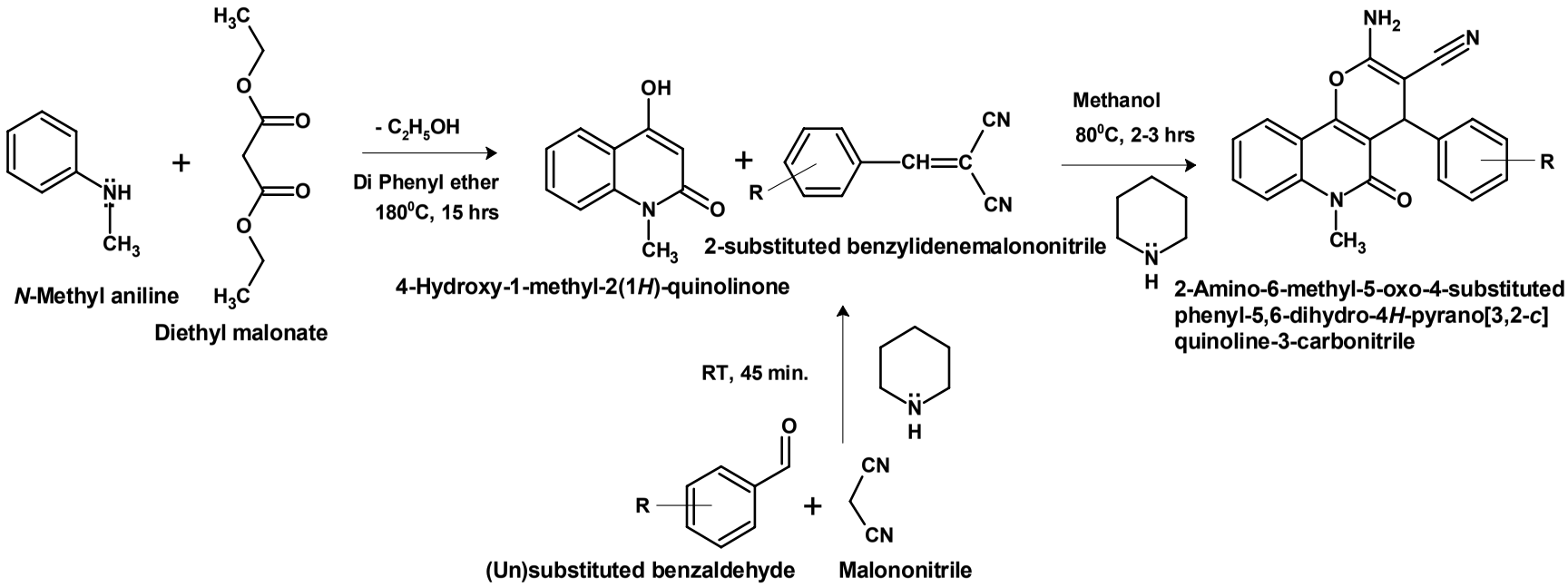
In another approach, the study of the reaction between secondary aromatic amine and malonic acid derivatives was elaborated with thermal condensation of one mole of N-benzylaniline with two moles of diethyl malonate at 220°C and was found to afford4-hydroxy-6-benzyl-5,6-dihydro-2,5-dioxo-2H-pyrano[3,2-c]quinoline[121,122].

When later was allowed to undergo Micheal addition followed by cyclocondensation with cinnamonitrile derivatives of aromatic and heteroaromatic aldehydes having various functional groups of different electronegativity and steric geometry , afforded the product was characterized to be 2-amino-6-benzyl-5-oxo-4-phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile derivatives on the basis of IR, NMR and Mass spectral and elemental analysis.

The final compounds 2-amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles(KUQ series) and 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles(KUQP series) synthezised in three and four steps respectively, as described in experimental part. For synthesis of 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile(KUQP series), it is important to mention that when 6-benzyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione treated with 2-substituted benzylidenemalononitriles under alkaline condition, instead of resulting into 3-amino-6-benzyl-5,12-dioxo-1-substituted phenyl-6,12-dihydro-1*H*,5*H*-pyrano[2',3':4,5]pyrano[3,2-*c*]quinoline-2-carbonitrile, pyran lacton undergoes hydrolytic ring opening along with subsequent decarboxylation to yield 2-amino-6-benzyl-5-oxo-4-substitutedphenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile, which were finally characterized by FT-IR, NMR and Mass Spectral and elemental analysis.

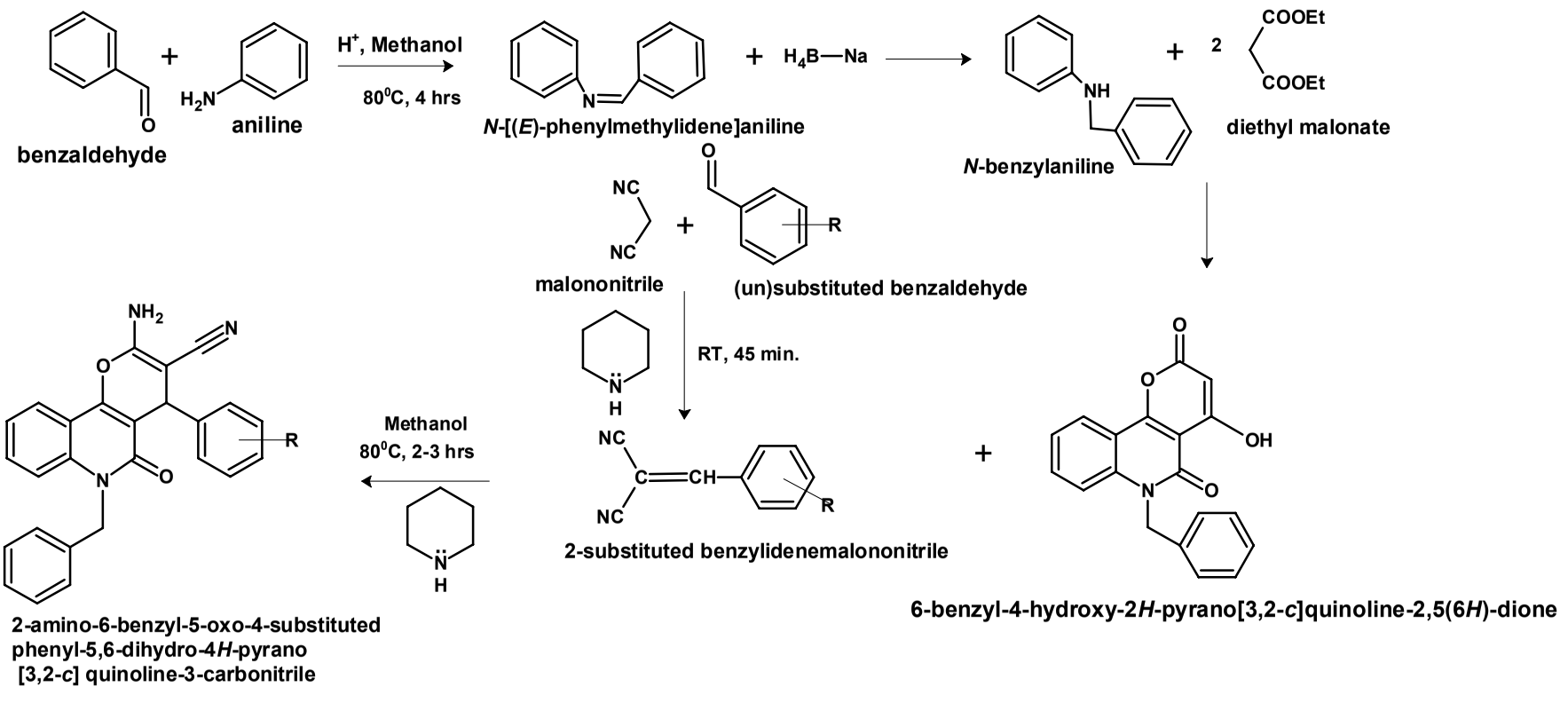
All the synthesized compounds were screened for different biological activities.

Reaction Scheme-1

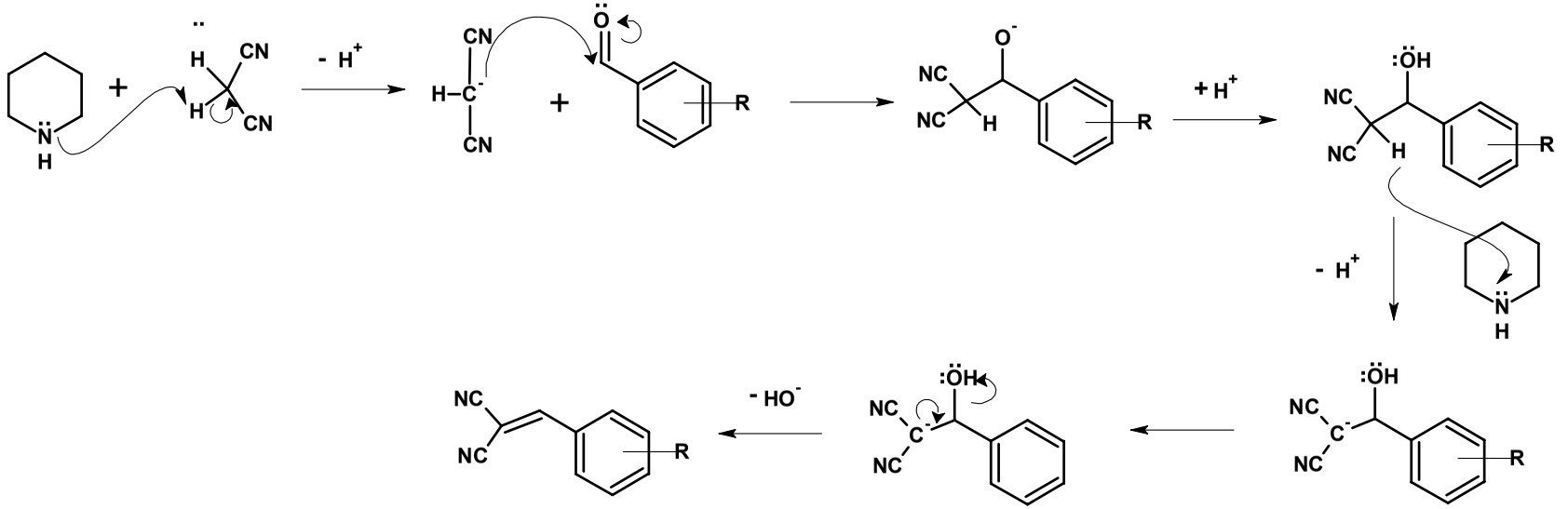


R = Various electron donating or electron withdrawing groups

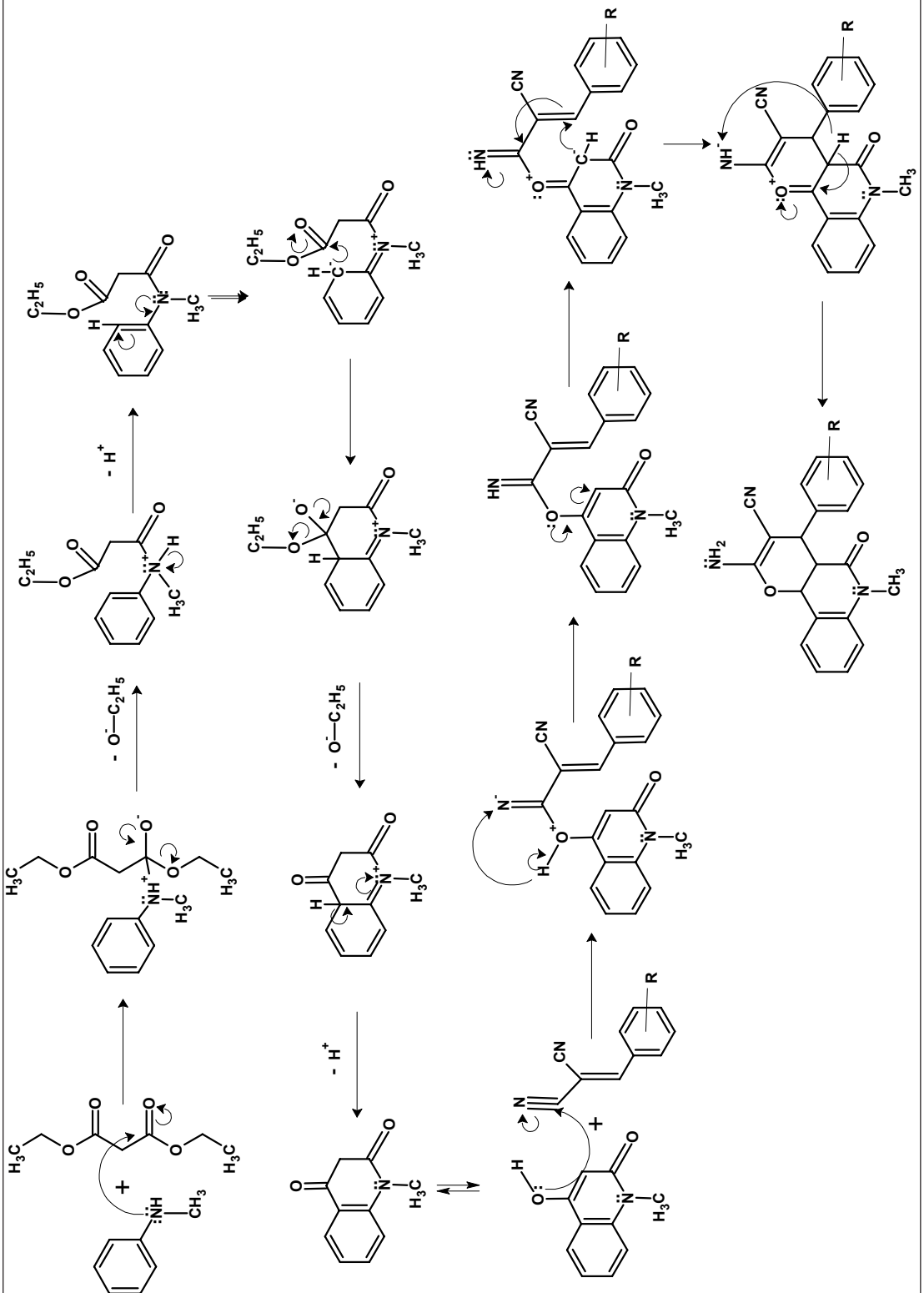
Reaction Scheme-2



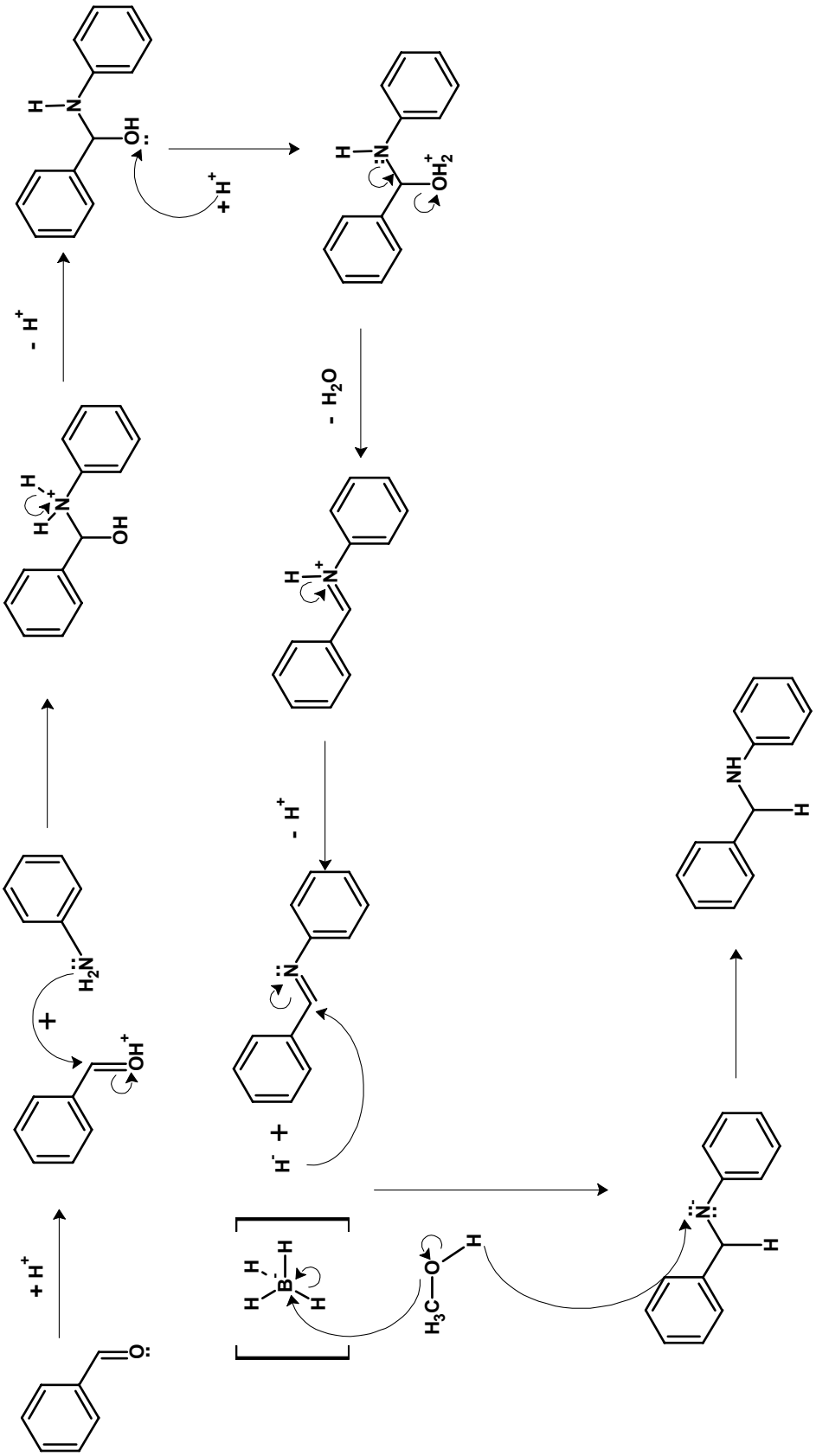
Reaction Mechanism

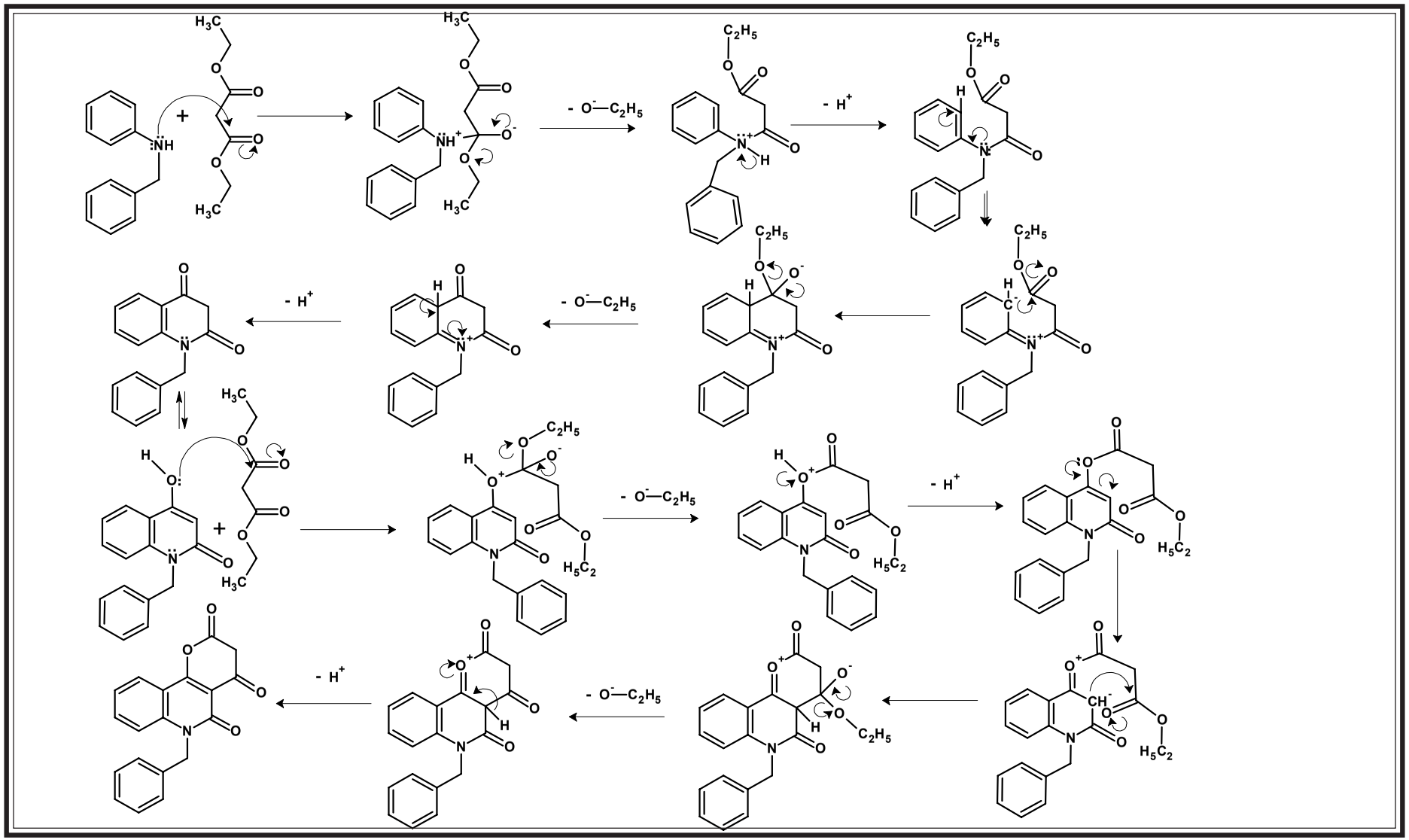


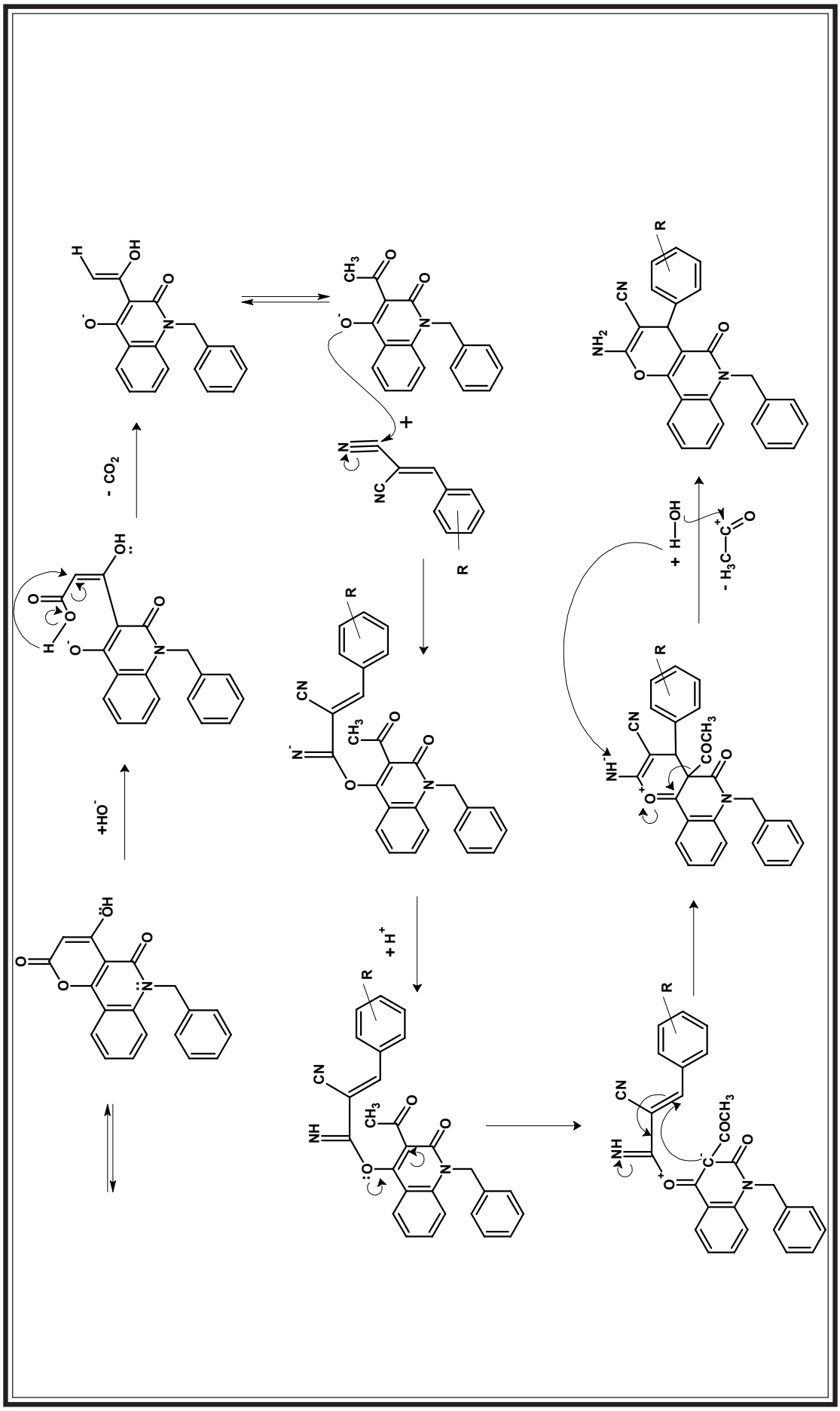
Reaction Mechanism (Scheme -1)



Reaction Mechanism (Scheme-2)




- O⁻—C₂H₅
- H⁺
- O⁻—C₂H₅
- H⁺
- O⁻—C₂H₅
- H⁺
- O⁻—C₂H₅
- H⁺



Experimental Protocols

All the starting materials, N-methylaniline, diethyl malonate, malononitrile and substituted benzaldehydes were obtained from the commercial sources commercial sources namely Spectrochem Ltd., s.d.fine chem. ltd., Sisco Research Lab., Ranbaxy Lab., Allied Chem. Ltd., etc. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature (using standard Zeal's thermometer) and are uncorrected. The commencement of the reaction and purity of the synthesized compounds were ensured using thin layer chromatography (TLC) silica gel-G, used as stationary phase, the TLC plates were purchased from Merck India Ltd. The ethyl acetate:hexane solvent system was used as the mobile phase. However other solvent systems like acetone:benzene and chloroform:methanol were also employed, but the best results observed with ethyl acetate:hexane system.

Experimental (Scheme - 1)

Synthesis of 4-hydroxy-1-methyl-2(1*H*)-quinolinone :

In 250ml RBF N-methyl aniline(0.1 mole), anhydrous malonic acid(0.11 mole), anhydrous zinc chloride(30gms) and phosphorous oxy chloride (40ml) were thoroughly mixed together. The content was heated on a water bath till the reaction mass become viscous. The reaction mass was poured into crushed ice. The crude product was filtered and thoroughly washed with water till become acid free. The crude product was treated with 30%NaOH solution and filter ed. The filterate was acidified with 30%HCl solution to give 4-hydroxy-1-methyl-2(1*H*)-quinolinone, which was filtered and dried and recrystallized from methanol. Yield 75%, m.p. 258-262^{o}C (Reported m.p. 256^{o}C[95]). Purity was checked by TLC using ethyl acetate:hexane(4:6) as mobile phase.

Synthesis of 2-amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (General Method) :

A substituted aromatic or heteroaromatic aldehyde(0.01 mole) was dissolved in minimum quantity of methanol and malanonitrile(0.03 mole) was added and mixed thoroughly. Catalytic amount of piperidine or triethyl amine was added and the reaction mixture was stirred at room temperature to afford crude 2-benzelidinemalononitriles. The crude product was crystallized from methanol. Yield 87-93%. Purity of each was checked by TLC using ethyl acetate:hexane (3:6) as mobile phase.

4-hydroxy-1-methyl-2(1*H*)-quinolinone(0.01mole) and 2-benzylidene malononitrile(0.01 mole) were dissolved in N,N-dimethyl formamide(17ml) along with catalytic amount of piperidine or triethyl amine. The reaction mixture was refluxed in a water bath for 1hour. The reaction mixture was cooled gradually to room temperature, to afford 2-amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles. The crystallized product was filtered and throughly washed with chilled N,N-dimethylformamide and recrystallized from hot N,N-dimethylformamide. The purity of each compound was check by TLC using ethyl acetate:hexane (5:5) as mobile phase.

The physical constants of the synthesized compounds are shown in **Table 6.1.**

Experimental (Scheme - 2)

Synthesis of N-Benzylidene aniline:

In a 100ml round bottomed flask fitted with a reflux condenser, a mixture of benzaldehyde(0.05 mole), aniline(0.05 mole) and rectified spirit(20ml) was refluxed in a water bath for 20minutes. A portion of water was added until a slight cloudiness persists and the solution was allowed to set on one side to cool. The oil which seperated may be induced to crystallise by rubbing with a glass rod. The solid deposited was collect by filteration and washed well with cold aqueous ethanol. Crude Schiff base obtained was recrystallized from small portion of aqueous methanol to give light yellow crystalls. Yield 88%, m.p. 52-54^{o}C (Reported m.p. 52^{o}C[123]). Purity was checked by TLC using ethyl acetate:hexane (5:5) as mobile phase.

Synthesis of N-Benzyl aniline:

A solution of N-Benzylidene aniline(0.044mole) in methanol(100ml) was warmed to about 40^{o}C and over a period of 30 minutes sodium borohydride(0.044mole) was added portionwise with stirring ; which led to a steady evolution of hydrogen gas. The solution was heated under reflux for 15 minutes, and the reaction mixture was poured into crushed ice. The solid amine deposited was collected by filteration and recrystallized from aqueous methanol. Yield 90%,m.p.36-38^{o}C, b.p.306-307^{o} C(Reported m.p. 35-38 ^{o}C[124], b.p.307^{o} C[124]). Purity was checked by TLC using ethyl acetate:hexane (5:5) as mobile phase.

Synthesis of 6-Benzyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione

A mixture of N-Benzyl aniline(1.0 mole) and diethyl malonate(2.0 mole) was heated in an oil bath equipped with a distilation apparatus. At about 160^{o}C liberation of ethanol took place and the temperature was increased slowly to 220^{o} C, where it was kept until no more ethanol was formed. Then the reaction mixture was allowed to cool to room temperature by remaining in the oil bath over night. The precipitate was filtered and washed with xylene or dioxane. Crystallized from toluene.Yield 57%, m.p. 238-240^{o}C. (Reported m.p. was 239^{o} C[126]). Purity was checked by TLC using ethyl acetate:hexane(7:3) as mobile phase.

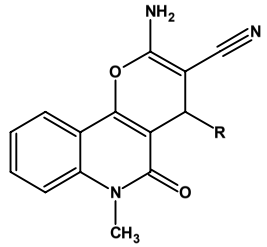
Synthesis of 2-Amino-6-benzyl-5-oxo-4-sub.phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (General Method) :

A mixture of 6-benzyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione (0.01mole) and 2-benzylidenemalononitrile(0.01 mole) in N,N-dimethyl formamide(17ml) along with catalytic amount of piperidine or triethyl amine was refluxed in a water bath for 1hr. After completion of reaction the reaction mixture was gradually cooled to room temperature, to afford 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles. The crystallized product was filtered and throughly washed with chilled N,N-dimethylformamide and recrystallized from hot N,N-dimethylformamide. The purity of each compound was checked by TLC using ethyl acetate:hexane (5:5) as mobile phase.

The elemental analysis of compounds performed by **PERKIN ELMER 470R** are in total agreement with the theoritical values.

The physical constants and elemental analysis of all compounds were shown in **Table no. 6.2**

Table 6.1 : Physical constants of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
1	KUQ-1	Phenyl	$C_{20}H_{15}N_3O_2$	329.3	153-155	72.94 72.92	4.59 4.58	12.76 12.77
2	KUQ-2	2-Cl Phenyl	$C_{20}H_{14}ClN_3O_2$	363.8	248-251	66.03 66.05	3.88 3.87	11.55 11.54
3	KUQ-3	3-Cl Phenyl	$C_{20}H_{14}ClN_3O_2$	363.8	267-270	66.03 66.05	3.88 3.87	11.55 11.54
4	KUQ-4	4-Cl Phenyl	$C_{20}H_{14}ClN_3O_2$	363.8	138-140	66.03 66.05	3.88 3.87	11.55 11.54
5	KUQ-5	2-NO_2 Phenyl	$C_{20}H_{14}N_4O_4$	374.3	262-264	64.17 64.19	3.77 3.76	14.97 14.99

Note:- The underline values denote the calculated percentage of composition

Table 6.1 : Physical constants of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C,H,N%)		
						C	H	N
6	KUQ-6	3-NO_2 Phenyl	$C_{20}H_{14}N_4O_4$	374.3	190-193	64.17	3.77	14.97
						64.19	3.76	14.99
7	KUQ-7	4-NO_2 Phenyl	$C_{20}H_{14}N_4O_4$	374.3	259-260	64.17	3.77	14.97
						64.19	3.76	14.99
8	KUQ-8	4-F Phenyl	$C_{20}H_{14}FN_3O_2$	347.3	88-89	69.16	4.06	12.10
						69.19	4.07	12.13
9	KUQ-9	3-Br Phenyl	$C_{20}H_{14}BrN_3O_2$	408.2	275-277	58.84	3.46	10.29
						58.83	3,47	10.27
10	KUQ-10	3-OC_6H_5 Phenyl	$C_{26}H_{19}N_3O_3$	421.4	263-264	74.10	4.54	9.97
						74.13	4.55	9.95
11	KUQ-11	4-SCH_3 Phenyl	$C_{21}H_{17}N_3O_2S$	375.4	249-252	67.18	4.56	11.19
						67.15	4.57	11.17
12	KUQ-13	3-OH Phenyl	$C_{20}H_{15}N_3O_3$	345.3	283-285	69.56	4.38	12.17
						69.53	4.37	12.19
13	KUQ-14	4-OH Phenyl	$C_{20}H_{15}N_3O_3$	345.3	129-132	69.56	4.38	12.17
						69.53	4.37	12.19
14	KUQ-15	4-N,N,DiCH_3 Phenyl	$C_{22}H_{20}N_4O_2$	372.4	278-280	70.95	5.41	15.04
						70.93	5.39	15.06

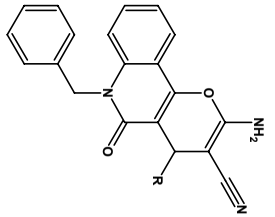
Note:- The underline values denote the calculated percentage of composition

Table 6.1 : Physical constants of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C,H,N%)		
						C	H	N
15	KUQ-16	2-OCH_3 Phenyl	$C_{21}H_{17}N_3O_3$	359.4	160-161	70.18 70.15	4.77 4.75	11.69 11.67
16	KUQ-17	4-OCH_3 Phenyl	$C_{21}H_{17}N_3O_3$	359.4	239-242	70.18 70.15	4.77 4.75	11.69 11.67
17	KUQ-18	3,4,5-TriOCH_3 Phenyl	$C_{23}H_{21}N_3O_5$	419.4	146-148	65.86 65.89	5.05 5.03	10.02 10.04
18	KUQ-19	3,4-DiOCH_3 Phenyl	$C_{22}H_{19}N_3O_4$	389.4	251-253	67.86 67.84	4.92 4.9	10.79 10.81
19	KUQ-21	9-Anthracyl	$C_{28}H_{19}N_3O_2$	429.5	236-239	77.94 77.92	4.91 4.9	9.74 9.72
20	KUQ-24	3-OC_2H_5, 4-OH Phenyl	$C_{22}H_{19}N_3O_4$	389.4	203-205	67.86 67.84	4.92 4.93	10.79 10.82
21	KUQ-25	3-Indolyl	$C_{22}H_{16}N_4O_2$	368.4	192-195	71.73 71.7	4.38 4.37	15.21 15.23
22	KUQ-26	3-OCH_3 Phenyl	$C_{21}H_{17}N_3O_3$	359.4	216-218	70.18 70.21	4.77 4.78	11.69 11.67

Note:- The underline values denote the calculated percentage of composition

Table 6.2: Physical constants of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C,H,N%)		
						C	H	N
1	KUQP-1	4-OCH_3 Phenyl	$C_{27}H_{21}N_3O_3$	435.4	255-257	74.47 74.45	4.86 4.87	9.65 9.64
2	KUQP-2	4-F Phenyl	$C_{26}H_{18}FN_3O_2$	423.4	267-269	73.75 73.73	4.28 4.26	9.92 9.9
3	KUQP-3	4-Cl Phenyl	$C_{26}H_{18}ClN_3O_2$	439.8	275-277	70.99 70.96	4.12 4.1	9.55 9.53
4	KUQP-4	4-NO_2 Phenyl	$C_{26}H_{18}N_4O_4$	450.4	187-189	69.33 69.35	4.03 4.05	12.44 12.41
5	KUQP-5	3,4-Di OCH_3 Phenyl	$C_{28}H_{23}N_3O_4$	465.5	207-209	72.24 72.21	4.98 5.0	9.03 9.05
6	KUQP-6	3,4,5-Tri OCH_3	$C_{29}H_{25}N_3O_5$	495.5	283-285	70.29 70.26	5.09 5.1	8.48 8.48
7	KUQP-7	2,5-Di OCH_3	$C_{28}H_{23}N_3O_4$	465.5	263-265	72.24 72.21	4.98 5.0	9.03 9.05

Note:- The underline values denote the calculated percentage of composition

Table 6.2: Physical constants of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point °C	Elemental Analysis (C,H,N%)		
						C	H	N
8	KUQP-8	2-NO_2 Phenyl	$C_{26}H_{18}N_4O_4$	450.4	195-197	69.33 69.35	4.03 4.05	12.44 12.41
9	KUQP-9	3-NO_2 Phenyl	$C_{26}H_{18}N_4O_4$	450.4	217-219	69.33 69.35	4.03 4.05	12.44 12.41
10	KUQP-10	2-OH	$C_{26}H_{19}N_3O_3$	421.4	211-213	74.10 74.13	4.54 4.55	9.97 9.99
11	KUQP-11	3,4-Di OH	$C_{26}H_{19}N_3O_4$	437.4	229-231	71.39 71.36	4.38 4.39	9.61 9.59
12	KUQP-12	2-OCH3	$C_{27}H_{21}N_3O_3$	435.4	183-185	74.47 74.45	4.86 4.87	9.65 9.64
13	KUQP-13	3-OCH3	$C_{27}H_{21}N_3O_3$	435.4	201-203	74.47 74.45	4.86 4.87	9.65 9.64
14	KUQP-14	3-Br	$C_{26}H_{18}BrN_3O_2$	484.3	197-199	64.47 64.45	3.75 3.74	8.68 8.66
15	KUQP-15	4-CH3	$C_{27}H_{21}N_3O_2$	419.4	198-201	77.31 77.28	5.05 5.06	10.02 10.05
16	KUQP-16	4-SCH3	$C_{27}H_{21}N_3O_2S$	451.5	241-243	71.82 71.85	4.69 4.67	9.31 9.33

Note:- The underline values denote the calculated percentage of composition

SPECTRAL CHARACTERIZATION

The constitution of newly synthesized compounds were supported by FT - IR, ^{1}H NMR and FAB-Mass spectral study.

IR Spectral Study

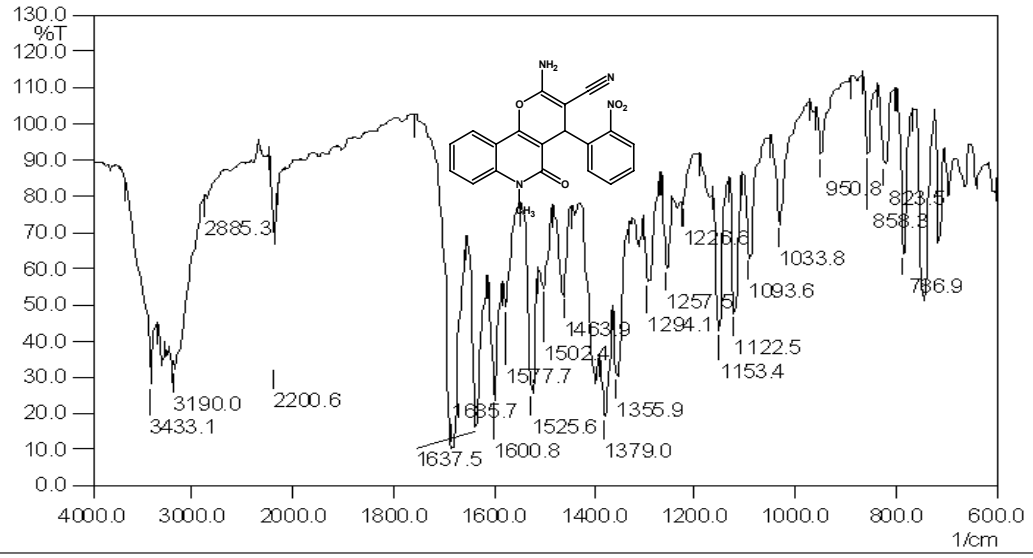
Instrument: SHIMADZU FT-IR 8400 Spectrophotometer

Sample technique: KBr pellet **Frequency range:** 400-4000 cm^{-1}

The 2-Amino-6-methyl-5-oxo-4-substitutedphenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles(KUQ Series) and 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles(KUQP Series) indicates the presence of very specific functional groups like cyano, amino, cyclic amide, aromatic ring skeleton etc. as well as N-methyl and N-benzyl substituents respectively, which absorb IR radiations of specific frequency and show sharp, medium or weak intense signals and hence supports in identifiction and confirmation of the molecule.

The IR spectrums of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles show common frequences as 3440-3300cm^{-1} for amino, 3114-3190cm^{-1} for aromatic C-H str.,2980-2830cm^{-1} for alkyl str., 2205-2180 cm^{-1} for cyano str., 1690-1665cm^{-1} for carbonyl str. of cyclic amide, aromatic ring skeleton observed between 1650-1480cm^{-1}, 1460-1400cm^{-1} for alkyl in plane deformation, 1380-1360cm^{-1} for tertiary N-C str. mono,di & tri phenyl substitutions observed between 890-740cm^{-1}. In case of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles that observed as 3440-3330cm^{-1} for amino, 3185-3050cm^{-1} for aromatic C-H str., 2950-2860cm^{-1} for methylene str., 2351-2175 cm^{-1} for cyano, 1680-1630cm^{-1} for carbonyl of cyclic amide, aromatic ring skeleton observed between 1600-1480cm^{-1}, 1460-1430$cm^{-1}$for methylene in plane deformation, 1400-1300cm^{-1} for 3^{o} N-C str. mono,di & tri phenyl substitutions observed between 870-730cm^{-1}. The IR spectral data of all compounds of KUQ and KUQP series are show in **Table 6.3** and **Table 6.4** respectively.

IR Spectrum of 2-Amino-6-methyl-5-oxo-4-(2-nitro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ- 5)



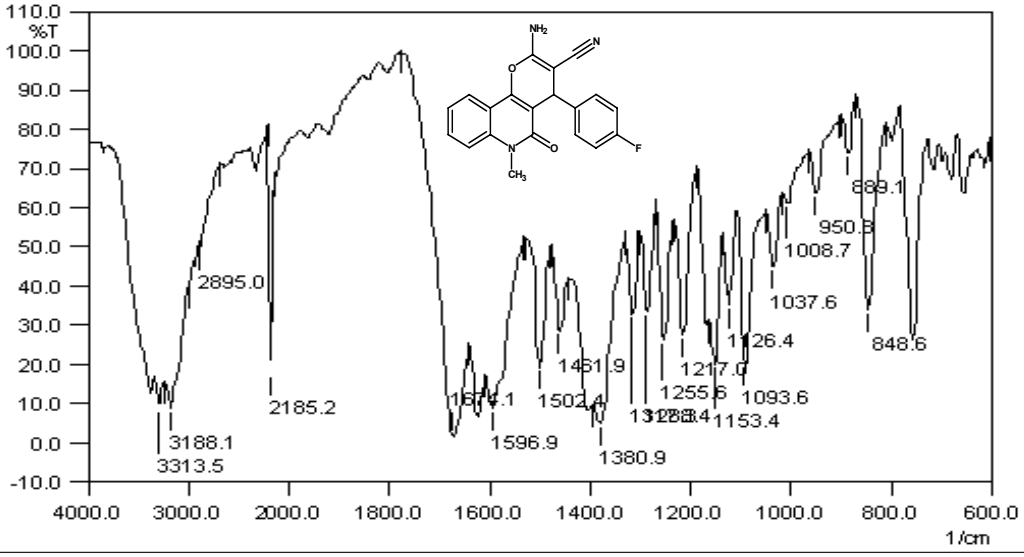
KUQ - 5

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3433
-CH Aromatic	-C-H str.	3190
$-CH_3$ Alkyl	-C-H str. -C-H i/p deformat.^{n}	2885 1463
-CN (cyano)	C=N str.	2200
-C=O	-C=O str.	1685
Aromatic Skeleton	-C=C- str.	1637,1600,1577
	1:2 di substitution	786
3°N-C	-N-C str.	1379
$-NO_2$	-N-O str.	1355

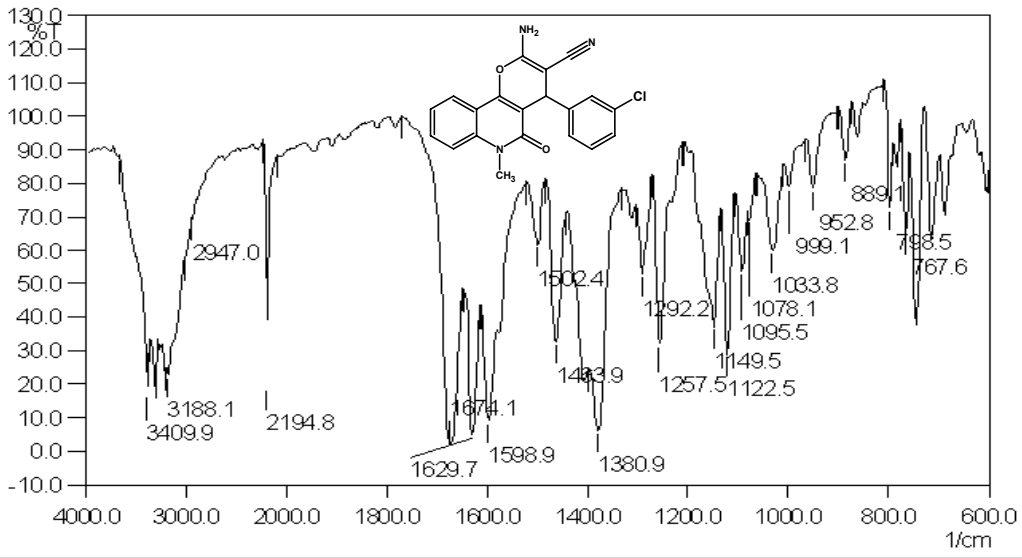
KUQ - 8

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3313
-CH Aromatic	-C-H str.	3183
$-CH_3$ Alkyl	-C-H str. -C-H i/p deformat.^{n}	2895 1461
-CN (cyano)	C=N str.	2185
-C=O	-C=O str.	1674
Aromatic Skeleton	-C=C- str.	1629,1596,1502
	1:4 di substitution	848
3°N-C	-N-C str.	1380
Fluoro	-C-Fstr.	1126

IR Spectrum of 2-Amino-6-methyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQ- 8)



IR Spectrum of 2-Amino-6-methyl-5-oxo-4-(3-chloro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ- 5)



KUQ - 3

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3409
-CH Aromatic	-C-H str.	3188
$-CH_3$ Alkyl	-C-H str. -C-H i/p deformat.^{n}	2947 1463
-CN (cyano)	C=N str.	2194
-C=O	-C=O str.	1674
Aromatic Skeleton	-C=C- str.	1629,1598,1502
	1:3 di substitution	889
3°N-C	-N-C str.	1380
Halogen	-C-Cl str.	767

KUQ - 25

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3317
-CH Aromatic	-C-H str.	3172
$-CH_3$ Alkyl	-C-H str. -C-H i/p deformat.^{n}	2965 1458
-CN (cyano)	C=N str.	2191
-C=O	-C=O str.	1676
Aromatic Skeleton	-C=C- str.	1631,1596,1577
	1:2 fused substitution	754
3°N-C	-N-C str.	1377
Indolyl	-N-H str. -C-N str(2°)	3420,1255

IR Spectrum of 2-Amino-4-(1*H*-indol-3-yl)-6-methyl-5-oxo-5,6-dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ- 25)

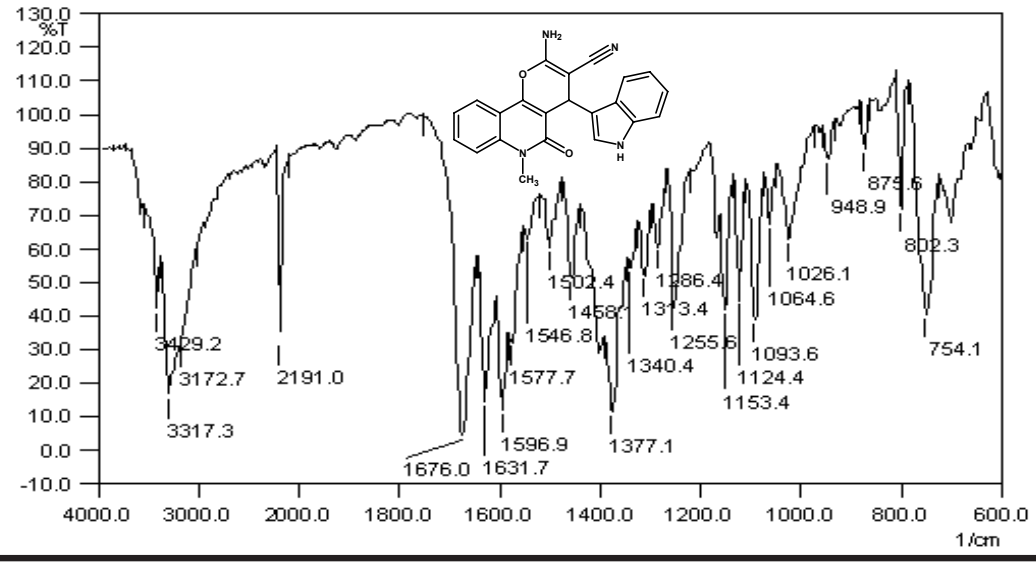
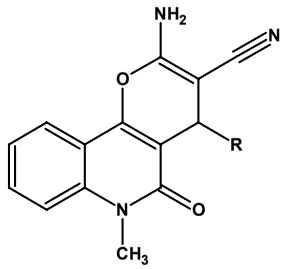
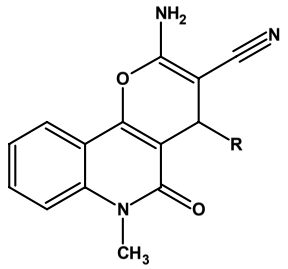


Table 3.1 :IR Spectral data of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



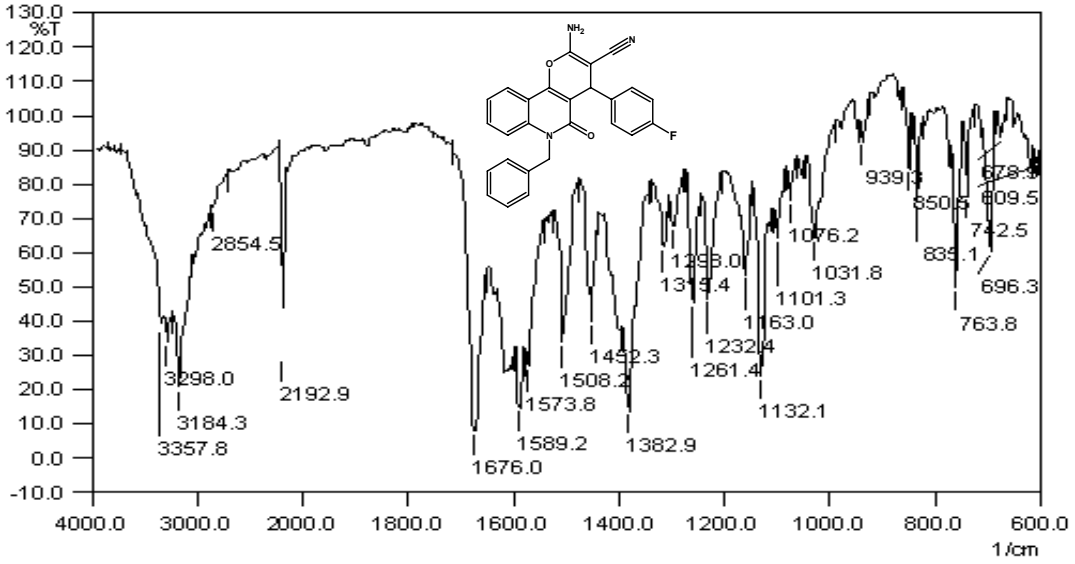
Code	Substitution (R)	-NH_2 Str.	Ar-H Str.	-CH_3 Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			-CH_3 deformation	>C-N< Str. (3° amine)	Phenyl Substitution	Substitution
KUQ-1	H	3392	3153	2900	2204	1678	1629	1598	1579	1452	1380	749 (mono sub)	-
KUQ-2	2-Cl	3357	3114	2858	2192	1687	1643	1600	1575	1465	1377	758 (1:2 di sub.)	747 (C-Cl str)
KUQ-4	4-Cl	3313	3188	2942	2185	1674	1625	1590	1503	1460	1379	840 (1:4 di sub.)	767 (C-Cl str)
KUQ-6	3-NO_2	3328	3172	2868	2191	1676	1611	1596	1525	1463	1386	964 (1:3 di sub)	1350 (-N=O str.)
KUQ-7	4-NO_2	3404	3188	2952	2185	1674	1632	1593	1514	1461	1379	821 (1:4 di sub)	1346 (-N=O str.)
KUQ-9	3-Br	3357	3199	2915	2196	1672	1624	1595	1504	1463	1380	848 (1:3 di sub)	717 (C-Br str)
KUQ-10	3-OC_6H_5	3313	3190	2931	2185	1674	1623	1596	1483	1446	1377	829 (1:3 di sub)	1238 (C-O-C str.)
KUQ-11	4-SCH_3	3357	3161	2977 2835	2187	1676	1629	1598	1579	1463	1477	833 (1:2 di sub)	697 (C-S str)
KUQ-13	3-OH	3309	3178	2916	2210	1670	1629	1600	1492	1461	1380	854 (1:3 di sub.)	1259 (C-O-C str.)

Table 3.3 :IR Spectral data of 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



Code	Substitution (R)	-NH_2 Str.	Ar-H Str.	-CH_3 Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			-CH_3 deformation	>C-N< Str. (3° amine)	Phenyl Substitution	Substitution
KUQ-14	4-OH	3328	3139	2916	2191	1668	1611	1595	1512	1400	1382	763 (1:4 di sub.)	3411,1255 (-OH str, C-O str)
KUQ-15	4-N,N-diCH_3	3358	3127	2922 2852	2190	1675	1591	1565	1520	1399	1363	812 (1:4 di sub)	-
KUQ-16	2-OCH_3	3419	3184	2885	2200	1678	1629	1596	1494	1468	1380	748 (1:2 di sub.)	1253 (C-O-C str.)
KUQ-17	4-OCH_3	3352	3163	2953 2853	2185	1672	1629	1598	1508	1436	1379	835 (1:4 di sub)	1251 (C-O-C str.)
KUQ-18	3,4,5-triOCH_3	3373	3186	2941 2833	2196	1670	1627	1596	1502	1460	1380	761(1:3:4:5 tetra sub.)	1257,1232,1225 (C-O-C str.)
KUQ-19	3,4-diOCH_3	3411	3166	2932 2833	2194	1670	1624	1589	1512	1450	1407	858 (1:3:4 tri sub.)	1201,1245 (C-O-C str.)
KUQ-21	9-Anthracyl	3436	3168	2956	2191	1670	1627	1595	1502	1460	1375	756 (mono sub)	-
KUQ-24	3-OC_2H_5, 4-OH	3404 (merge with OH)	3153	2962	2192	1672	1631	1585	1512	1460	1380	947 (1:3:4 tri sub.)	1257(C-O-C str) 1039(C-OH str)
KUQ-26	3-OCH_3	3344	3172	2945	2200	1691	1637	1601	1581	1436	1380	896 (1:3 di sub.)	1093(C-O-C str)

IR Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQP-2)



KUQP-2

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3357(d)
-CH Aromatic	-C-H str.	3184
$-CH_2$ Methylene	-C-H str. -C-H i/p deformat.^{n}	2854 1452
-CN (cyano)	C=N str.	2192
-C=O	-C=O str.	1676
Aromatic Skeleton	-C=C- str.	1589,1573,1580
	1:4 di substitution	835
3°N-C	-N-C str.	1382
Halogen	-C-F str.	1132

KUQP-1

Functional Group	Vibration Mode	Frequency (cm^{-1})
$-NH_2$	N-H str.	3358(d)
-CH Aromatic	-C-H str.	3180
$-CH_3$ Alkyl	-C-H str. -C-H i/p deformat.^{n}	2928 1462
-CN (cyano)	C=N str.	2185
-C=O	-C=O str.	1675
Aromatic Skeleton	-C=C- str.	1597,1575,1508
	1:4 di substitution	830
3°N-C	-N-C str.	1386
Methoxy	C-O-C str.	1250

IR Spectrum of 2-Amino-6-methyl-5-oxo-4-(4-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-1)

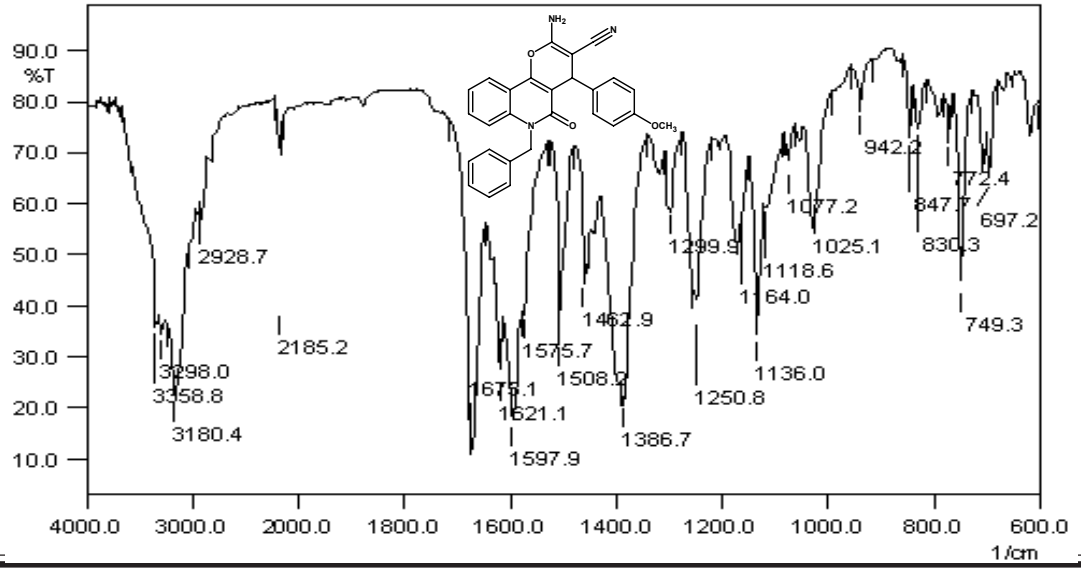
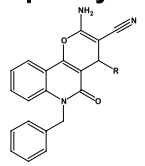
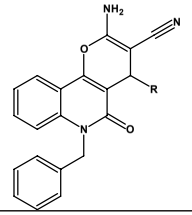


Table 3.4: IR spectral data of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



Code	Substitution (R)	-NH_2 Str.	Ar-H Str.	>CH_2 Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			>CH_2 deformation	>C-N< Str. (3° amine)	Phenyl Substitution	Substitution
KUQP-3	4-Cl	3330	3129	2930	2186	1675	1626	1597	1578	1455	1377	754 (mono sub) 838 (1:4 di sub.)	754 (C-Cl)
KUQP-4	4-NO_2	3343	3178	2925	2191	1678	1596	1578	1522	1451	1381	752 (mono sub.) 827 (1:4 di sub.)	1347 (-N=O str.)
KUQP-5	3,4-diOCH_3	3435	3126	2952	2163	1644	1590	1571	1515	1452	1400	758 (mono sub) 815 (1:3:4 tri sub.)	1263 (C-O-C str.)
KUQP-6	3,4,5-triOCH_3	3435	3124	2934	2208	1666	1633	1563	1497	1452	1402	758 (mono sub) 850 (1:3:4:5 tetra sub.)	1264,1247,1225 (C-O-C str.)
KUQP-7	2,5-diOCH_3	3374	3178	2925	2188	1677	1591	1575	1499	1460	1385	753 (mono sub) 798 (1:2:5 tri sub.)	1265,1245 (C-O-C str.)
KUQP-8	3-NO_2	3435	3118	2929	2245	1656	1631	1583	1529	1450	1400	759 (mono sub) 829 (1:3 di sub.)	1350 (-N=O str.)
KUQP-9	2-NO_2	3419	3114	2863	2259	1646	1571	1530	1496	1433	1308	743 (mono sub) 758 (1:2 di sub)	1357 (-N=O str.)

Table 3.4: IR spectral data of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitriles



Code	Substitution (R)	-NH_2 Str.	Ar-H Str.	>CH_2 Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			>CH_2 deformation	>C-N< Str. (3° amine)	Phenyl Substitution	Substitution
KUQP-10	2-OH	3325	3118	2928	2175	1633	1571	1530	1496	1433	1308	740 (mono sub) 755 (1:2 di sub	3411, 1314 (OH str., C-O str)
KUQP-11	3,4-diOH	3419 (merge with OH)	3089	2923	2351	1673	1610	1553	1500	1427	1400	766 (mono sub) 810 (1:3:4 tri sub.)	1301 (C-O str)
KUQP-12	2-OCH_3	3343	3128	2926	2188	1672	1633	1591	1511	1452	1400	757 (mono sub) 762 (1:2 di sub.)	1248 (C-O-C str.)
KUQP-13	3-OCH_3	3419	3130	2950	2260	1645	1589	1531	1492	1452	1398	759 (mono sub) 829 (1:3 di sub.)	1265 (C-O-C str.)
KUQP-14	3-Br	3419	3053	2927	2260	1676	1631	1571	1512	1431	1382	745 (mono sub) 810 (1:3 di sub)	729 (C-Br str)
KUQP-15	4-CH_3	3348	3126	2925	2194	1681	1591	1569	1512	1452	1400	756 (mono sub) 773 (1:4 di sub.)	-
KUQP-16	4-SCH_3	3352	3173	2924	2189	1675	1635	1622	1495	1452	1401	734 (mono sub) 754 (1:4 di sub.)	697 (C-S str)

^{1}H NMR Spectral Study

^{1}H NMR Spectrum of the 2-amino-6-methyl-5-oxo-4-(3-chloro)phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile(KUQ) and 2-amino-6-benzyl-5-oxo-4-(3-chloro)phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile(KUQP) show signals relevant to the number of protons and their electronic environment known as chemical shift. Chemical shift have been observed either to upfield (Shielding) or down field(deshielding), according to the electronic environment of the corresponding proton. In addition to this each ^{1}H NMR signal further splitted into number of subpeaks according to the number of neighbouring protons present in the skeleton of molecule. The splitting of the NMR signal further provides signification about the degree of interaction between neighbouring protons by means of spin-spin coupling constant J.

Instrument : BRUKER AC 300MHz FT-NMR

Internal Reference : TMS Solvent : $CDCl_3$ or DMSO d_6

2-Amino-6-methyl-5-oxo-4-(3-methoxy)phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile (KUQ-26)

^{1}H NMR Spectrum of KUQ-26 shows a singlet of N-CH_3 protons appears at δ2.886 ppm, where as a singlet of methyne proton of the pyran ring appears at δ 2.726, a singlet of methoxy protons of phenyl residue appears at δ3.703. Two protons of an amine group resonate at δ4.497. Protons $H_{(e)}$ & $H_{(g)}$ appear as multiplet due to close electronic environment at δ6.754, while proton $H_{(c)}$ appears at δ 7.298 as singlet and $H_{(f)}$ appears at δ7.194 splitted into triplet with J value of 8.1 & 7.8Hz, which reveals that methoxy substitution situated at meta position at phenyl residue. Protons $H_{(5)}$, $H_{(6)}$, $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ8.02 for with J value 7.8Hz, a triplet at δ 7.394 with J value of 7.5 & 8.1Hz, a triplet at δ7.715 with J value of 7.8 & 7.2Hz and a doublet δ7.583 with J value 8.4Hz respectively, which reveal that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

2-Amino-6-methyl-5-oxo-4-(3-phenoxy) phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile (KUQ-10)

^{1}H NMR Spectrum of KUQ-10 shows a singlet due to three protons of N-CH_3 group at chemical shift value of δ3.561ppm, a singlet of methyne proton appears at 3.389δ, while two amino protons resonate at δ 4.531as singlet. In aromatic chemical shift region two multipletes corresponding to m-phenoxy phenyl residue appears at chemical shift range between 6.895-7.006δ ppm and 7.248-7.422δ ppm. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ8.012 with J value 7.8Hz, a triplet at δ 7.120 with J value of 7.5 & 7.2Hz, a triplet at δ 7.364 with J value of 8.1& 7.5Hz and a doublet at δ 7.583 with J value 8.7Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

2-Amino-6-methyl-5-oxo-4-(3,4,5-trimethoxy)phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile (KUQ-18)

In case of ^{1}H NMR Spectrum of KUQ-18 a singlet is observed due to N-CH_3 protons at chemical shift δ 2.724, a singlet of methyne proton of the pyran ring appears at δ2.884, two singlets due to three methoxy groups appeared at δ 3.563 & 3.685. While two amino protons resonate at δ4.539. Phenyl residue having two chemically equivalent protons $H_{(c)}$ show a singlet at δ6.6478. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ 8.02 with J value 7.8Hz, a triplet at δ7.394 with J value of 7.5 & 8.1Hz, a triplet at δ 7.715 with J value of 7.8 & 7.2Hz and a doublet at δ7.583 with J value 8.4Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

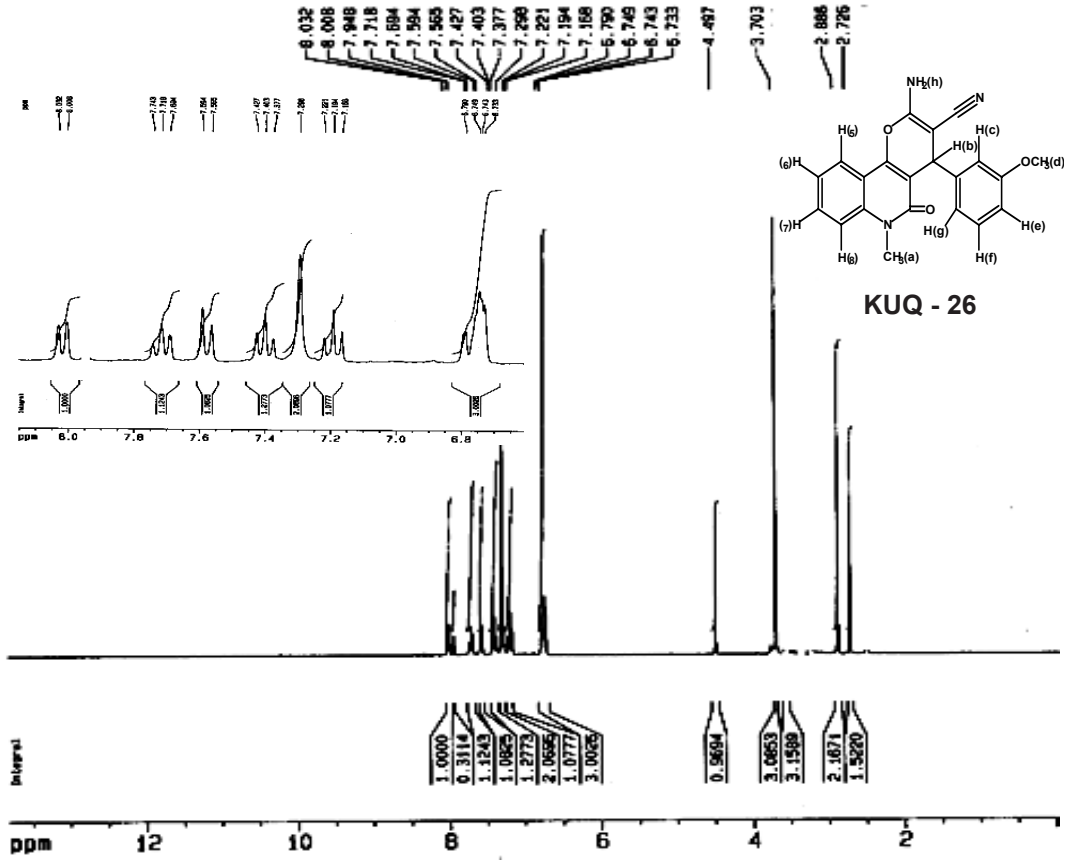
2-Amino-6-benzyl-5-oxo-4-(4-methoxy)phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile (KUQP-1)

^{1}H NMR Spectrum of KUQP-1 shows a singlet due to methyne proton at δ2.502, while a singlet of methoxy protons of phenyl substitution appears at δ3.7103. Two amine protons resonate at δ 4.565. Protons $H_{(a)}$ & $H_{(b)}$ of methylene group of benzyl residue show two doublet at δ 5.3406 with J value of 16.19Hz and δ5.537 with J value of 16.22Hz respectively which reveals that these two protons undergo methylene gem coupling with each other, evidenced by it's higher coupling constant value. Among the five protons of phenyl ring of the benzyl residue, the signals due to protons of type $H_{(2)}$ & $H_{(3)}$ are merged because of close electronic environment, hence appear as multiplet at chemical shift range between δ7.102-7.172 ppm, while $H_{(1)}$ shows a doublet at δ 6.857 with J value of 8.55Hz. Where as phenyl residue substituted at pyran ring possess two pair of chemically equivalent but magnetically non-euivalent protons viz. $H_{(d)}$ & $H_{(e)}$ appear as two doublet at δ7.270 with J value of 8.14Hz and δ7.223 with J value of 4.5Hz respectively. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part show a doublet at δ8.04 with J value 7.6Hz, a triplet at 7.35 with J value of 7.6 & 7.8Hz, a triplet at δ7.58 with J value of 7.4 & 7.5Hz and a doublet at δ 7.423 with J value 8.5Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano [3,2-*c*]quinoline-3-carbonitrile (KUQP-2)

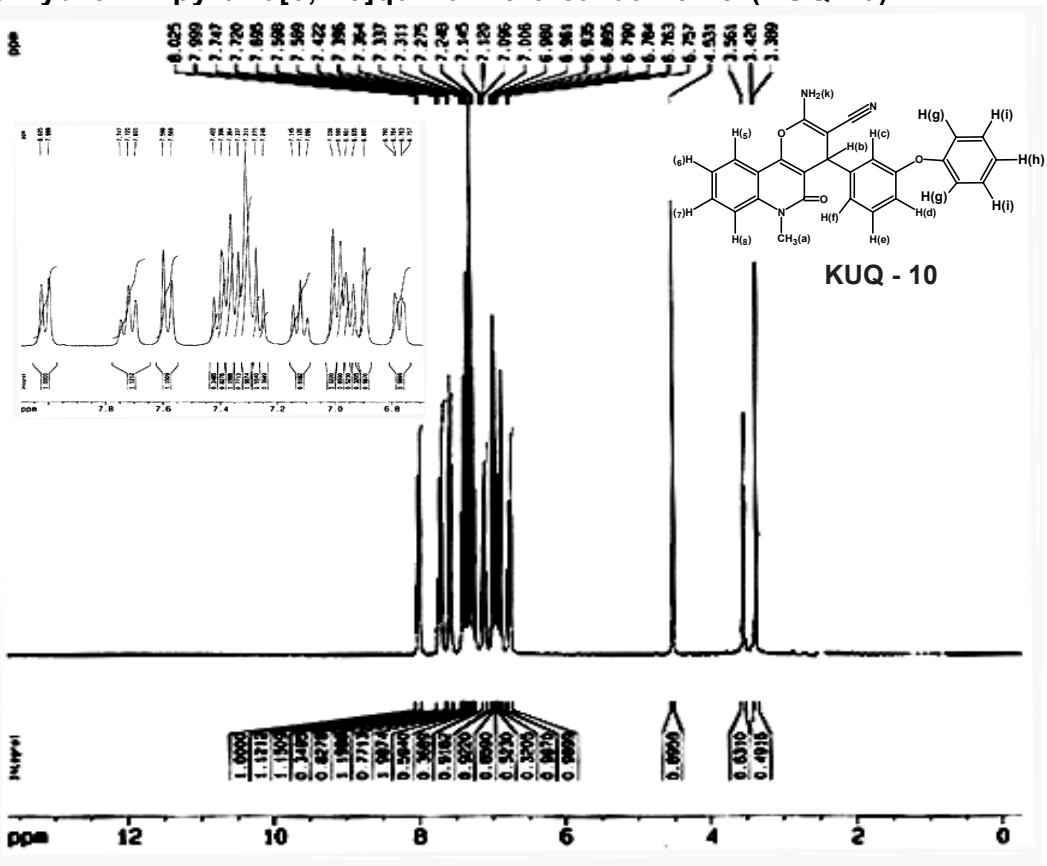
^{1}H NMR Spectrum of KUQP-2 shows a singlet due to methyne proton of the pyran ring at δ 2.502. Two amino protons are resonate at δ 4.565. Protons $H_{(a)}$ & $H_{(b)}$ of methylene group of benzyl residue show two doublets at δ5.352 with J value of 16.22Hz and δ5.527 with J value of 16.27Hz respectively which reveals that these two protons undergo methylene gem coupling with each other, evidenced by it's higher coupling constant value. Among the five protons of phenyl ring of the benzyl residue, the signals due to protons of type $H_{(1)}$, $H_{(2)}$ & $H_{(3)}$ are merge because of close electronic environment, hence appear as multiplet at chemical shift range between δ7.275-7.172 ppm. Where as phenyl residue substituted at pyran ring possess two pair of chemically equivalent but magnetically non-euivalent protons viz. $H_{(d)}$ & $H_{(e)}$ appear as two doublet at δ7.102 with J value of 6.8Hz and δ7.135 with J value of 9.01Hz respectively. Protons $H_{(5)}$, $H_{(6),}$ $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows a doublet at δ8.05 with J value 7.2Hz, a triplet at δ7.364 with J value of 7.85 & 4.97Hz, a triplet at δ 7.583 with J value of 7.4 & 7.5Hz and a doublet at δ7.437 with J value 8.5Hz respectively, which reveals that $H_{(5)}$ & $H_{(6)}$, $H_{(6)}$ & $H_{(7)}$ and $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other, while $H_{(5)}$ & $H_{(7)}$ and $H_{(6)}$ & $H_{(8)}$ are relatively in meta position.

NMR Spectrum of 2-Amino-6-methyl-5-oxo-4-(3-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQ-26)



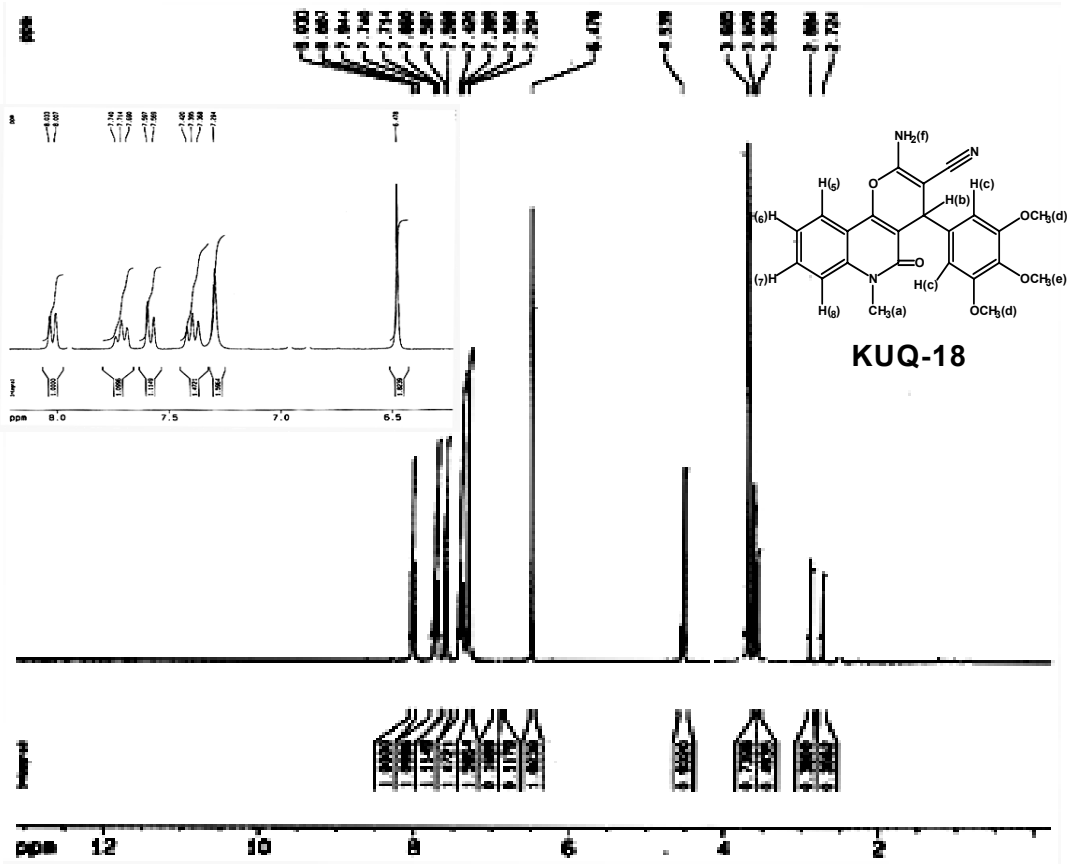
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
$N\text{-}CH_{3\,(a)}$	2.886	3	s	-
$-\,CH_{(b)}$	2.726	1	s	-
$-OCH_{3(d)}$	3.703	3	s	-
$NH_{2(h)}$	4.497	2	s	-
$Ar\text{-}H_{(e,g)}$	6.754	2	m	-
$Ar\text{-}H_{(c)}$	7.298	1	s	-
$Ar\text{-}H_{(f)}$	7.194	1	t	8.1 & 7.8
$Ar\text{-}H_{(6)}$	7.402	1	t	7.2 & 7.8
$Ar\text{-}H_{(8)}$	7.58	1	d	8.7
$Ar\text{-}H_{(7)}$	7.72	1	t	7.5 & 7.2
$Ar\text{-}H_{(5)}$	8.02	1	d	7.2

NMR Spectrum of 2-Amino-6-methyl-5-oxo-4-(3-phenoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQ-10)



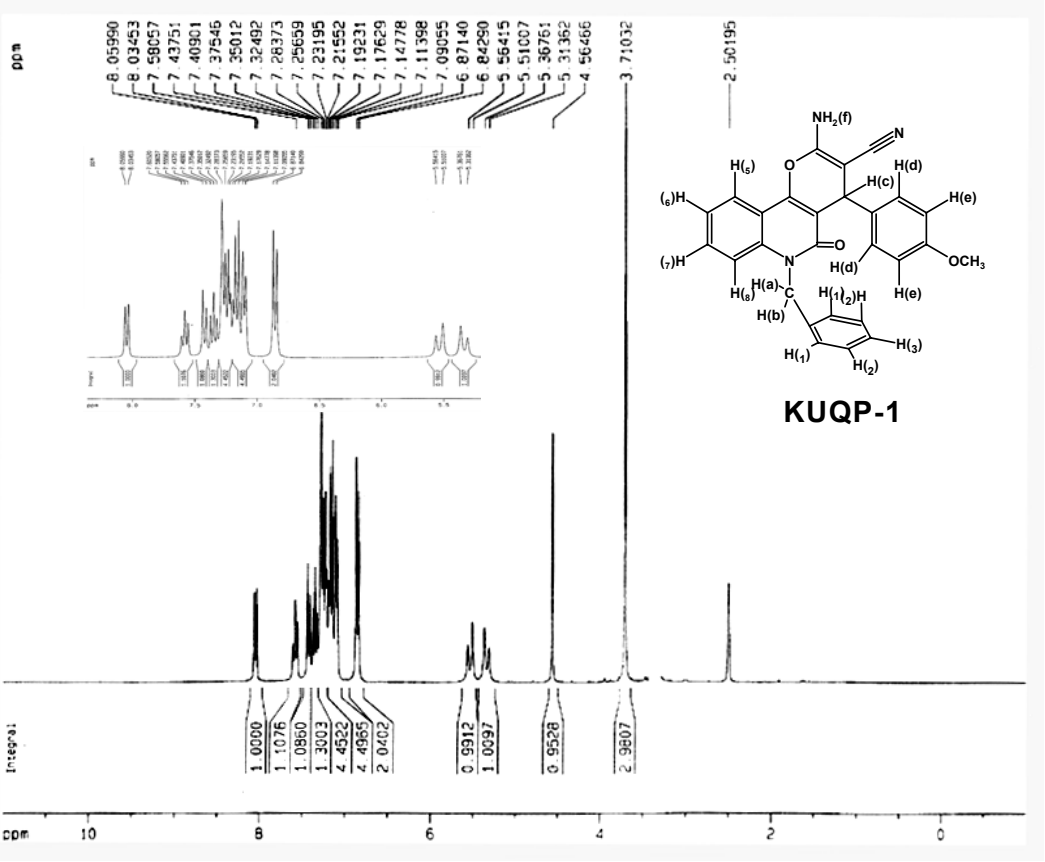
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
N-$CH_{3\ (a)}$	3.561	3	s	-
- $CH_{(b)}$	3.389	1	s	-
$NH_{2(k)}$	4.531	2	s	-
Ar-$H_{(d)}$	6.773	1	dd	1.8,6.3 & 1.8
Ar-$H_{(c,e,f)}$	6.895-7.006	3	m	-
Ar-$H_{(g-k)}$	7.248-7.422	1	m	
Ar-$H_{(6)}$	7.12	1	t	7.5 & 7.2
Ar-$H_{(8)}$	7.583	1	d	8.7
Ar-$H_{(7)}$	7.364	1	t	8.1 & 7.5
Ar-$H_{(5)}$	8.012	1	d	7.8

NMR Spectrum of 2-Amino-6-methyl-5-oxo-4-(3,4,5-trimethoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQ-18)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
$N\text{-}CH_{3\,(a)}$	2.724	3	s	-
$-\,CH_{(b)}$	2.884	1	s	-
$2\text{-}OCH_{3(d)}$	3.685	6	s	-
$-OCH_{3(e)}$	3.563	3	s	-
$NH_{2(f)}$	4.539	2	s	-
$Ar\text{-}H_{(c)}$	6.478	2	s	-
$Ar\text{-}H_{(6)}$	7.394	1	t	7.5 & 8.1
$Ar\text{-}H_{(8)}$	7.583	1	d	8.4
$Ar\text{-}H_{(7)}$	7.715	1	t	7.8 & 7.2
$Ar\text{-}H_{(5)}$	8.02	1	d	7.8

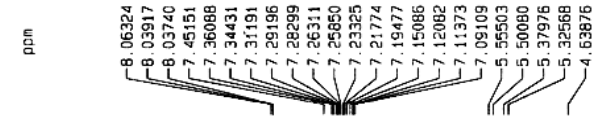
NMR Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-1)

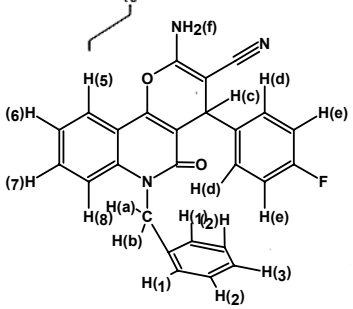


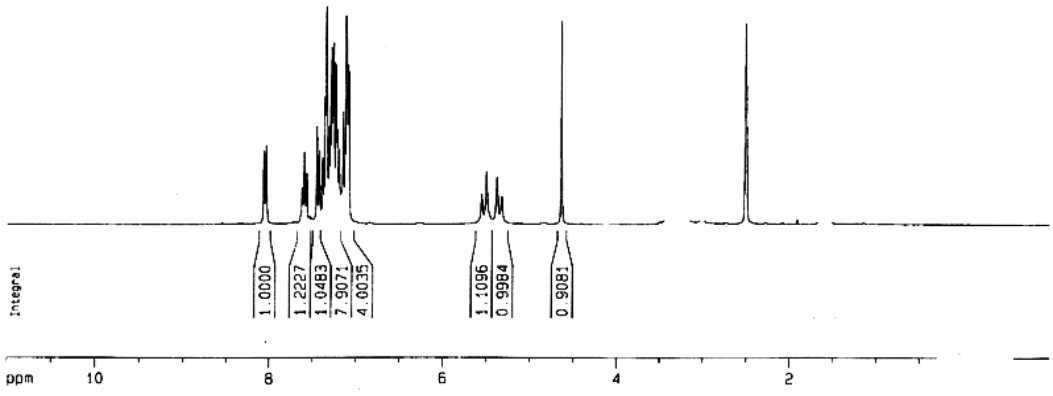
KUQP-1

Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
$-OCH_3$	3.7103	3	s	-
$-CH_{(c)}$	2.5019	1	s	-
$-CH_{(a)}$	5.3406	1	d	16.19
$-CH_{(b)}$	5.5371	1	d	16.22
$-NH_2$	4.565	2	s	-
$Ar-H_{(d)}$	7.2702	2	d	8.14
$Ar-H_{(e)}$	7.224	2	d	4.5
$Ar-H_{(1)}$	6.857	2	d	8.5
$Ar-H_{(2,3)}$	7.135	3	m	-
$Ar-H_{(6)}$	7.3502	1	t	7.6 & 7.6
$Ar-H_{(8)}$	7.423	1	d	8.5
$Ar-H_{(7)}$	7.5804	1	t	7.4 & 7.5
$Ar-H_{(5)}$	8.047	1	d	7.6

NMR Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-2)







Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{(c)}$	2.502	1	s	-
- $CH_{(a)}$	5.353	1	d	16.22
- $CH_{(b)}$	5.528	1	d	16.27
$-NH_2$	4.639	2	s	-
Ar-$H_{(d)}$	7.1024	2	d	6.8
Ar-$H_{(e)}$	7.136	2	d	9.01
Ar-$H_{(1,2,3)}$	7.276	5	m	-
Ar-$H_{(6)}$	7.364	1	t	7.85 & 4.97
Ar-$H_{(8)}$	7.437	1	d	8.5
Ar-$H_{(7)}$	7.584	1	t	7.4 & 7.5
Ar-$H_{(5)}$	8.05	1	d	7.2

Mass Spectral Study

The MASS spectral data have reveald sigificant clues in the strucure characterization of all the newly synthesized 2-Amino-6-methyl-5-oxo-4-substituted phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitriles(KUQ series) and 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitriles(KUQP series). By both the Fast Atomic Bombartment an Electron Impact Mass spectral analysis of the products obtained by the Micheal addition and cyclocondensation reaction between 6-Benzyl-4-hydroxy-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione and 2-benzelidinemalononitrile derivatives, the molecular ion peak generated of m/z value correspond to the molecular weight of 2-Amino-6-benzyl-5-oxo-4-substituted phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitriles, which indicated that rupture of the α-pyranone ring takes place during the reaction. However, ^{1}H NMR spectral analysis was failed to interpret this data as the α-pyranone ring of the predicted tetracylic derivative was in lack of proton.

The molecular ion peak(M+) of the compounds are in total agreement with it's molecular weight.

FAB - MASS Spectral analysis

Instrument: JEOL SX 102/DA-6000 Spectrograph for FAB

2-Amino-6-methyl-5-oxo-4-(3-methoxy) phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-26)

In the case of FAB Mass spectrum of KUQ-26 the Molecular ion peak appears at m/z 360, base peak of the fragmentation is M+1peak appears at m/z 360, other ions generated are m/z307, m/z 289, m/z 252, m/z 242, m/z 227, m/z 156, m/z 136 etc.

2-Amino-6-methyl-5-oxo-4-(3-phenoxy) phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-10)

In the case of FAB Mass spectrum of KUQ-10 the Molecular ion peak appears at m/z 421, M+1peak appears at m/z 422, base peak of the fragmentation appears at m/z 307, other ions generated are m/z391, m/z 350, m/z 289, m/z 252, m/z 194, m/z 167 etc.

2-Amino-6-methyl-5-oxo-4-(3,4,5-trimethoxy) phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-18)

In the case of FAB Mass spectrum of KUQ-18 the Molecular ion peak appears at m/z 419, M-1peak appears at m/z 418, base peak of the fragmentation appears at m/z 252, other ions generated are m/z391, m/z 388, m/z 350, m/z 307, m/z 289, m/z 194 etc.

Comparative Study of FAB Mass and EI Mass Spectral analysis

2-Amino-6-benzyl-5-oxo-4-(4-methoxy) phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQP-1)

In the case of FAB Mass spectrum of KUQP-1 the Molecular ion peak appears at m/z 435, M+1peak appears at m/z 436, base peak of the fragmentation appears at m/z 307, other ions generated are m/z391, m/z 344, m/z 328, m/z 289, m/z 251, m/z 120 etc.

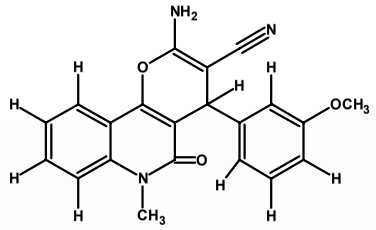
On the other hand EI Mass spectrum of KUQP-1 shows the Molecular ion peak appears at m/z 435, M+1peak appears at m/z 436, base peak of the fragmentation appears at m/z 93, other ions generated are m/z368, m/z 344, m/z 328, m/z 301, m/z 278, m/z 221 etc.

2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6-dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQP-2)

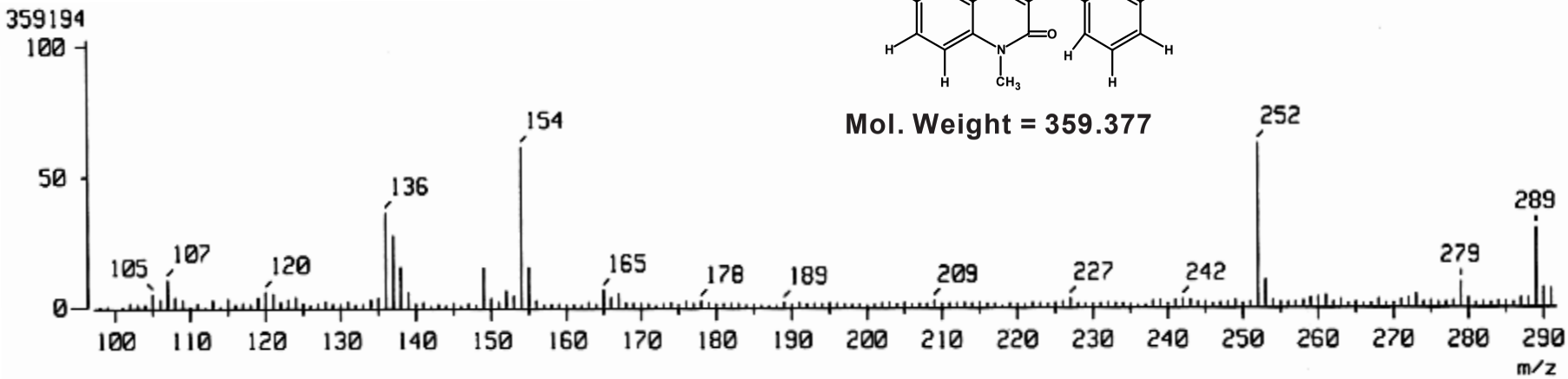
In the case of FAB Mass spectrum of KUQP-1 the Molecular ion peak appears at m/z 423, base peak of the fragmentation is M+1peak appears at m/z 424, other ions generated are m/z409, m/z 397, m/z 358, m/z 344, m/z 328, m/z307, m/z289, m/z289, m/z273, m/z251, m/z238 etc.

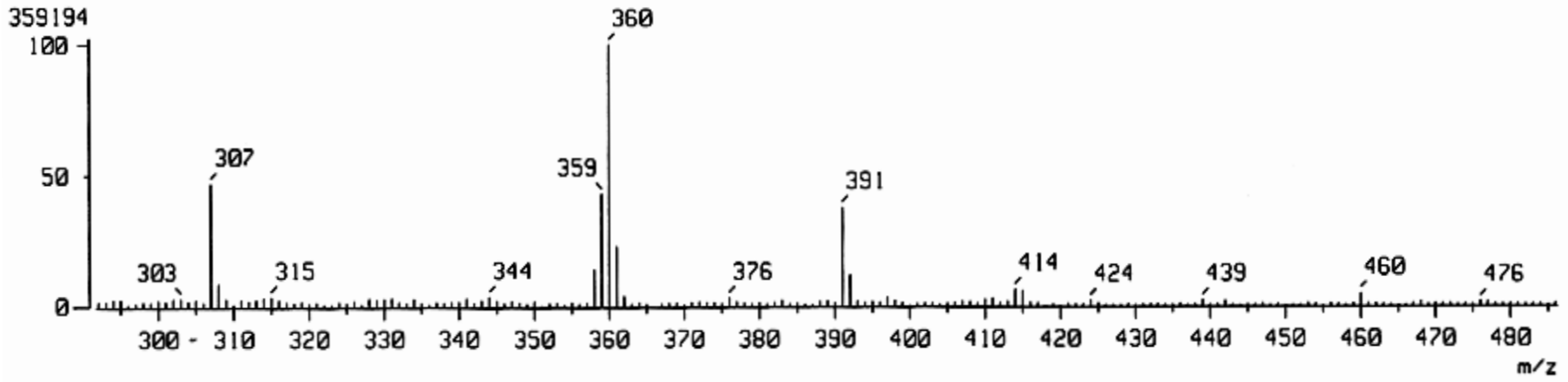
Where as EI Mass spectrum of KUQP-1 shows the Molecular ion peak appears at m/z 435, M+1peak appears at m/z 436, base peak of the fragmentation appears at m/z 93, other ions generated are m/z357, m/z 332, m/z 328, m/z 266, m/z 237, m/z 209, m/z180, m/z163, m/z163, m/z144, m/z132, m/z120 etc.

FAB - MASS Spectrum of 2-Amino-6-methyl-5-oxo-4-(3-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-26)

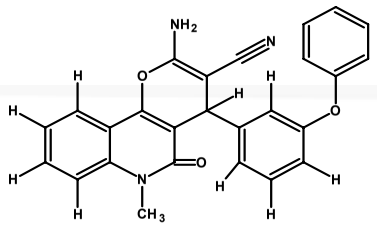


Mol. Weight = 359.377



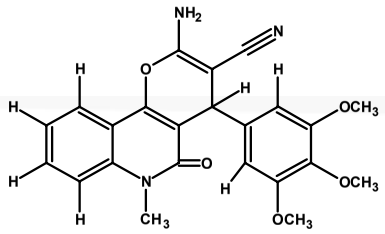


FAB - MASS Spectrum of 2-Amino-6-methyl-5-oxo-4-(3-phenoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-10)



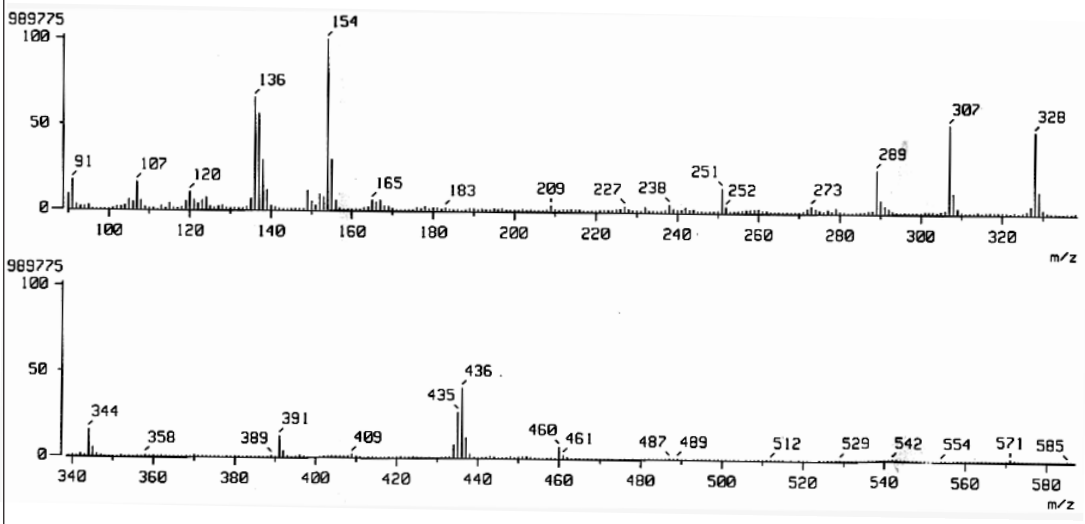
Mol. Weight = 421.44

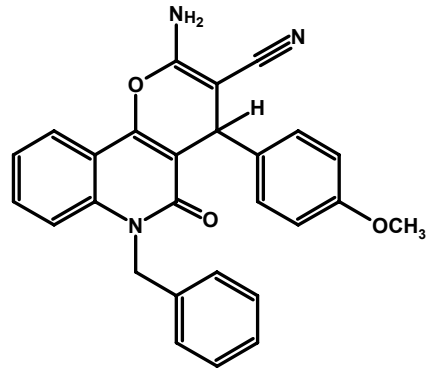
FAB - MASS Spectrum of 2-Amino-6-methyl-5-oxo-4-(3,4,5-trimethoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*] quinoline-3-carbonitrile (KUQ-18)



Mol. Weight = 419.43

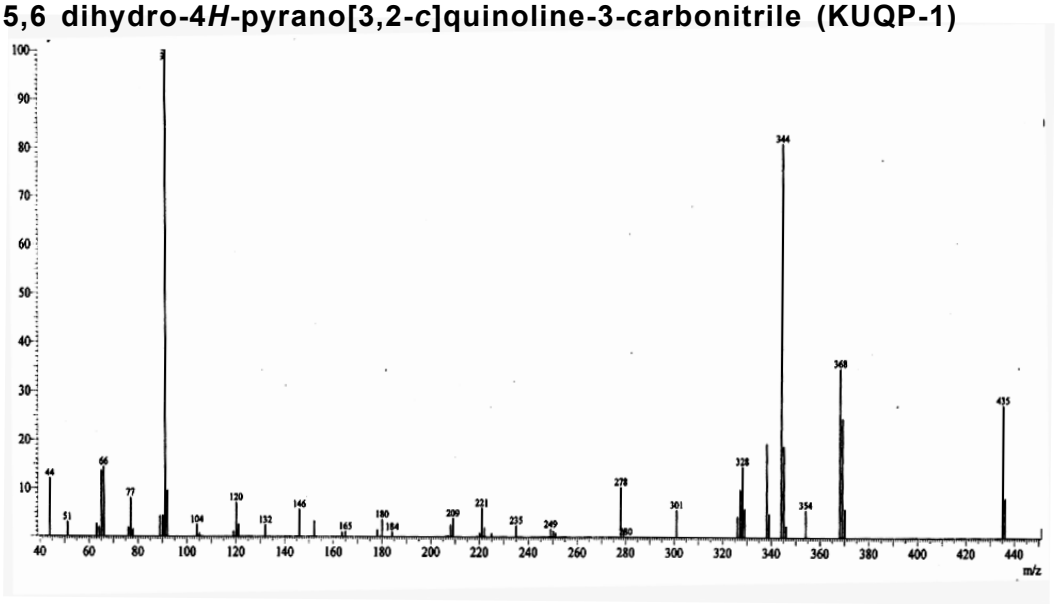
FAB MASS Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-1)



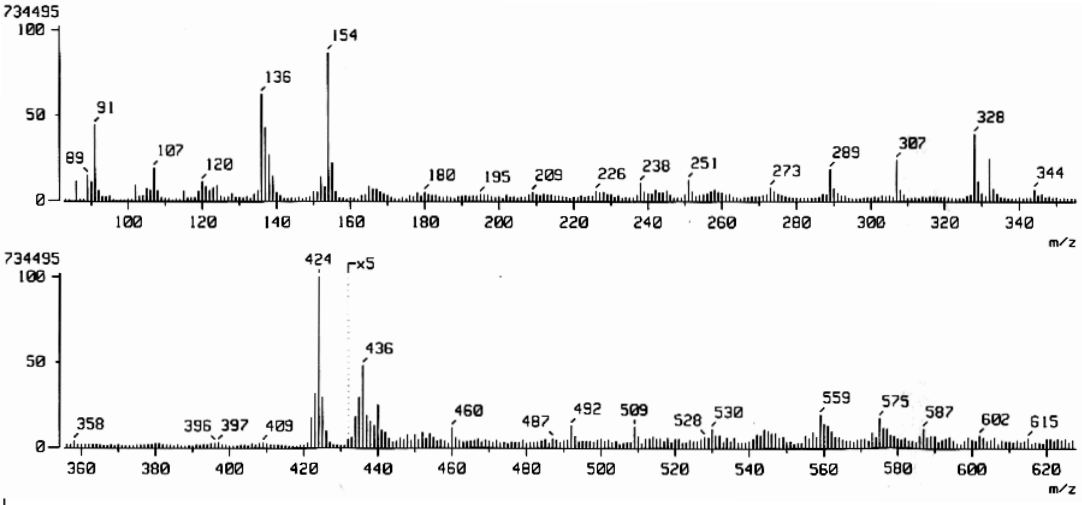


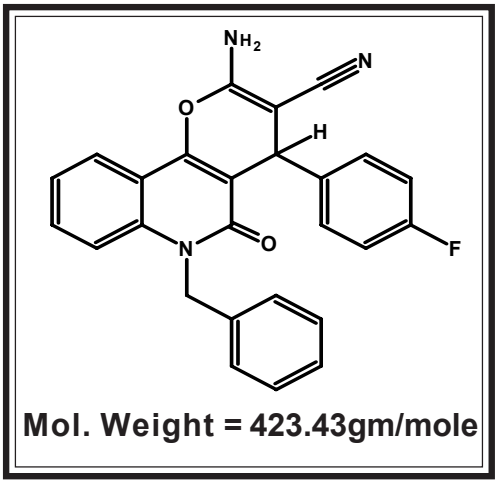
Mol. Weight = 435.47gm/mole

EI MASS Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-methoxy)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-1)



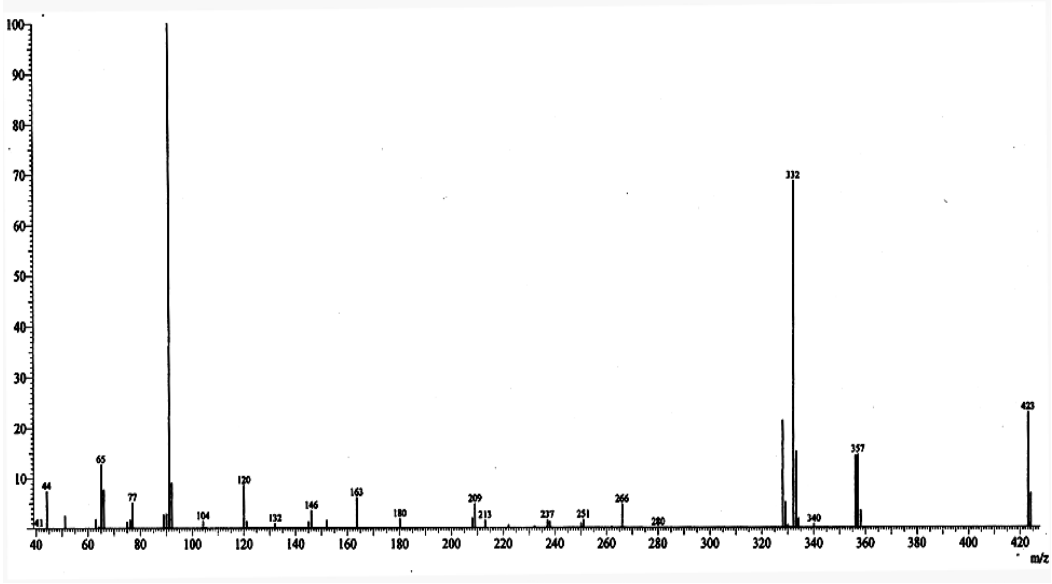
FAB MASS Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-2)



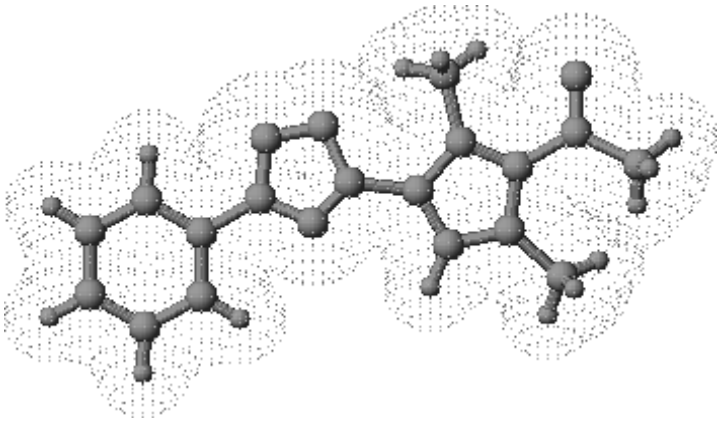


Mol. Weight = 423.43gm/mole

EI MASS Spectrum of 2-Amino-6-benzyl-5-oxo-4-(4-fluoro)phenyl-5,6 dihydro-4*H*-pyrano[3,2-*c*]quinoline-3-carbonitrile (KUQP-2)



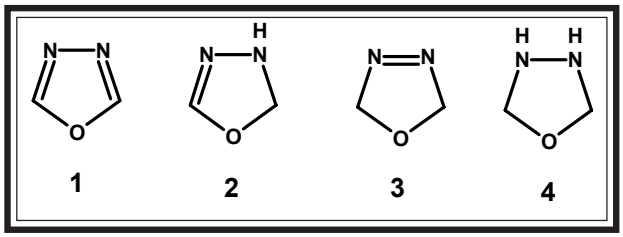
CHAPTER-4



Synthesis and Characterization of Some 1-[2,4-Dimethyl-5-(5-(un)substituted phenyl-1,3,4-oxadiazol-2-yl)-1H-pyrrol-3-yl]ethanones

Part-A. 1,3,4-Oxadiazole

1,3,4-oxadiazole(1) is a thermally stable aromatic heterocycle and exist in two partially reduced forms; 2,3-dihydro-1,3,4-oxadiazole(Ä^2 - 1,3,4-oxadiazoline)(2) and 2,5-dihydro-1,3,4-oxadiazole(Ä^3 - 1,3,4-oxadiazoline)(3) depending on the position of the double bond. The completely reduced form of the 1,3,4-oxadiazole is known as 2,3,4,5-tetrahydro-1,3,4-oxadiazole (1,3,4-oxadiazolidine)(4)[1]



Bactericidal and/or fungicidal activity was reported for oxadiazole(5a), amino-oxadiazole(5b)[2] and oxadiazolinethiones(6a)[3]. The tin derivatives (6b) is an effective fungicide and antimicrobial activity is shown by thiones(6c)[4]. Anti-inflammatory, sedative and analgesic properties were reported for aryloxadiazoles(5c)[5]. Amino-oxadiazoles(5d) show analgesic activity and amono-oxadiazoles(5e) exhibit both antiinflammatory and antiproteolytic properties[6]. Anticonvulsant and nervous system depressant activity was reported for amino-oxadiazoles(5f), where R is quinazolin-3-yl group[7]. Amino-oxadiazole(5g) show local anaesthetic activity[8]. The oxadiazolinone(6d) is an orally active antiallergic agent, for example in the treatment of asthma and allergy disease and is claimed to be more potent than sodium cromoglycate[9]. Examples of the many oxadiazolones for the many herbicidal activity(week killers) are(6e,6f) and “oxadiazon”(6g), which is the subject of many regular reports in the literature. Insecticidal activity is shown by oxadiazolones(6h,6i the later is an aphicide), and oxadiazole(5h)

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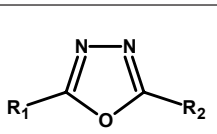
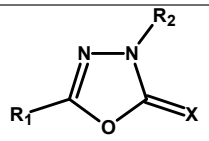


5 (a-h)

	R^1	R^2
5a	Ar	$CH_2CONHCONHR$
5b	$AROCH_2$	NHCOR
5c	trimethoxy or	3,4-dimethoxyphenyl
5d	2-pyridyl	NR_2HCl
5e	4-biphenylylmethyl	NHAr
5f	Ar	$NHCH_2CONHR$
5g	Ar	$NHCO(CH_2)_nNRR'HCl(n=2or3)$



6 (a-i)

	R^1	R^2	X
6a	$heteroarylOCH_2$	H	S
6b	1-methylcyclopropyl	$Sn(Ph)_3$	O
6c	5-Cl-2-phenylindol-3-ylNH	H	S
6d	3-Cl-benzo[b]thiophen-2-yl	H	O
6e	4-cyclohexylphenoxy	H	O
6f	2,4-diCl-phenoxymethyl	Bn	O
6g	t-Bu	2,4-diCl--5-isopropoxyphenyl	O
6h	OCH_3	o-methoxyphenyl	O
6i	CH_3NH	2,3-diH-2,2,4-triMebenzofuran-7-yl	O

Pharmacology

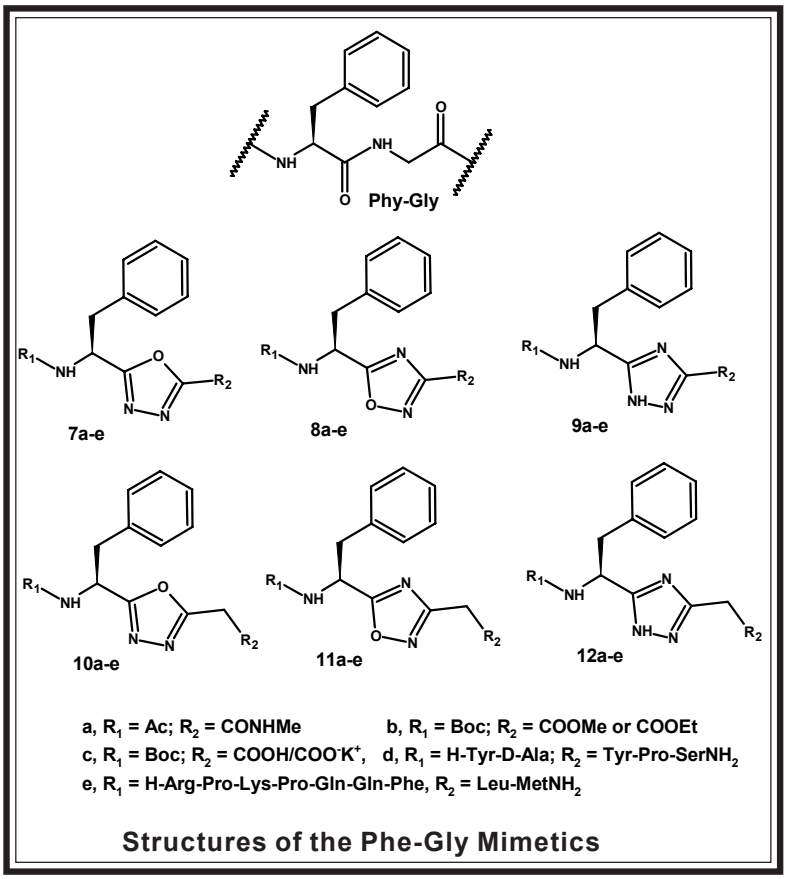
❖As Phenylene-Glycine dipeptidomimetics

Although several different strategies have been used in the development of peptidomimetic compounds, it is still not fully understood how to rationally convert a peptide into a nonpeptide while maintaining the desired biological activity. The structural and biological effects caused by replacement of important dipeptide fragments in biologically interesting peptides with nonpeptidic moieties were reported[10,11]. Frequently, only a small part of the peptide(4-8 amino acids), the binding epitope, is responsible for the recognition and binding to the receptor. These key amino acids can be used as the starting point for the design of nonpeptidic receptor ligands.

Susana Borg et al[12] have synthesized enantiopure, Phe-Gly dipeptidomimetics containing 1,3,4-oxadiazole, 1,2,4-oxadiazole, and 1,2,4-triazole ring systems as building blocks in the synthesis of pseudopeptides. Three derivatives (7-9) have the carboxylic acid function directly bound to the heterocyclic ring, and three derivatives (10-12) have an extra methylene group between the heterocyclic ring and the acid function to allow for an increased conformational flexibility. The three heterocycles are known as efficient ester and/or amide isosteres,[13] but only the 1,2,4-triazole has previously been used in peptide mimicry.[14,15] Although similar in size and shape, the ring systems show variations in aromatic, electrostatic, and hydrogenbonding properties: (i) all three heterocycles can participate in hydrogen bonding, the oxadiazoles as acceptors and the triazole as both acceptor and donor; (ii) the triazole and the 1,3,4-oxadiazole rings are aromatic, whereas the 1,2,4-oxadiazole

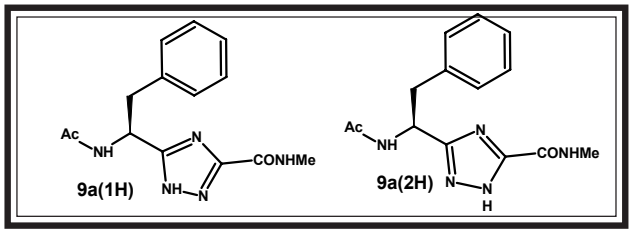
is better described as a conjugated diene;[16] and (iii) only the 1,2,4-triazole displays any acidic or basic properties with p*K*a values of 2.19 (as base) and 10.26 (as acid).[17] These variations provide an opportunity to evaluate properties of importance for amide bond mimicry and to determine the characteristics of the peptide backbone that are crucial for bioactivity.

The mimetics were used as Phe-Gly replacements in the biologically active peptides dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) and substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH_2, SP). The biological evaluation was performed by testing the µ- and ä-opioid receptor affinities of the dermorphin pseudopeptides and the NK1 receptor affinities of the SP pseudopeptides.



Structures of the Phe-Gly Mimetics

In order to carry out appropriate conformational searches, molecular mechanics calculations were performed on the N-acetylated and C-terminally methyl-amidated (Ac- Phe-Gly-NHMe) derivatives, to better simulate the Phe-Gly moiety within a peptide chain. Two tautomers of the 1,2,4-triazole derivatives were used for further calculations and comparisons [9a(1*H*), 9a(2*H*) and 12a(1*H*), 12a(2*H*)].[13d] Low-energy conformations of all derivatives were identified and imported for further analysis.



Only 12 unique conformers were found by the conformational search of Ac-Phe-Gly-NHMe. Among these three stretched conformations (maximum distance between terminal ends), two ,â-turn conformations, four conformations with a ã-turn in the Gly part, and one conformation with a ã-turn were identified in the Phe part. The later conformation was highest in energy. The stretched conformations were among the low-energy conformations, whereas the ,â-turn conformations were higher in energy.

To study the geometrical similarities, all individual low-energy conformations of each mimetic were superimposed on the 12 low-energy conformations of Ac-Phe-Gly-NHMe. In total six atoms were fitted, the amide oxygen and nitrogen atoms of the dipeptide and the corresponding atoms in the mimetics. Thus, the 12 conformations of Ac-Phe-Gly-NHMe were superimposed on 48 conformations each of 7a and 8a, 36 conformations of 9a(1*H*), 38 conformations of 9a(2*H*), 116 conformations of 10a, 74 conformations of 11a, 72 conformations of 12a(1*H*), and 108 conformations of 12a(2*H*). Comparisons of fits to one stretched and one ,-turn low-energy conformation of Ac-Phe-Gly-NHMe, to which the peptidomimetics have energetically ‘allowed’ conformations with close resemblance (good fits), are shown in Figure(A-D). The geometric comparisons of 7a-9a with Ac-Phe-Gly-NHMe in the stretched conformation show good fits (Figure A), but the same comparisons with the turn conformation are less attractive (Figure C). The geometric comparison using the mimetics containing a methylene spacer (10a-12a) produce considerably better fits with the turn conformation of Ac-Phe-Gly-NHMe (Figure D) compared to the stretched conformation (Figure B), demonstrating that the additional conformational flexibility inherent in these mimetics can improve the similarity of certain geometric properties.

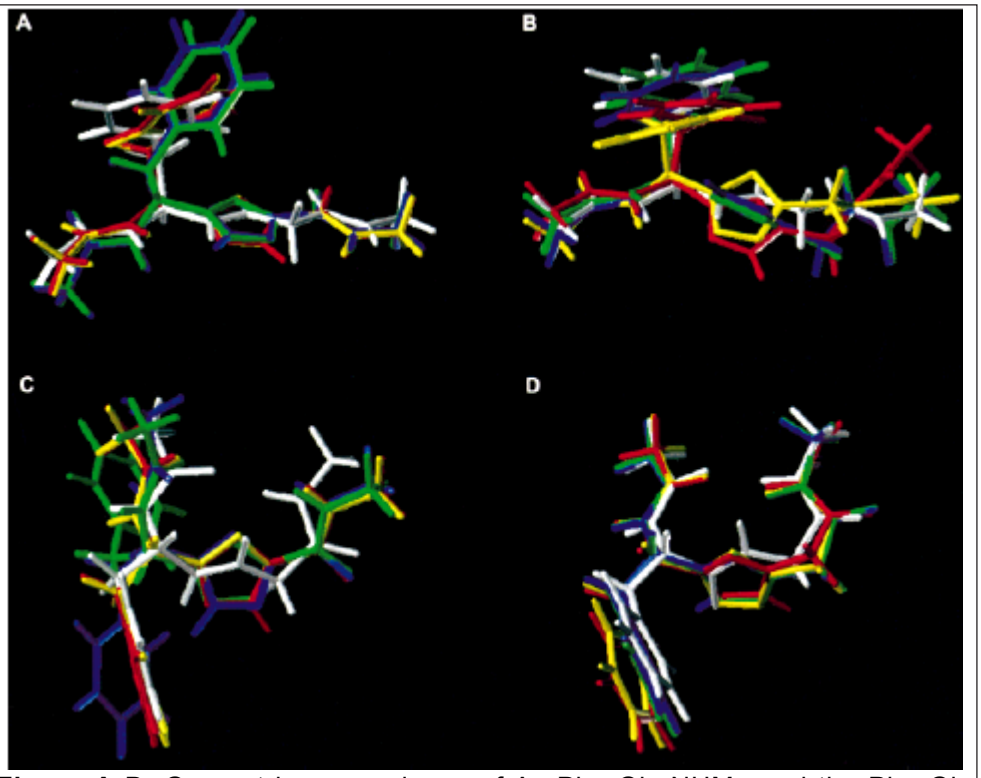


Figure A-D. Geometric comparisons of Ac-Phe-Gly-NHMe and the Phe-Gly mimetics 7a-9a in a stretched conformation **(A)** and in a ,â-turn conformation **(C)** and the corresponding comparisons of Ac-Phe-Gly-NHMe and 10a-12a **(B and D, respectively)**. Six atoms were fitted in the comparisons: the amide oxygen and nitrogen atoms of the dipeptide and the corresponding atoms of the mimetics. Ac-Phe-Gly-NHMe is shown in white,7a and 10a in yellow, 8a and 11a in green, 9a(1*H*) and 12a(1*H*) in blue, and 9a(2*H*) and 12a(2*H*) in red.

In order to consider electrical properties along with geometrical and confirmational similarities, the confirmations used in the fits were subjected to electrostatic potential calculations from AM1 atomic charges[18]. In Figures E and F the electron density surfaces of the compounds are displayed and color-coded according to magnitude. The results obtained for Ac-Phe-Gly-NHMe and 10a (Figure F) were compared with ab initio calculations[19]. The energy density surfaces obtained by both levels of calculations were in good agreement thereby providing support for the use of the less timeconsuming semiempirical calculations. None of the mimetics perfectly matched the electrostatic propertiesof the parent dipeptide. However, the oxadiazole derivatives seem to be better mimetics of Ac-Phe-Gly-NHMe than the triazole derivatives in terms of electrostatics, the 1,3,4-oxadiazoles being the best in both series of mimetics.

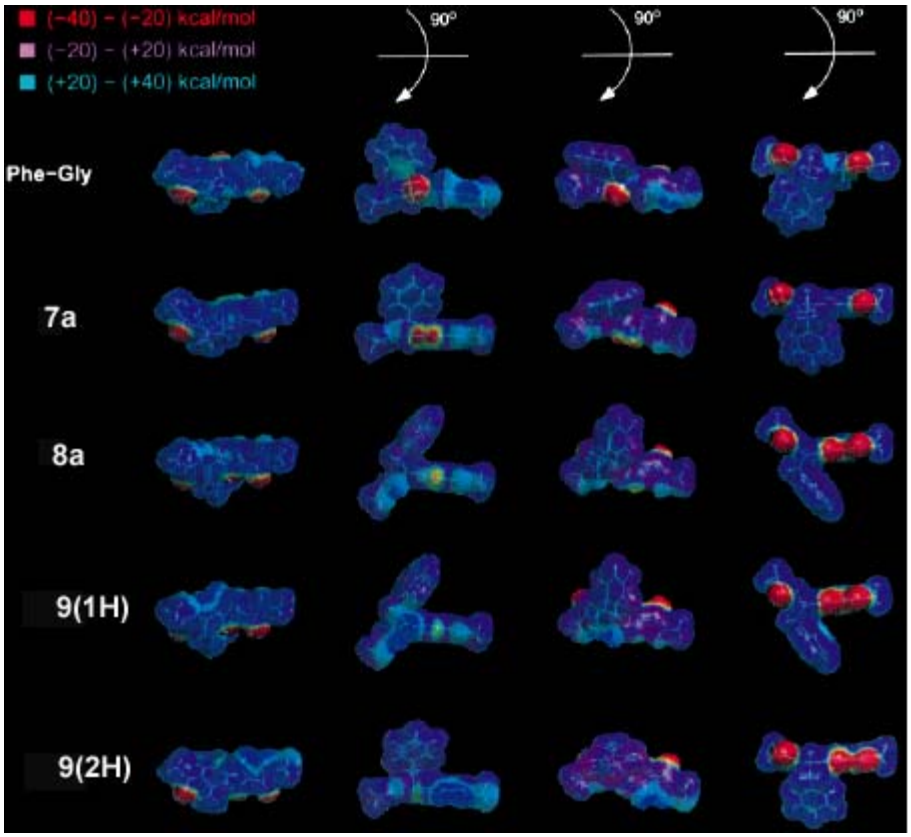


Figure E. Comparisons of electron density surfaces of Ac-Phe-Gly-NHMe and 7a-9a in stretched conformations (see **Figure A**), displayed and color-coded according to magnitude. Four different orientations are shown, to allow for accurate comparisons (rotation in 90° increments around the *x*-axis). Red areas represent potentials ranging from -40 to -20 kcal/mol, purple areas represent potentials ranging from -20 to +20 kcal/mol, and blue areas represent potentials ranging from +20 to +40 kcal/mol.

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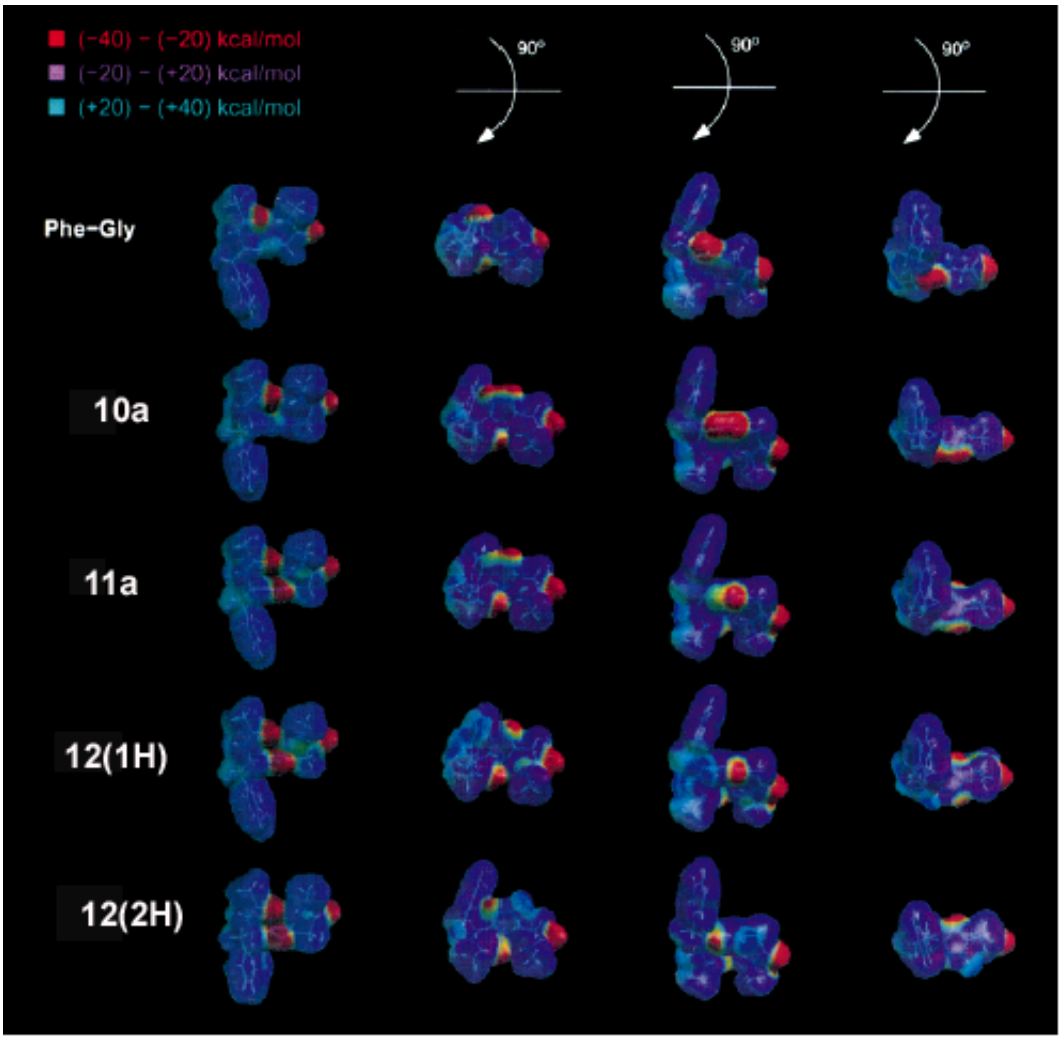


Figure F. Comparisons of electron density surfaces of Ac-Phe-Gly-NHMe and 10a-12a in ,â-turn conformations as shown in (**Figure D**), displayed and color-coded according to magnitude (see legend to **Figure E** for color coding).

The results showed that all mimetics except 3 were excellent replacements of Phe-Gly in dermorphin since they displayed affinities for the ì-receptor (IC_{50} 12-31 nM) in the same range as dermorphin itself (IC_{50} 6.2 nM).The agonist activity of three pseudopeptides at human ì-receptors was also evaluated. It was shown that the tested compounds retained their agonist activity. The SP pseudopeptides showed considerably lower affinities (IC_{50} > 1 ìM) for the NK1 receptor than SP itself (IC50 1.5 nM) indicating that the Phe-Gly replacements prevent the pseudopeptides from adopting bioactive conformations. Insertion of the novel dipeptide mimetics into SP led a dramatic loss in affinity for the rat NK1 receptor, the most potent pseudopeptide being 150-fold less potent than SP in terms of NK1 receptor affinity. Hence, the heterocyclic mimetics

used herein are not useful as mimics of Phe-Gly in SP. The reduction in affinity of the pseudopeptides might be due to undesirable steric, electrostatic, or other properties such as an inability to adopt energetically favorable bioactive conformations. However, during the design process, force-field calculations were used to establish that the conformational preferences of the synthesized Phe-Gly mimetics were quite similar to those of Phe-Gly. Therefore, it was more likely that other properties of the Phe-Gly mimetics were detrimental to the activity of the SP pseudopeptides. In contrast to the above, insertion of the dipeptide mimetics into dermorphin led to potent and selective pseudopeptides as they had high affinities for ì-receptors and low affinities for ä-receptors.

❖As Tyrosinase Inhibitors

Tyrosinase, also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme, widely distributed in plants and animals. It catalyses the o-hydroxylation of monophenols and also the oxidation of o-diphenols to o-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Therefore, tyrosinase inhibitors should be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn.

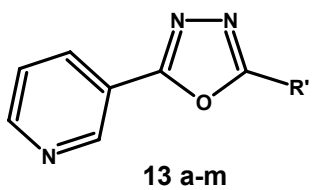
In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms.[20] In insects, several functions of this enzyme have been reported in the generation of o-diphenols and quinones for pigmentation, wound healing, parasite encapsulation, and sclerotization and the enzyme is an alternative target site for the control of insect pests. In food industry, tyrosinase is responsible for the enzymatic browning reactions in damaged fruits during post-harvest handling and processing. Control of enzymatic browning during processing is important in fruit pulp manufacturing. In addition, tyrosinase inhibitors are becoming important constituents of cosmetic products that relate to hyperpigmentation.

Therefore, there is a concerted effort to search for naturally occurring tyrosinase inhibitors from plant, because plants constitute a rich source of bioactive chemicals and many of them are largely free from harmful adverse effects.[21]

Ahmad et al. in 2004 reported that, a new coumarinolignoid 80-epi-cleomiscosin A together with the new glycoside 8-O-â-D- lucopyranosyl-6-hydroxy-2-methyl-4H-1-benzopyrane-4-one, exhibited strong inhibition against the enzyme tyrosinase, when compared to the standard tyrosinase inhibitors kojic acid and L-mimosine. The new coumarinolignoid exhibited two times more potency than that of the standard potent inhibitor L-mimosine.[22] Karbassi et al. reported the inhibition kinetics of two new synthetic bi-pyridine molecules, [1,4']bipiperidinyl-10-yl-naphthan- 2-yl-methanone and [1,4']bipiperidinyl-10-yl-4- methylphenyl-methane of the catecholase activity of mushroom tyrosinase. The kinetics studies indicated that these are uncompetitive inhibitors and the values of the Ki are 5.87 and 1.31 μM, respectively, which showed high potency. Fluorescent studies confirmed the uncompetitive type of inhibition for these two inhibitors. They also suggested that, the inhibition mechanism presumably coming from the presence of a particular hydrophobic site which can accommodate these inhibitors. This site could be formed due to a probable conformational change that was induced by binding of substrate with the enzyme.[23]

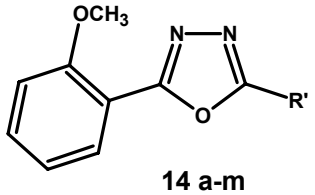
Very recently, Mahmud Tareq hassan Khan, Atta-ur-Rahmann and co-researchers[24] have demonstrated tyrosinase inhibition studies of library of 2,5-disubstituted-1,3,4-oxadiazoles along with their structure activity relationship. There were 26 derivatives of two types of basic oxadiazole skeleton have been studied to explain their inhibition patterns and structure activity relationship against tyrosinase enzyme. In one type of compounds, substitutions were changing at different positions of the phenyl ring at C-5 while keeping the pyridine ring constant at C-2 (13a-m),

while another type of compounds, substitutions were changing at different positions of the phenyl ring while keeping the o-methoxy phenyl ring constant at C-2 position (14a-m).



13 a-m

13a, R' = C_6H_5, 13b, R' = o-$NO_2C_6H_4$, 13c, R' = o-BrC_6H_4, 13d, R' = m-BrC_6H_4 , 13e, R' = p-BrC_6H_4, 13f, R' = 3-pyridyl, 13g, R' = CH_2Cl, 13h, R' = $CHCl_2$, 13i, R' = CCl_3, 13j, R' = p-$CH_3C_6H_4$, 13k, R' = 3,4,5-tri$OCH_3C_6H_2$, 13l, R' = 2-naphthyl, 13m, R' = 1-naphthyl



14 a-m

14a, R' = C_6H_5, 14b, R' = o-$NO_2C_6H_4$, 14c, R' = o-BrC_6H_4, 14d, R' = m-BrC_6H_4 , 14e, R' = p-BrC_6H_4, 14f, R' = 3-pyridyl, 14g, R' = CH_2Cl, 14h, R' = $CHCl_2$, 14i, R' = CCl_3, 14j, R' = p-$CH_3C_6H_4$, 14k, R' = 3,4,5-trimethoxyphenyl, 14l, R' = 2-naphthyl, 14m, R' = 1-naphthyl

The biological screening results suggested that compound 13a exhibited potent tyrosinase inhibition and the IC_{50} value was 5.15ìM, where the IC_{50} value of reference tyrosinase inhibitor kojic acids (KA) was 16.67 ìM. This compound was totally unsubstituted. Substitution with nitro group the resulting 3b was showing highly potent (IC_{50} =3.18 ìM) inhibition against tyrosinase, when compared with highly potent reference tyrosinase inhibitor L-mimosine (LM) (IC_{50} = 3.68 ìM). Due to the substitution of this nitro group the resulting compound exhibited potent inhibition. But when the same phenyl ring was found to have bromine atom at ortho position (13c,IC_{50} = 5.23 ìM) and meta position (13d, IC_{50} = 6.04 ìM) , the activities were decreased although the potency was much better than the KA. Again when the bromination was done at para position, the resulting compound 13e exhibited highest potency against the enzyme tyrosinase and IC_{50} value is 2.18 ìM, which is 1.96 times more potent than the LM. When pyridine was attached at C-5 position, the resulting

compound 13f was showing highly potent (IC_{50} = 3.29 ìM) inhibition against tyrosinase, even better than the LM.When chloromethyl group was present at the C-5 position, the resulting compound 13g was showing highly potent

(IC_{50} = 4.18 ìM) inhibition against tyrosinase, if compared with the KA, which is 3.99 times more potent. At the same position one more chlorine was attached, the resulting compound 13h exhibited little more potency (IC_{50} = 4.01 ìM) than the previous one. Finally when all chlorine atoms are attached, the resulting 13i exhibited more potency (IC_{50} = 3.98 ìM) than the previous two compounds. These compounds confirmed that, for the better inhibition of tyrosinase, electronegativity is necessary and the tyrosinase inhibition is directly proportional to the electronegativity of substituent(s). When the compound was substituted with 2-naphthyl group at C-5 position, the resulting compound 13l was showing potent (IC_{50} = 3.23 ìM) inhibition, if compared with LM.

In the second series of the library of compounds, substitutions introduced at C-5 position of the oxadiazole ring, while keeping the methoxy group at C-2. When bromine was present at meta position, the resulting compound 14d was showing potent inhibition (IC_{50} = 7.18 ìM) when compared with KA. When bromine was present at para position as in the case of compound 14e, then the potency of the compound decreased (IC_{50} = 7.82 ìM). Again it has been noted that when bromine is present at ortho rather than at meta, as in the case of compound 14c, the potency has been found to increase (IC_{50} = 5.16 ìM) and exhibit better inhibition than the 14d and 14e. When the bromine was totally replaced by phenyl ring, as in the case of compound 14a, then the potency of the compound decreased (IC_{50} = 8.71 ìM). It means that, the presence and the position of the –Br is essential for tyrosinase inhibition, which is also supporting the .ndings of Wang et al.[25] When the tricholoromethyl group is present at C-5 position 14i of oxadiazole ring the compound showed better inhibition (IC_{50} = 6.21 ìM), when compared with the compound 14h (IC_{50} = 7.28 ìM), where one –Cl was replaced with proton. The compound 14j, containing methyl group at aromatic ring, exhibited excellent potency (IC_{50} = 6.45 ìM).

The comparative tyrosinase inhibition study of each candidate summerized graphically in Figure-G. It can be concluded from this study that for the better inhibition of enzyme tyrosinase, electronegative changeover is crucial and the location of the group is also imperative in support of the inhibition. If the electronegativity is increased the inhibition also increases. The compound 13e (3-[5-(4-bromophenyl)-1,3,4-oxadiazol- 2-yl]pyridine) exhibited most potent inhibition against the enzyme tyrosinase, while compared with both of the reference inhibitors.

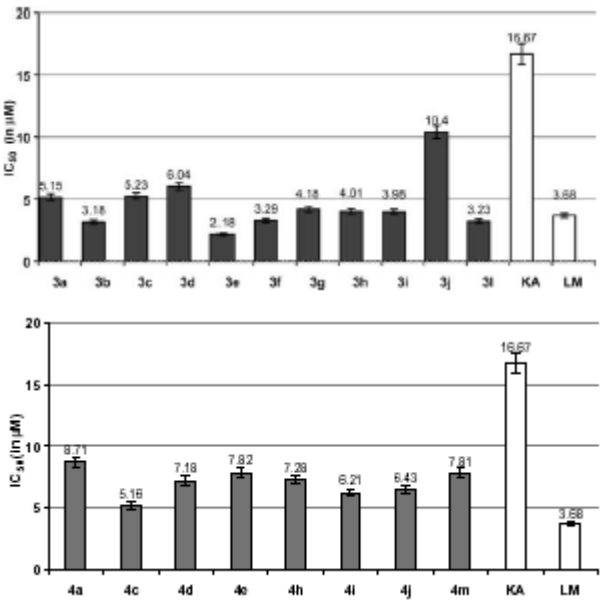


Figure - G

Graphical presentation of the comparative IC_{50} values of the series 3 (a–m) against the enzyme tyrosinase.

Graphical presentation of the comparative IC_{50} values of the series 4 (a–m) against the enzyme tyrosinase.

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❖As TNF-á Inhibitors & Cyclic Nucleotide Phospodiesterase Inhibitors

Tumor necrosis factor-á is a cytokine which is released preliminary by cells of immune systems in response to certain immunostimulators. When administered to animals or humans, it causes inflammations, fever, cardiovascular effects, haemorrhage, coagulation, cachexia and acute phase responses similar to those seen during acute infections, inflammatory diseases and shock states. Excessive or unregulated TNF-á production has been implicated in a number of disease conditions. These include endotoxamia and/or toxic shock syndrome[35.1,35.2], rheumatoid arthritis, inflammatory bowel disease, cachexia[35.3] and lupus. TNF-á concentration in excess of 12,000pg/ml have been detected in pulmonary aspirates from Adult Respiratory Distress Syndrom(ARDS) patients[35.4]. Systemic infusion of recombinant TNF-á resulted in changes typically seen in ARDS[35.5].

TNF-á appears to be involved in a number of bone resorption diseases, including arthritis. When activated, leukocytes will produce bone resorption. TNF-á apparently contributes to this mechanism[35.6,35.7]. TNF-á also has been shown to stimulate bone resorption and inhibit bone formation *in vitro* and *in vivo* through stimulation of osteoclast formation and activation combined with inhibition of osteoblast functions. Another compelling link with disease is the association between production of TNF-á by tumor or host tissues and malignancy associated hypercalcemia[35.8]. In Grant versus Host Reactions, increased serum TNF-á levels have been associated with major complications following acute allogenic bone marrow transplants[35.9]. High levels of TNF-á are associated with Crohn's disease[35.10].

Cerebral malaria is a lethal hyperacute neurological syndrome associated with high blood levels of TNF-á and the most severe complications occuring in malaria patients. Elevated levels of serum TNF-á correlated directly with the severity of disease and the prognosis in patients with acute malaria attacks [35.11]

TNF-á plays a role in the area of chronic pulmonary inflammatory diseases. The deposition of silica particles leads to silicosis, a disease of progressive respiratory failure caused by a fabrotic reaction. Antibodies to TNF-á completely blocked the silica induced lung fibrosis in mice[35.12]. High levels of TNF-á production(in the serum and isolated macrophages have been demonstrated in animal models of silica and asbestos induced fibrosis[35.13]. Alveolar macrophages from pulmonary sarcoidosis patients have also been found to spontaneously release massive quantities of TNF-á as compared with macrophages from normal donars[35.14].

Elevated levels of TNF-á are implicated in reperfusion injury, the inflammatory response which follows reperfusion and is a major cause of tissue damage after blood loss[35.15]. TNF-á also alters the properties of endothelial cells and has various pro-coagulant activity, supressing the anticoagulant protein-c path way and down regulating the expression of thrombomodulin[13.16]. TNF-á has proinflammatory activities which together with its early production(during the initial stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to, myocardial infarction, strock and circulatory shock.

It has been reported that TNF-á is apotent activator of retrovirus replication including activation of HIV-1[35.17 - 35.21]. Cytokines, specifically TNF-á, are implicated in activated T-cell mediated HIV protein expression and/or virus replication by playing a role in maintaining T-Lymphocyte activation. Therefore, interference with cytokine activity such as prevention or inhibition of cytokine production, notably TNF-á, in an HIV infected individual assist in limiting the maintanance of T-lymphocyte caused by HIV infection. Additional studies have identified TNF-á as a common factor in the activation of HIV *in vitro* and have provided a clear mechanism of action via nuclear regulatory protein found in the cytoplasm of cells[35.22]. AIDS viral replication of latent HIV in T-cell and macrophage lines can be induced by TNF-á[35.23]. A molecular mechanism for the virus inducing

activity is suggested by TNF-á's ability to activate gene regulatory protein. (transcription factor, NFkB) found in the cytoplasm of the cells, which promotes HIV replication through binding to a viral regualtory gene sequence(LTR)[35.22]. TNF-á in AIDS associated cachexia is suggested by eleveted serum TNF-á and high levels of spontaneous TNF-á production in peripheral blood monocytes from patients[35.24]. TNF-á has been implicated in various roles with other viral infections, such as the cytomegalia virus(CMV), influenza virus, adeno virus and the herpes family of viruses for similar reasons as those noted.

Many cellular functions are mediated by levels of adenosine-3',5'-cyclic monophosphate(cAMP). Such cellular functions can contribute to inflammatory conditions and diseases including asthma, inflammation and other conditions[35.25]. It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-á and NFkB. Increased level of cAMP also leads to the relaxation of airway smooth muscle.

The primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes refered to as cyclic nucleotide phosphodiesterase (PDE)[35.26]. There are ten known members of the family of PDEs. It is well documented that the inhibition of PDE type IV (PDE4) enzyme is particularly effective in both the inhibition of inflammatorymediator release and the relaxation of airway smooth muscle[35.27]

Decreasing in TNF-á levels constitutes a valuable therapeutic strategy for the treatment of many inflammatory, infectious, immunological and malignant diseases. These include but are not restricted to: septic shock, endotoxic shock, hemodynamic shock and sepsis syndrome, post ischemic reperfusion injury, malaria, mycobacterial infections, meningitis, psoriasis and other dermal diseases, congestive heart failure, fibrotic disease, opportunistic infections in AIDS, rheumatoid arthritis, rheumatoid

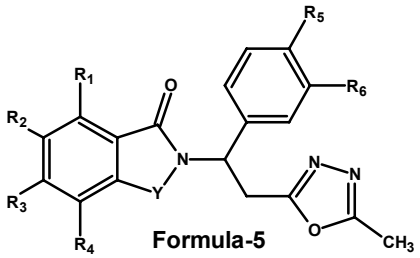
spondylitis, osteoarthritis, other arthritic conditions, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus, erythrematosis ENL in leprosy, radiation damage and hyperoxic injury.

Prior efforts directed to the supression of the effects of TNF-á have ranged from the utilization of steroids such as dexamethasone and prednisolone to the use of both poly clonal and monoclonal antibodies[35.28]. Validation of TNF-á inhibition as a clinical therapy has been demonstrated by use of TNF-á antibodies and soluble TNF-á receptors.TNF-á blockage with monoclonal antiTNF-á antibodies has been shown to be beneficial in rheumatoid arthritis[35.29].

Hon-Wah Man and George W.Muller[35.30] have patented substituted 1,3,4-oxadiazole of formula-5 based on the discovery that certain classes of non-polypeptide compounds descreasesthe level of TNF-á and/or inhibit PDEs particularly PDE4 and/or inhibit angeiogenesis and/or useful in the treatment of cancer, inflammatory and autoimmune diseases.

Compounds that selectively inhibit PDE4 specifically would atleast partially inhibit inflammation and relaxation of airway smooth muscle with minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. The compound of the invention are useful in the inhibition of phosphodiesterases, particularly PDE4 and in the treatment of disease states mediated thereby. More over the compound described in the invention can inhibit the action of NFkB in the nucleus and thus are useful in the treatment of a variety of diseases including but not limited to rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, septic shock, sepsis, endotoxic shock, graft versus host disease, wasting, inflammatory bowel disease, Crohn's disease, ulserative colitis, multiple sclerosis, systemic lupus, erythrematosis, ENL in leprosy, HIV-AIDS and opportunistic infections in AIDS.

The compounds of invention can also be used topically in the treatment of prophylaxisof topical disease states including, but not limited to atopic dermatitis, psoriasis, viral infections such as those caused by the herpes viruses or viral conjuctivitis.



Formula-5

Y= C=O, CH_2, SO_2 or CH_2C=O, X = H, H-$(CH_2)_n$-H where n=1 to 4, R_1, R_2, R_3, R_4 = H, halogen, CF_3, acetyl, alkyl of 1 to 6 carbons, NO_2, CN, OH, -CH_2NR'R, -NR'R, fused benzo, fuzed naphtho, fused quinoxaline, fused benoimidazole etc
R_5, R_6= H, alkyl of 1 to 4 carbons, alkoxy of 1 to 6 carbons, CN, benzocycloalkoxy, cycloalkoxy and bicycloalkoxy and tricycloalkoxy of upto 18 carbons, cycloalkylalkoxy of upto 18 carbons.

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❖As Monoamine OxidaseType-B Inhibitors

Monoamine oxidase (MAO; EC 1.4.3.4) is a FAD-containing enzyme[35.31] located in the outer membrane of mitochondria[35.32]. On the basis of specificity for substrates and sensitivity to inhibitors, two MA0 forms, called MAO A and MAO B, have been distinguished[35.33,35.34]. The two forms catalyze the oxidative deamination of a wide variety of biogenic monoamines[35.35], as well as xenobiotics.[35.36]. It has been shown that the two isoenzymes are distinct with about 70% homology in their primary sequence[35.37] and are coded by two different genes located on the X chromosome[35.38].

Both enzymes present a considerable pharmacological interest because of their key role in the metabolism of monoamine neurotransmitters like serotonin, catecholamines, and dopamine and their possible involvement in many neuropsychiatric disorders[35.39]. For example, motor disability in Parkinsonism is linked to a deficit of dopamine striatum, resulting from degeneration of the dopamine nigrostriatal neurones which project to striatum.

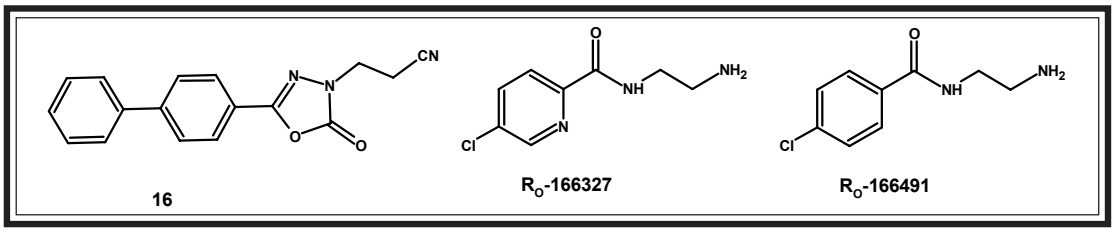
According to a concept which is gaining acceptance, MAO's of glial cells neighbouring nerve terminals play an important role in the metabolism of certain amine neurotransmitters as well as other amines such as false transmitters or intruding monoamines[35.40].

The origin of Parkinsonism is not yet known. It has been suggested that hydroxyl radicals •OH are implicated in the genesis of the disease.[35.41] Due to a deficit of glutathione in substantia nigra in Parkinsonian brains, accumulation of H_2O_2 from dopamine deamination with concommitant increase of Fe^{2+} would lead, via Fenton reaction, to an overproduction of •OH and nigral degeneration. Alternatively, proliferation of glial cells in replacement of dead neurons[35.42] may contribute to the breakdown of the neurotransmitter.

In modern Parkinson therapy, one major approach to overcome the lack of dopamine is substitution by L-dopa with adjunction of a MAO B inhibitor[35.43], either alone or with an antioxidant[35.44]. From controlled clinical

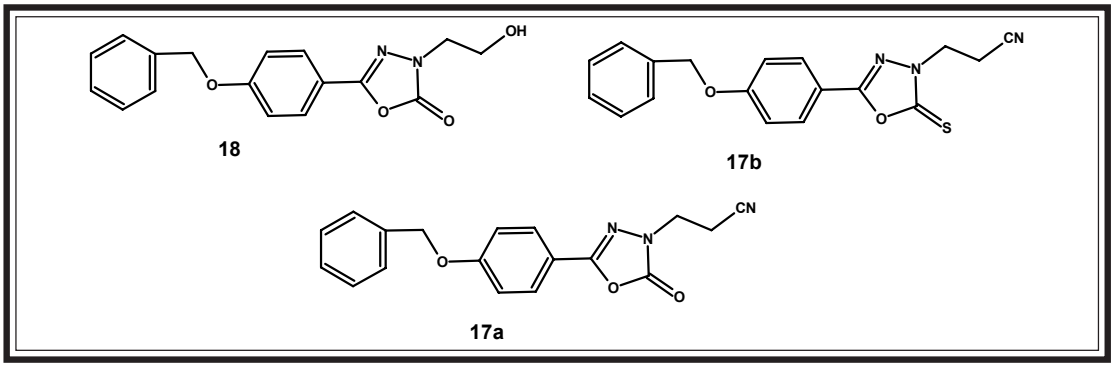
trials with L-deprenyl, a preferential MAO B inhibitor,[35.34] it has been confirmed that selective inhibitors of MAO B either alone[35.45] or as adjuncts to L-dopa[35.46] are beneficial in Parkinson management, especially in the early stages of the disease.

During decade of ninties, more attention has been focused on the development of reversible inhibitors, believed to be advantageous in therapeutic uses.[35.47]. The previously developed Ro 166327[35.48] and its less potent analogue Ro 166491[35.49] were the only two reversible MAO B inhibitors available. Interestingly, Ro 166327 has even proved to surpass the "suicide" inhibitors both in activity and selectivity.[35.40, 35.50]. Another new promising class of reversible MAO B inhibitors are the 1,3,4-oxadiazol-2(3H)-one derivative[35.51], with 16 as the representative compound.



On the basis of this findings, F.Mazouz, et al[35.52] have synthesized thirty-three new 5- [4-(benzyloxy)phenyl] - 1,3,4-oxadiazol-2(3H)-one derivatives including related analogues, designed as inhibitors of monoamine oxidase type B (MAO B) and investigated both *in vitro* and *ex vivo* for their abilities to inhibit selectively rat brain MAO B over MAO A. Three inhibitors were found to act as reversible, highly potent, and selective MAO B inhibitors, namely the nitrile derivative 5- [4-(benzyloxy)phenyll-3-(2-cyanoethyl)-l,3,4-oxadiazol-2(3H)-one (17a) and two closely related homologues, the corresponding oxadiazolethione 17b and the alcohol 18. Their IC_{50}(MAO B) values are in the low nanomolar range of 1.4-4.6 nM and their selectivities, estimated by the ratio of IC_{50} values (A/B), are from 3200 to >71400. Compound 17a exhibited the highest activity against MAO B. Its IC_{50} was evaluated to be 1.4 nM with a quasitotal selectivity (>71400) toward this enzyme. In *ex vivo* studies, 17a showed a reversible and short duration of action. MAO B was markedly inhibited with the

oral dose of 1 mg/kg without any alteration of MAO A, and the inhibition almost did not exceed 24 h. Its ED_{50} (1 h after oral administration) was evaluated to be 0.56 mg (1.7 μmol)/kg. Remarkably, MAO A was not affected at doses as high as 1500 mg/kg. In addition, no apparent toxicity or behavioral anomaly was observed during the treatment even at the maximum administrated dose. SAR studies emphasize the existence of three binding sites to the enzyme with a special importance of the terminal phenyl. Analysis of the inhibition kinetics indicated that 17a acts in a two-step mechanism as a competitive, slow, and tight-binding inhibitor of MAO B with a Ki value of 0.22 μM and an overall Ki* value at equilibrium of 0.7 nM.



❖As Antidepressants

Various substituted 2-pyrrolidine-2-yl-[1,3,4]-oxadiazole derivatives are known from literature. Common to all of them is the fact that they are used for the treatment of neuronal diseases. Hagen-Heinrich Hennies and coresearchers[35.53] have patented 2-pyrrolidin-2-yl-[1,3,4]-oxadiazole of general formula 19 have a pronounced anto-depressive and analgesic effect

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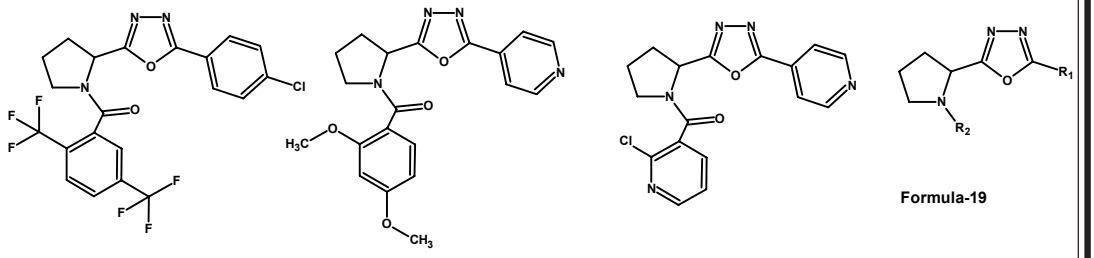


(2,5-bistrifluoromethylphenyl)-{2-[5-(4-chlorophenyl)-[1,3,4]-oxadiazol-2-yl)-pyrrolidin-1-yl)methanone

(2,4-dimethoxyphenyl)-[2-(5-pyridin-4-yl-[1,3,4]-oxadiazol-2-yl)-pyrrolidin-1-yl]methanone

(2-chloro-pyridin-3-yl)-[2-(5-pyridin-4-yl-[1,3,4]-oxadiazol-2-yl)-pyrrolidin-1-yl]methanone

Formula-19

The biological screening result suggested that the three compounds were found to be most potent serotonin uptake inhibitors namely (2,5-bistrifluoromethylphenyl)-{2-[5-(4-chlorophenyl)-[1,3,4]-oxadiazole-2-yl]-pyrrolidin-1yl}-methanone, (2,4-dimethoxyphenyl)-[2-(5-pyridin-4-yl-[1,3,4]-oxadiazol-2-yl)-pyrrolidin-1yl]-methanone and (2-chloro-pyridin-3-yl)-[2-(5-pyridin-4-yl-[1,3,4]-oxadiazol-2-yl)-pyrrolidin-1yl]-methanone with Serotonine reuptake inhibition of 80%, 81% and 84% respectively.

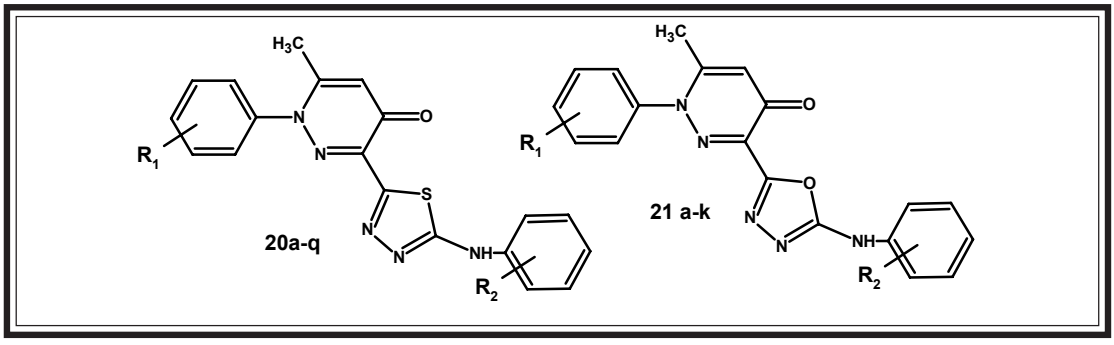
❖Fungicidal activity

The idea of bioisosterism is one of the most successful techniques of bioactive compound design[26]. The substitution of sulfur for oxygen in the heterocyclic ring represents an example of an approach that is commonly known as bioisosterism. The 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring.

Zu-Xing Zhang et al[27,28] reported that pyridazinone-substituted 1,3,4-thiadiazole exhibited highly fungicidal activity against wheat leaf rust, *Puccinia recondita*. On the other hand, 1,3,4-oxadiazoles exhibit a broad spectrum of biological activity [29-31]. In view of these facts and on the basis of chemistry of pyridazinones [32-34], Xia-Juan Zou et al[35] have synthesized bioisosterism based series of novel 5-[1-aryl-1,4-dihydro-6-methylpyridazin-4-one-3-yl] -2-arylamino-1,3,4-oxadiazoles, and tested *in vivo* against wheat leaf rust, *Puccinia recondita*.

These compounds were shown to be fungicidally active, and their activity was influenced by the nature of the substituents. By using the three-dimensional quantitative structureactivity relationships (3D-QSAR) method of comparative molecular field anaysis (CoMFA), the structure and activity relationship of the compounds containing both pyridazinonesubstituted 1,3,4-thiadiazoles(20a-q) and pyridazinone-substituted 1,3,4-oxadiazoles(21a-k) was established.

The fungicidal screening results of the compounds studied are summarized in Table-1. The results indicate that pyridazinone-substituted 1,3,4-oxadiazoles exhibited the same fungicidal activity as that of pyridazinone substituted 1,3,4-thiadiazoles. The fungicidal activity varies with the substituents of the phenyl moiety



Compd.	R_1	R_2	X	EA[a]	CoMFA	PA[b]	Residue[c]
20a	*o*-Cl	*m*-CF_3	S	0.7	185	0.838	−0.138
20b	*o*-Cl	*o*-F	S	0.1	172	0.099	0.001
20c	*o*-Cl	H	S	0.1	165	0.032	0.068
20d	H	*o*-F	S	0.5	164	0.609	-0.109
20e	H	*m*-CF_3	S	0.6	180	0.544	0.056
20f	2,6-Cl_2	*m*-CF_3	S	0.5	184	0.489	0.011
20g	2,6-Cl_2	*o*-F	S	0.3	174	0.364	-0.064
20h	*p*-Cl	*m*-CF_3	S	0.6	192	0.574	0.026
20i	*p*-Cl	*o*-F	S	0.5	169	0.532	-0.032
20k	2,4,5-Cl_3	*o*-F	S	0.6	180	0.619	-0.019
20l	2,4,5-Cl_3	*m*-CF_3	S	0.7	179	0.681	0.019
20m	2,4-2CH_3	*m*-CF_3	S	0.9	199	0.909	-0.009
20n	2,4-2CH_3	*o*-F	S	0.9	181	0.800	0.100
20o	2,4-Cl_2	*o*-F	S	0.8	172	0.680	0.120
20p	2,4-Cl_2	*m*-CF_3	S	0.9	185	0.851	0.049
20q	2,4-Cl_2	H	S	0.2	171	0.280	-0.080

Compd.	R_1	R_2	X	EA[a]	CoMFA	PA[b]	Residue[c]
21a	*o*-Cl	*m*-CF_3	O	0.9	181	0.770	0.130
21b	*o*-Cl	*o*-F	O	0.8	165	0.768	0.032
21c	*o*-Cl	H	O	0.1	161	0.128	-0.028
21d	H	*o*-F	O	0.9	161	0.949	-0.049
21e	2,6-Cl_2	*o*-F	O	0.8	172	0.828	-0.028
21f	*p*-Cl	*m*-CF_3	O	0.9	172	0.967	-0.067
21g	*p*-Cl	*o*-F	O	0.7	160	0.681	0.019
21h	2,4,5-Cl_3	*o*-F	O	0.9	181	0.881	0.019
21i	2,4-2CH_3	*m*-CF_3	O	0.7	196	0.727	-0.027
21j	2,4-2CH_3	*o*-F	O	0.8	185	0.792	0.008
21k	*p*-Cl	*p*-OCH_3	O	0.8	187	0.810	-0.010

[a]Experimental activities (percent inhibition × 10^{-2}). [b]Predictive activities(percent inhibition × 10^{-2}). [c]Residue = EA − PA.

Table-1. Experimental & Calculated Activities and Residual Values of the Title Compounds

In CoMFA study, compound 20m was selected as the template molecule because of its scaffold being involved in all compounds and its high activity. Nine features were selected for the alignment of all compounds. These atoms are numbered 1-9 in Figure H. The alignments of the bioactive conformation are showed in Figure I.

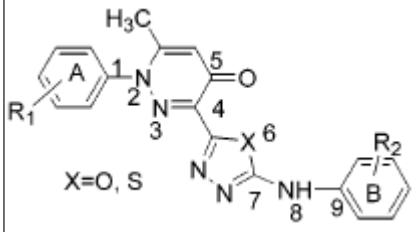


Figure H. Superposition modes.

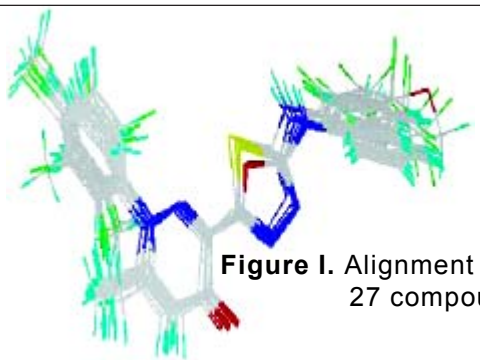


Figure I. Alignment of 27 compounds

The predictive activity versus the experimental activity is graphically represented in Figure J. The analyses results are presented in Tablel-1.

The 3D-QSAR modes gave good correlation between the variations on percent inhibition and the steric-electrostatic properties. The CoMFA contour maps of this series of compounds are presented in Figures K and L, respectively. The green-yellow steric contour in Figure K describes

that region in space where increasing (green) or decreasing (yellow) steric bulk is consistent with enhanced percent inhibition. The red-blue electrostatic contour in Figure L illustrates that the large blue areas are the regions where positive charge is favorable for the percent inhibition; red areas are unfavorable. One red polyhedra near the *para*phenyl ring B moiety indicates that electron rich groups are beneficial to the activity. Six blue contours in the molecules suggest that positive-charged substituents are favorable to increase the activity.

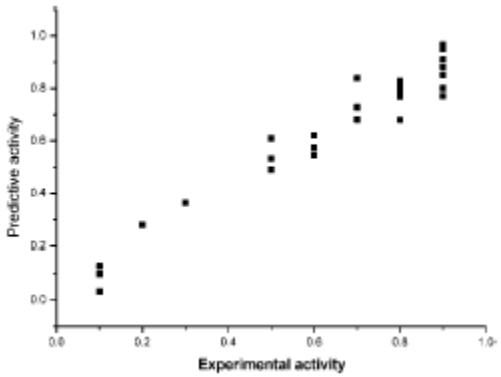


Figure J. Predictive and experimental activities for the CoMFA model.

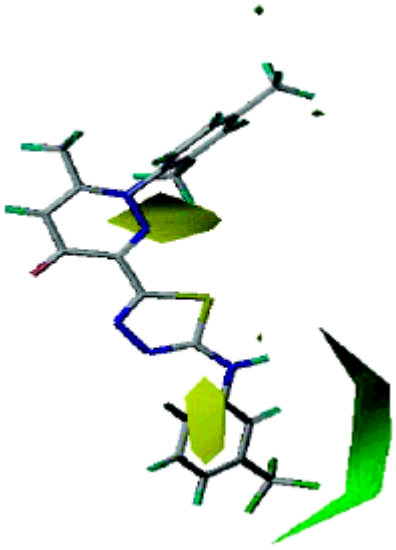


Figure K. Steric field contour map of CoMFA.

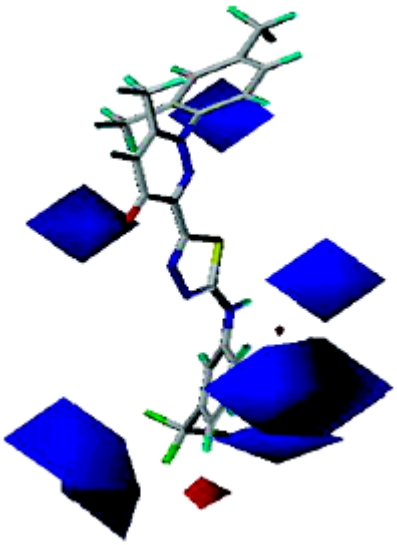
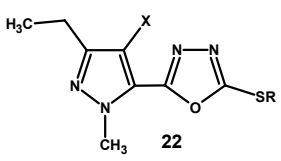


Figure L. Electrostatic field contour map of CoMFA

These results are consistent with a common mode of action for the pyridazinone- substituted 1,3,4-thiadiazoles and the pyridazinone-subsituted 1,3,4-oxadiazoles, which further confirms that the 1,3,4-oxadiazole ring is a bioisosteric analogue of the 1,3,4-thiadiazole ring.

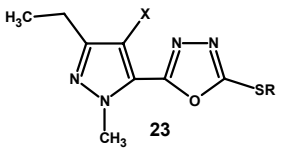
In 1994 Ashour et al[31] have sugeested that 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives being fungicidal. If they are linked to pyrazole ring, the resulting biheterocyclic compounds could have better fungicidal activity. In continuation with this, Lee H.S. et al[21] have synthesized some series of 2-alkyl (alkythio)-5-((4-chloro)-3-ethyl-1-methyl-1H-pyrazole-5-yl)-1,3,4-oxadiazoles (thiadiazoles) as potential fungicides. Their fungicidal activity was evaluated against rice sheath blight, which is a major disease of rice in China.

The Structure-Activity Relationship derived from fungicidal screening results, revealed that 5-(4-chloro-3-ethyl-1-methyl-1H-pyrazole-5-yl)-1,3,4-thiadiazole-2-thione has the higher fungicidal activity. The screening results indicated that compounds 23 had significant potency against *Rhizoctonia solani*, and compounds 22 had hardly any inhibition against *R.solani*, that is to say, the activity of compounds that were substituted by alkylthio at the 2-position of the 1,3,4-oxadiazole ring was higher than the activity of those compounds that were substituted by mercapto. The activity was found to fall off with increasing size of the alkyl group (*R*). The preferred substituent for *R* was found to be methyl. Loss of biological activity was observed if the chloride atom at the 4-position of the pyrazole ring was substituted by hydrogen. Oxidation of the sulfide group of compound **3** to 2-one analogue almost eliminated the activity. Removal of the sulfur atom, by replacement of the alkylthio group with an alkyl group, resulted in some decrease in activity. A sulfur atom at the 2-position of 1,3,4-oxadiazole is necessary for fungicidal activity to occur.



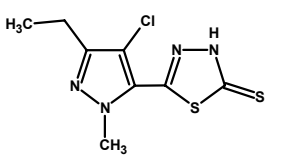
22

X = H, Cl R = H



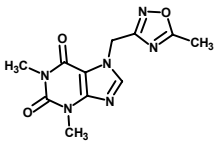
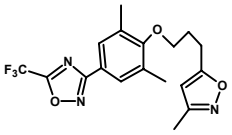
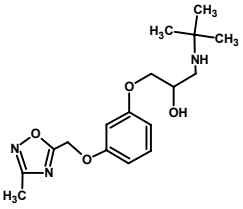
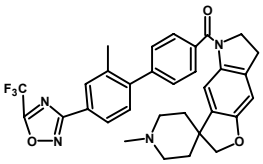
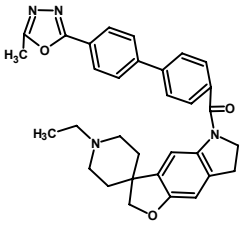
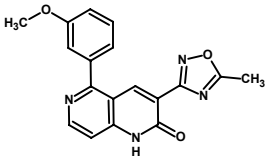
23

X = H, Cl
R = CH_3, n-C_3H_7, n-C_5H_{11}, n--C_7H_{15}, n-C_9H_{17}, $CH(CH_3)COOEt$, C_2H_5

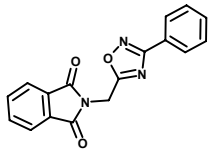
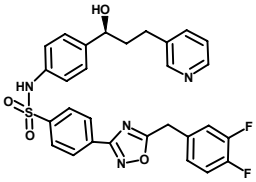
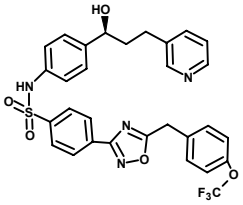
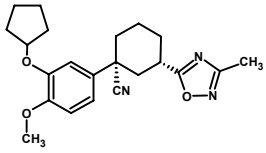
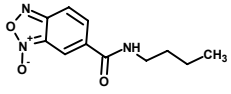
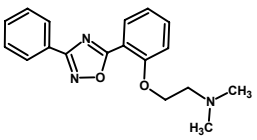


5-(4-chloro-3-ethyl-1-methyl-1*H*-pyrazol-5-yl)-1,3,4-thiadiazole-2(3*H*)-thione

Some Oxadiazole drugs & derivatives under Preclinical/Phase clinical trials.

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
Chinoin-170		Antitussive, Bronchodilator	Phase-I	Sanofi-Synthlabo
Pleconaril		Antirhinoviral, Antiviral	Phase-III	Viro pharma
Proxodolol		Antihypertensive, Antianginal, Antiglaucoma agent, Beta-adrenoceptor antagonist	Phase-II	Center for Chemistry of Drugs
SB-224289		Antidepressants, Anxiolytic, 5-HT_1D Antagonist	Biological testing	Smithkline Beecham
SB-236057		Antidepressants, Anxiolytic, 5-HT_1D Inverse agonist	Preclinical	Smithkline Beecham
SX-3933		Cognition enhancing drug, GABA(A) receptor modulator, GABA(A) B_2 site inverse agonist	Preclinical	Dainoppon pharma

Some Oxadiazole drugs & derivatives under preclinical/Phase clinical trials.

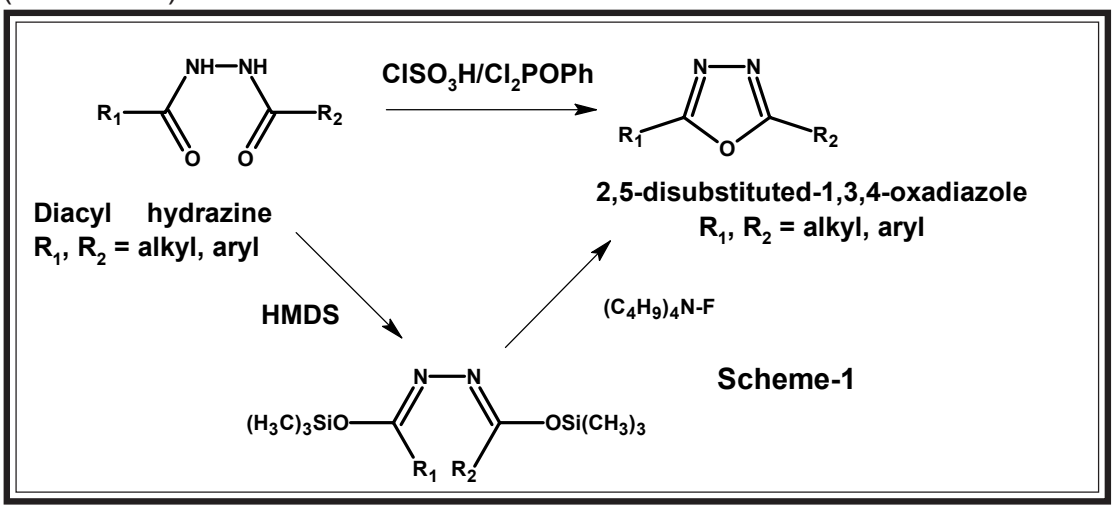
Drug Name/Code	Chemical Structure	Activity	Phase	Originator
-		Analgesic	Preclinical	Universidade federal pernambuco
-		Antiobesity drug, Antidiabetic drug, Beta3 adrenoceptor agonist	Preclinical	Merck
-		Antiobesity drug, Antidiabetic drug, Beta3 adrenoceptor agonist	Preclinical	Merck
-		Bronchodilator, Phosphodiesterase Inhibitor	Preclinical	Smithkline Beecham
-		Antitrypanosomal	Preclinical	Universidad de la republica
-		Antiepileptic drug, Neuronal Injury Inhibitor, AMPA antagonist, Sodium channel blocker	Preclinical	Boehringer Ingelaeim

Synthetic Approaches

There were several routes for the synthesis of 1,3,4-oxadiazoles reported in the literature among which the most important aspects of synthesis were discussed as under.

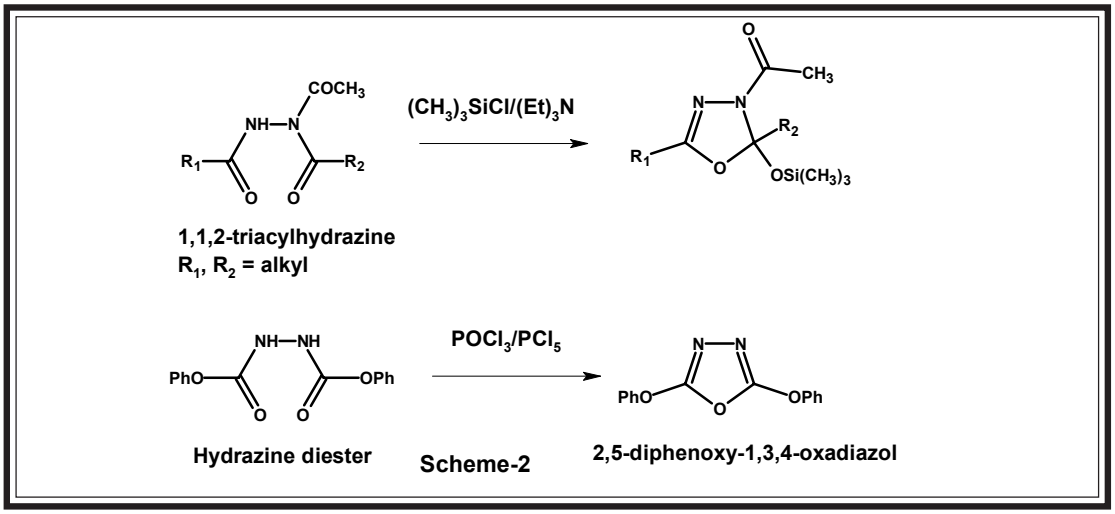
❖Cyclization with formation of one Bond

2,5-disubstituted 1,3,4-oxadiazole can be accompleshed by cyclodehydration of 1,2-diacylhydrazine either by using chlorosulphonic acid[36] or phenyl dichorophosphite[37]in dimethylformamide. A nonaqueous, nonacedic, route involves treatment of hydrazine with hexamethyl disilazane(HMDS) and tetrabutylammoniumfluoride, the last step presumably being fluoride catalysed cyclization of intermediate bis silyl ether[37] (Scheme-1)



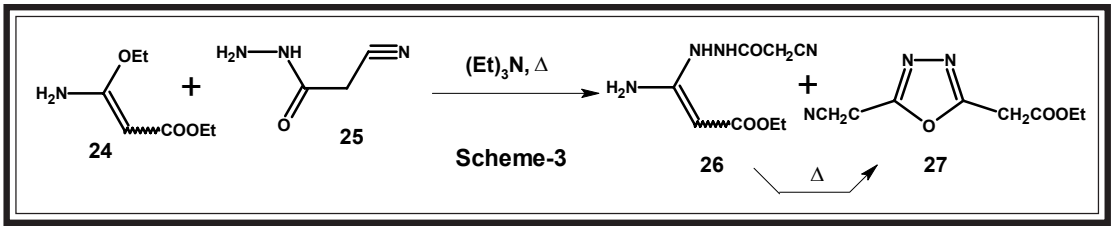
Scheme-1

In a related reaction, 1,1,2-triacetylhydrazine with trimethylsilylchloride/triethylamine gave oxadiazolinyl silylether[39]. Cyclodehydration(PCl_5/$POCl_3$) of hydrazinyl diester gave the diphenyloxyoxadiazole[40]. (Scheme-2)

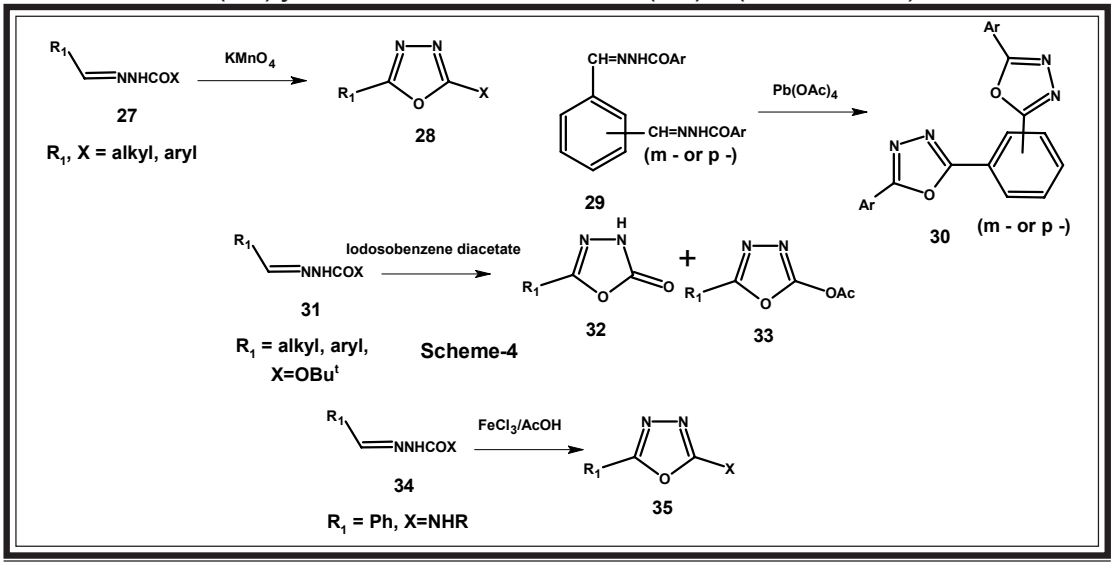


Scheme-2

The malonate derivative(24) reacted with acylhydrazine(25) to give a mixture of diacylhydrazine monoamine(26) and oxadiazole(27). The later was also formed from (26) on heating[41]. (Scheme-3)



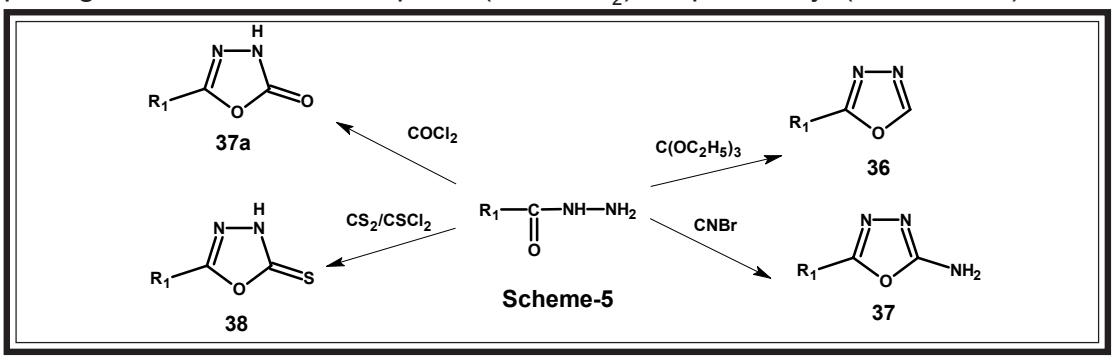
Oxidation of acylhydrazones derived(27) from aldehydes has been developed into a useful route to disubstituted oxadiazoles(28). The use of potassium permangante with acetone as solvent was claimed to give yields than the use of other oxidizing agents (e.g.halogens)[42]. An improved synthesis of bis-oxadiazolylbenzenes (30) involved oxidation of bishydrazones (29) with lead tetraacetate[43]. Acylhydrazones(31) were oxidized by iodosobenzene diacetate to oxadiazolinones (32), with acetates (33) also being formed in some cases. A similar oxidation of ethyl esters(9, X=OEt) gave oxadiazolyl ethers(11,X=OEt)[44]. Oxidative cyclization($FeCl_3$/AcOH) of semicarbazone(34) yielded amino-oxadiazoles(35)[45].(Scheme-4)



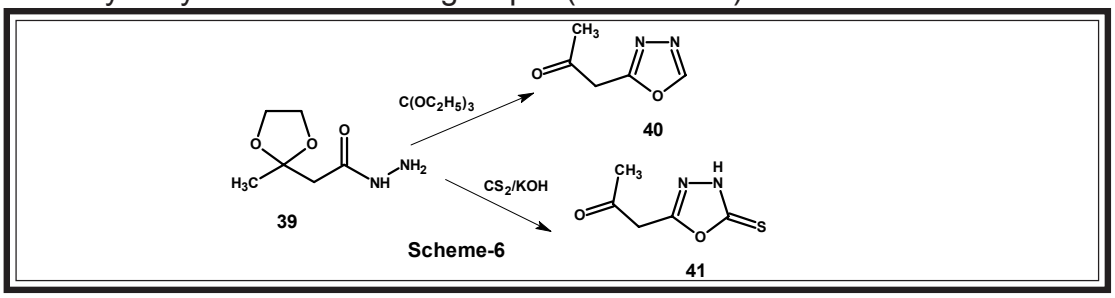
36. C.Chiriac, *Rev.Chim.*,(Bucharest),**34**,1131(1983), *Chem.Abstr.*,**100,**174735(1984)
37.C.Chiriac, *Rev.Chim.*,(Bucharest),**27**,935(1982), *Chem.Abstr.*,**98,**107216(1983)
38.B.Rigo, P.Cauliez, D.Fasseur, D.Couturier, *Synth.Commun.*,**16**,1665(1986)
39.A.Kalinin, B.Khasapov, E.Aposav, I.Kalikhman, S.Ioffe, *Izv.Akad Nauk SSSR Ser.Khim.,*694(1984),*Chem.Abstr.*,**101,**91045(1984)
40.A.Theocharis, N.Alexandrou,*J.Heterocycl.Chem.*, **27**,1685(1990)
41.M.Elnagdi, N.Ibrahim, F.Abdelrazek, A.Erian, *Liebigs Ann.Chem.*,909,1988
42.P.Reddy, P.Reddy, *Ind.J.Chem.Sect-B,* **26**,890(1987)
43.S.A.Rekkas, N.A.Rodias, N.E.Alexandrou,*Synthesis*, 411(1986)
44.H.Baumgarten, D.Hwang, T.Rao, *J.Heterocycl.Chem.*,**23**, 945(1986)
45.S.Hiremath, N.Goudar, M.Purohit, *Ind.J.Chem.Sect-B,* **21**,321(1982)

❖Cyclization with formation of Two Bonds

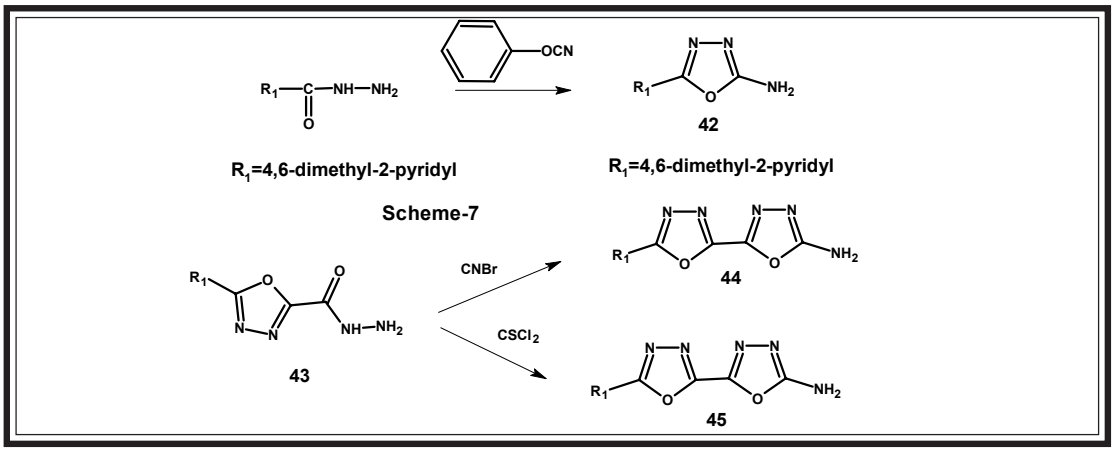
Important routes to monosubstituted oxadiazoles (36), aminooxadiazoles (37), oxadiazolinones(37a) and oxadiazolinethiones(38) involve reaction of hydrazides($R_1CONHNH_2$) with triethyl orthoformate, cyanogen bromide, phosgene, or carbon disulphide(or $CSCl_2$) respectively. (Scheme-5)



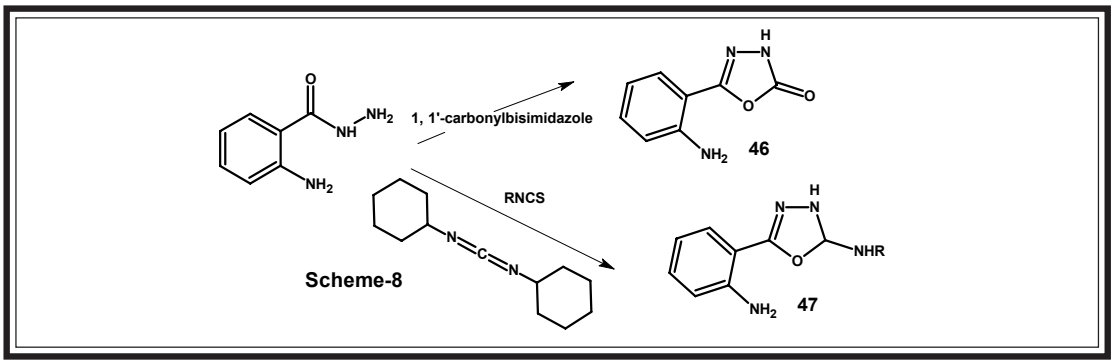
Reaction of hydrazide(39) with triethylorthoformate, or with CS_2/KOH, allowed the synthesis of oxadiazolyl methyl ketones(40) and (41) respectively after hydrolysis of the acetal group[46]. (Scheme-6)



An alternative to cyanogenbromide is phenyl cyanate(PhOCN), which reacted with hydrazines($R_1CONHNH_2$) to give aminooxadiazoles(42, R_1 = 4,6-dimethyl-2-pyrimidyl)[47]. From oxadiazol-2-carbohydrazides(43) bioxadiazolyls(44) and(45) were prepared using cyanogen bromide[48] or thiophosgene[49] respectively. (Scheme-7)

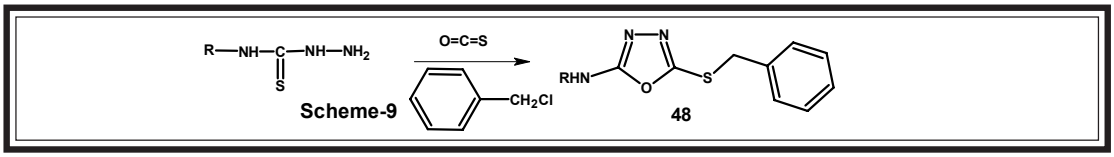


It has been shown that o-aminobenzoylhydrazine reacted with (i) 1,1'-carbonyl-bis-imidazole(a variation of the use of phosgene) to give oxadiazolinone(46)[50] and (ii) 1,3-dicyclohexylcarbodiimide and an isothiocyanate RNCS to give aminooxadiazole(47)[51]. (Scheme-8)



Scheme-8

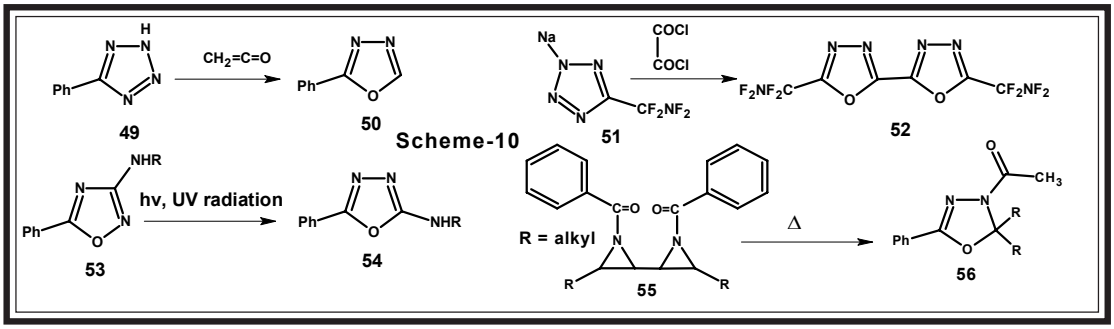
A variation of the oxidative cyclization of acyl-thiosemicarbazides to amino-oxadiazoles[52]. A variation of the reaction of acylhydrazines and carbon disulfide forming oxadiazolinethiones, is the reaction of thiosemicarbazide ($RNHCSNHNH_2$) with carbon oxysulfide and benzyl chloride, which yields amino-oxadiazolyl thioethers(48)[53]. (Scheme-9)



Scheme-9

❖Ring Synthesis by Transformation of another Ring

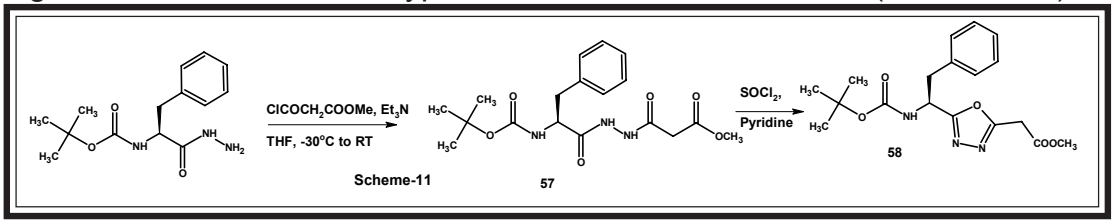
Two variations of the useful routes to 2,5-disubstituted-1,3,4-oxadiazoles via loss of nitrogen from 5-substituted tetrazoles were reported as (i) the reaction of tetrazole (49) with diketene to give the oxadiazolylmethyl ketone(50)[54] and (ii) the reaction of oxaloyl chloride with the sodium salt of tetrazole(51, prepared from F_2NCF_2CN and NaN_3) to give the bi-oxadiazolyl(52)[55]. Ultraviolet irradiation of 1,2,4-oxadiazole(53) gave 1,3,4-oxadiazoles(54)[56]. On heating 3,3-dialkyl -N,N'-dibenzoyldiazeridines(55) rearrange to oxadiazolines(56)[57].(Scheme-10)



Scheme-10

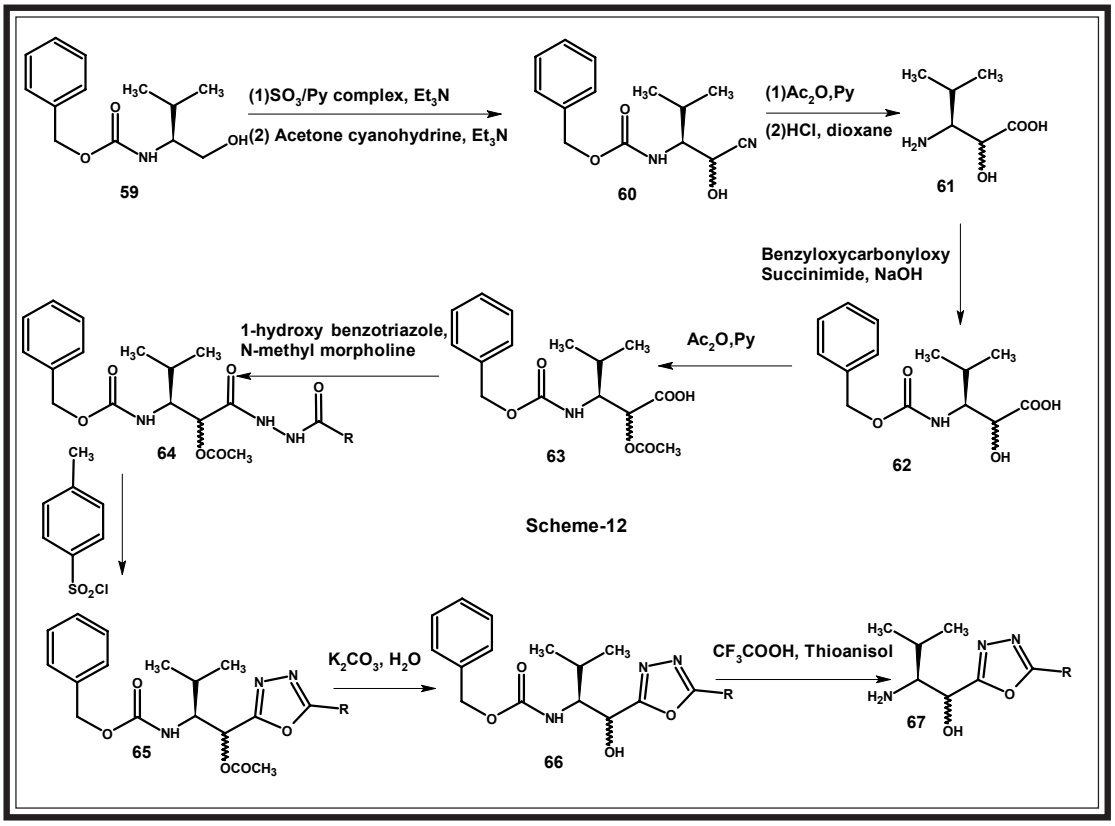
❖Miscellaneous apects

Susanna Borg et. al[12] have suggested that dehydration of the appropriate diacylhydrazines(57)(which were derived from Boc-L-phenylalanine hydrazide and methyl malonyl chloride) with thionyl chloride and pyridine afforded a 1,2,3,4-oxathiadiazole S-oxide intermediate, by heating the crude intermediate resulting into 1,3,4-oxadiazole of type 58 with loss of sulfur dioxide.(Scheme-11)



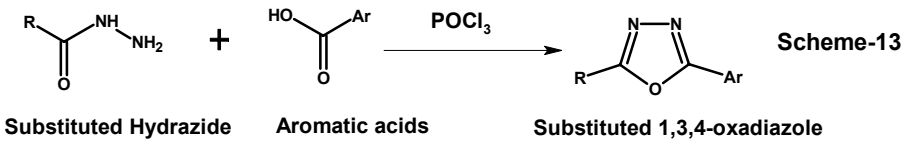
Tohru Miyazaki, et al[58] have synthesized 1,3,4-oxadiazole derivatives by cyanurization of benzyloxy carbonyl protected amino alcohol(59) with either sulfur trioxide-pyridine complex and triethyl amine or acetone cyanohydrine, the resulting cyano derivative (60) was hydrolysed by either acetic anhydride in pyridine or by hydrochloric acid to yield corresponding acid derivative(61) along with benzyloxy carbonyl deprotection of amino group. Which was again protected with Benzyloxycarbonyloxysuccinimide in the presence of base to give á-hydroxy carboxylic acid derivative(62) which was o-acetylated with acetic anhydride to protect hydroxy group, the resulting compound (63) was fused with appropriate hydrazide to produce diacetyl hydrazine derivative(64) and later upon cyclized with p-toluenesulfonyl chloride gave 1,3,4-oxadiazole derivative (65) was deprotected with base (K_2CO_3) and acid(CF_3COOH) to give corresponding hydroxy-1,3,4-oxadiazole(66) and amino-1,3,4-oxadiazole(67) derivatives.(Scheme-12)

46.B.Kubel, *Monatsh Chem.*, **113**, 793(1982)
47.A.Hetzheim, G.Mueller, P.Vainilavicius, D.Girdziunaite,*Pharmazie*, **40**, 17(1985)
48.J.Dost, M.Heschel, J.Stein, *J.Prakt.Chem.*, **327**,109(1985)
49.E.Beriger, W.Eckhardt, *Eur.Pat.*364396(1990),*Chem.Abstr.*,**113**, 152432(1990)
50.E.Tihanyi, M.Gal, P.Dvortsak, *Heterocycles,* **20**, 571(1983)
51.N.Peet and S.Sunder, *J.Heterocycl.Chem.*, **21**, 1807(1984)
52.J.Hill,*Comp.Heterocycl.Chem.,*1stEdn.,**6**, 427(1984)
53.M.Chande, A.V.Karnik, I.N.Inamdar, S.D.Damle, *Ind.J.Chem.*Sect-B.,**30**,430(1991)
54.T.Kato, T.Chiba, M.Daneshtaleb, *Chem.Pharm.Bull.*, **24**, 2549(1976)
55.E.John, R.Kirchmeier, J.M.Shreeve,*J.Fluor.Chem.,* **47**, 333(1990)
56.S.Buscemi, M.Cicero, N.Vivona, T.Carrona, *J.Heterocycl.Chem.*, **25**, 931(1988)
57.L.Somagyi, *Chem.Ber.*, **119**,2963(1986)
58.T.Miyazaki, T.Sugiura, T.Horiuchi, *US Patent Appl.*, **130326**



Scheme-12

Atta-ur-Rahmann et al[24] have synthesized a library of 2,5-disubstituted-1,3,4-oxadiazoles by the reaction of commercially available hydrazine with different aromatic carboxylic acids in the presence of phosphorous oxychloride.(Scheme-13)

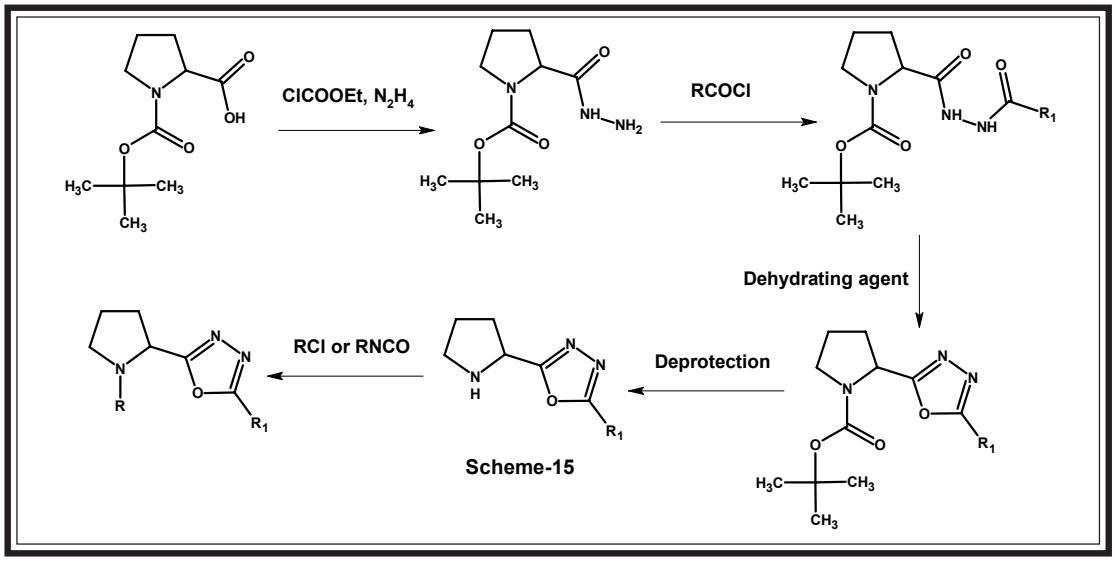


Substituted Hydrazide + Aromatic acids → Substituted 1,3,4-oxadiazole

R = 3-pyridyl, 2-methoxyphenyl

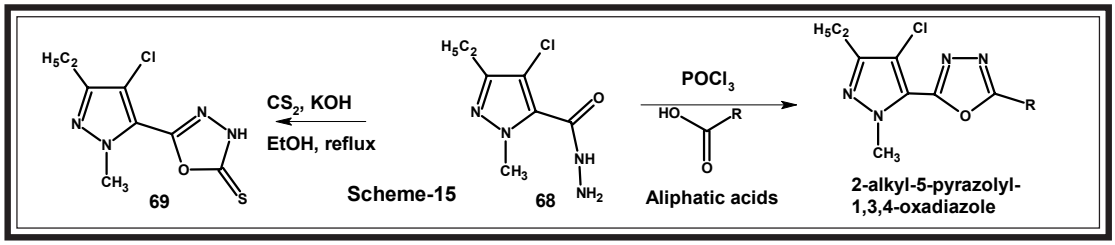
Scheme-13

Hennies, et al[35.53] have suggested that to produce 2-pyrrolidine-2-yl-[1,3,4]-oxadiazole derivatives, tertiary butoxy carbonyl protected proline was reacted with ethyl chloroformate and hydrazine in a suitable solvent like tetrahydrofuran. The resulting hydrazide was reacted with an acid chloride to afford diacyl hydrazide. For ring closure, acid and and a dehydrating reagent like phosphorous pentoxide, methanesulfonic acid, pyridine and/or thionyl chloride were added to di acyl hydrazide. The cyclized derivative allowed for deprotection and resulting product was substitued with appropriate acid chloride or isocyanate.(Scheme-14)



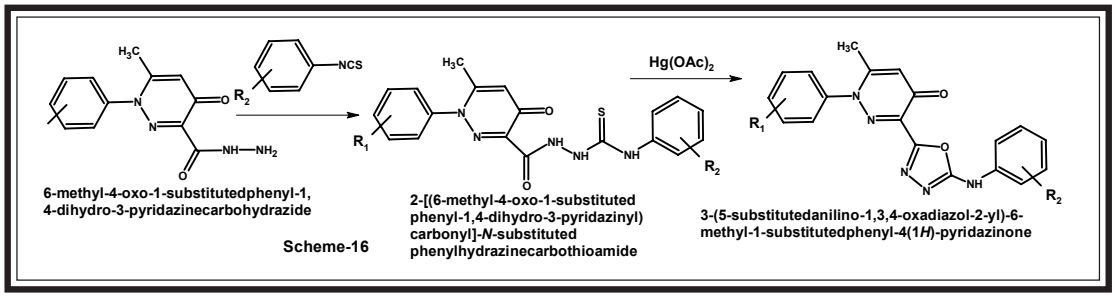
Scheme-15

Hansong Chen et al[30] have descibed that 5-pyrazole formic acid hydrazide (68) when reacted with carbon disulfide and potassium hydroxide in ethanol, afforded 5-pyrazolyl-1,3,4-oxadiazole-2-thiones(69). The chemical literature records several synthetic routes leading to the formation of 2,5-disubstituted-1,3,4-oxadiazoles(Yang J., Hua, W., Review: Synthesis of 2,5-substituted-1,3,4-oxadiazole derivatives, *Chemistry*, **9**, 18(1996)). 2-alkyl-5-pyrazolyl-1,3,4-oxadiazoles were synthesized from (68) and aliphatic acid in the presence of phosphorous oxychloride via one pot synthesis.(Scheme-15)



Scheme-15

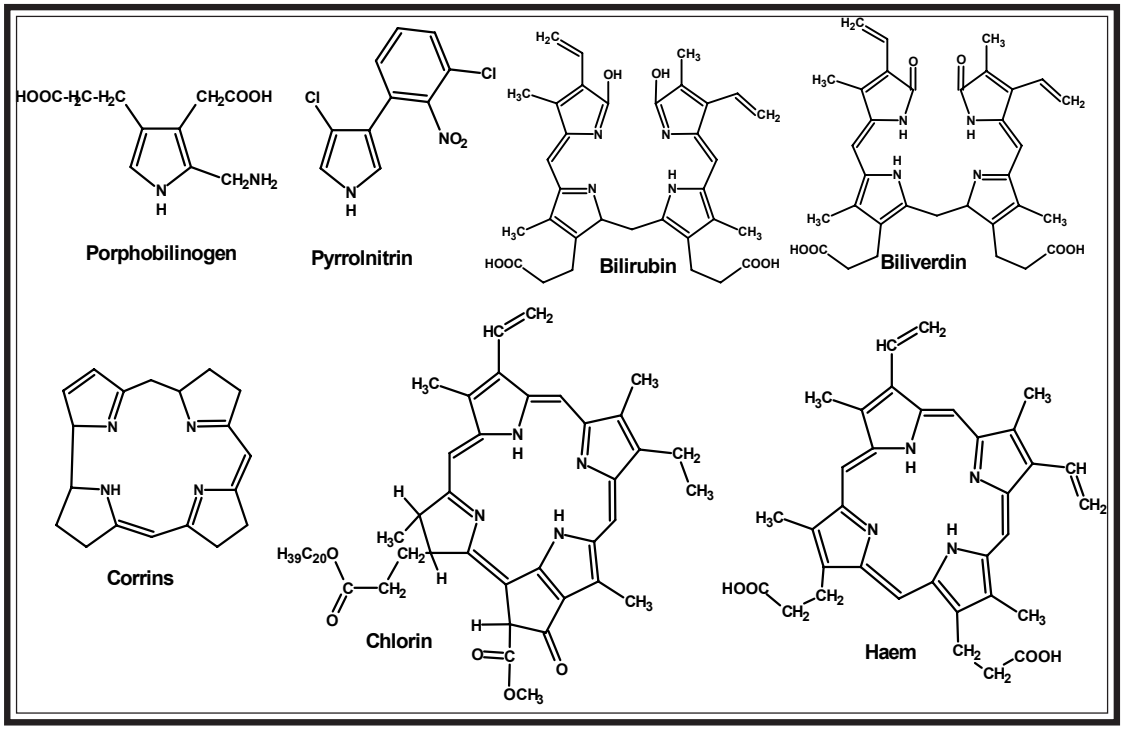
Xia-Juan Zou et al[28] revealed that treatment of 2-[(6-methyl-4-oxo-1-substituted phenyl-1,4-dihydro-3-pyridazinyl)carbonyl]-*N*-substituted phenylhydrazinecarbothioamide with mercuric acetate yields 3-(5-substitutedanilino-1,3,4-oxadiazol-2-yl)-6-methyl-1-substitutedphenyl-4(1*H*)-pyridazinone.(Scheme-16)



Scheme-16

Part-B. Pyrrole

Pyrrole is an important ð-excessive aromatic heterocycle because this ring system is incorporated in as a basic structural unit in porphyrins;porphin (heam) and chlorin(chlorophyll) and corrins (vitamin B_{12}). More over the presence of pyrrole ring in system in porphobilinogen (intermediate in bio-synthesis of porphyrins and vitamin B_{12}), biliverdin and bilirubin(pyrrole-based bile pigments) and pyrrolnitrin(with antibiotic activity) has provided an impetus to the pyrrole chemistry.



Pyrrole has a planar pentagonal structure with four carbon atoms and nitrogen sp^2-hybridized. Each ring atom forms two sp^2-sp^2 ó -bonds to its neighbouring ring atoms and one sp^2-só -bond to a hydrogen atom. The remaining unhybridized p-orbitals, one on each ring atom(with one electron on each carbon and two electron on nitrogen), are perpendicular to the plane of ó -bondsand overlap to form a ð-molecular system with three bonding orbitals. The six ð-electrons form an aromatic sextet which is responsible for aromaticity and renders stability to the pyrrole ring. The $C_{á}$-N and $C_{â}$-$C_{â}$ bonds are shroter than normal single bonds, where as $C_{á}$-$C_{â}$ bonds are normal than normal double bonds. The molecular dimentions of the pyrrole reflect cyclic delocalization with the environment of lone pair of electrons on the nitrogen atom.

Pyrrole is extremely weak base because the lone pair of electrons on the nitrogen atom involved in the cyclic delocalization and is, therefore, less available for protonation. Moreover, pyrrole is a weaker base than pyridine and even than aniline in which lone pair on the nitrogen atom is involved in the resonance and not essentially contributes to the aromatic sexlet. The protonation of pyrrole at nitrogen or C_2 or C_3 atom of the ring reduces its basicity and destroys its aromaticity. However, C- and N- alkyl substituents enhance the basicity of pyrrole but the electron withdrawing substituents on the ring make pyrrole a weaker base[59-69].

Pharmacology

❖As slective Mono Amine Oxidase type A Inhibitors

Monoamine oxidase (MAO, EC 1.4.3.4) is a flavoprotein located at the outer membranes of mitochondria in neuronal, glial, and other cells. It catalyses the oxidative deamination of monoamine neurotransmitters such as serotonin (5-hydroxytryptamine, 5-HT), norepinephrine, and dopamine, and appears to play important roles in several psychiatric and neurological disorders [70.71]. In addition, it is also responsible for the biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into 1-methyl-4-phenylpyridinium (MPP), a Parkinson producing neurotoxin [72-74]. Recently, it has been shown that MAO contributes to the apoptotic process because inhibition of MAO activity suppresses cell death [75].

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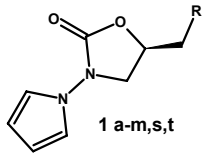
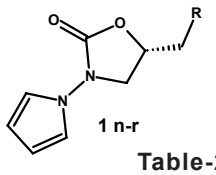
MAO exists in two forms, namely, MAO-A and MAO-B, distinguishable by their molecular cloning,substrate and inhibitor selectivity, and tissue distribution [76-79]. MAO-A preferentially oxidizes serotonin and is irreversibly inhibited by low concentrations of clorgyline[77]. MAO-B preferentially oxidizes b-phenylethylamine (PEA) and benzylamine, and it is irreversibly inactivated by low concentrations of L-deprenyl [80]. Dopamine, tyramine, and tryptamine are commonsubstrates for both MAOs.

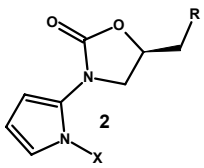
Due to the key role played by monoamine oxidases (MAOs) in the metabolism of neurotransmitters, MAO inhibitors (MAOIs) represent an useful tool for the treatment of several neurological diseases. Among selective MAOIs, MAO-A inhibitors (e.g.clorgyline) are used as antidepressant and antianxiety drugs and are claimed to protect neuronal cells against apoptosis, and selective MAO-B inhibitors (e.g. L-deprenyl) can be used in the treatment of Parkinson's disease either alone or in combination with L-DOPA. However, they engender covalent bonds with the active site of the enzyme and induce irreversible inhibition; moreover, they tend to lose their initial selectivity at high dosages or with repeated administrations. Phenyloxazolidinones belong to thirdgeneration-MAOIs, characterized by a selective and reversible inhibition of the enzyme. Among these molecules, the most representative are toloxatone and befloxatone, two selective and reversible MAO-A inhibitors used in therapy as antidepressant drugs.

Going on searches on CNS potentially active compounds containing pyrrole moiety A. Mai, et.al[81] have synthesized 3-(1H-pyrrol-1-yl)-2-oxazolidinones (70) and isomeric 3-(1H-pyrrol-2-and -3-yl)-2-oxazolidinones (71 and 72)(Table-2) as anti-MAO agents.The majority of compounds showed inhibitory activity against the A isoform of MAO enzyme higher than that exerted against the MAO-B.

Among compounds 70, derivatives 70a-e, g, j, k, p, s, t were the most active with concentration values of MAO-A inhibitory activity in the nanomolar range. In particular, (R/S)-toloxatone and the related pyrrole analog (racemic mixture of 70a and 70n) were equipotent as MAO-A inhibitors,







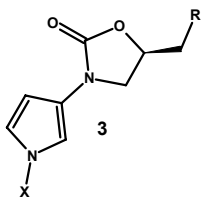
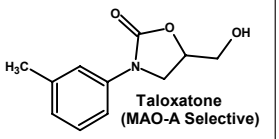
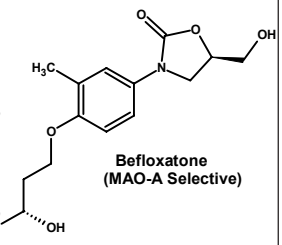


Table-2

Compd.	C_5 configuration	R
70a	*R*	OH
70b	*R*	OCH_3
70c	*R*	OCH_2CH_3
70d	*R*	$OCH_2CH_2CH_3$
70e	*R*	$OCH(CH_3)_2$
70f	*R*	$OCH_2CH_2CH_2CH_3$
70g	*R*	N_3
70h	*S*	NH_2
70i	*S*	$N(CH_3)_2$
70j	*S*	$NHCH_3$
70k	*S*	$NHCH_2CH_3$
70l	*S*	$NHCH(CH_3)_2$
70m	*S*	$NHNH_2$
70n	*S*	OH
70o	*S*	OCH_3
70p	*R*	$NHCH_3$
70q	*S*	N_3
70r	*R*	NH_2
70s	*R*	$OCOCH_3$
70t	*R*	OCOPh

Compd.	X	R
71a	CH_3	OH
71b	CH_3	OCH_3
71c	CH_3	N_3
71d	CH_3	NH_2
71e	CH_2CH_3	OH
71f	CH_2CH_3	OCH_3
71g	CH_2CH_3	N_3
71h	CH_2CH_3	NH_2
71i	$CH_2CH{=}CH_2$	OH
71j	$CH_2CH{=}CH_2$	OCH_3
71k	$CH_2CH{=}CH_2$	N_3
71l	$CH_2CH{=}CH_2$	$NHCH_3$
71m	$CH_2{-}Ph$	OCH_3
71n	$CH_2{-}Ph$	N_3
71o	$CH_2{-}Ph$	NH_2
71p	$CH_2{-}Ph$	$NHCH_3$
72a	CH_3	OH
72b	CH_3	OCH_3
72c	CH_3	N_3
72d	$CH_2CH{=}CH_2$	OH
72e	$CH_2CH{=}CH_2$	$NHCH_3$
72f	$SO_2{-}Ph$	OH





the later being 6-fold less MAO-A selective than the former. Furthermore, the assays performed on the R (70a) and S (70n) enantiomers showed 70a to possess the best activity and selectivity. O-alkylation of this compound afforded, i.e. (R)-5-methoxymethyl-3-(1H-pyrrol-1-yl)-2-oxazolidinone (70b), a MAO-A inhibitor endowed with very high potency and A-selectivity. In fact, compound 70b (Ki_{MAO-A} 4.9nM), was equipotent to befloxatone (R,R form) (Ki_{MAO-A} 2.5 nM), a new toloxatone analog, and it was characterized by very high selectivity towards the MAO-A isoenzyme (A-selectivity 10,200, about 116-fold greater than that of befloxatone).

The potency abatement is strictly dependent on the steric hindrance exerted by N_1-alkyl substituents: N_1-methyl-pyrrole derivatives represented the products belonging to the most active series, followed by the N_1-ethyl and N_1-allyl analogues. N_1-Benzyl derivatives are practically inactive as anti-MAO agents, with the exception of the R-5-methylamino-3-(1-benzyl-1H-pyrrol-2-yl)-2-oxazolidinone (71p), which was endowed with both anti-MAO-A and anti-MAO-B activities (Ki_{MAO-A} 0.14 ìM; Ki_{MAO-B} 0.5 ìM).Among derivatives 71 and 72, the most active compound resulted the R-5-

azidomethyl-3-(1-methyl-1H-pyrrol-2-yl)-2-oxazolidinone 71c (Ki against MAO-A 0.004 mM;Ki against MAO-B 4 ìM; A-selectivity 1000), being as potent as befloxatone and 10 times more A-selective.

X-ray diffraction-crystallographic studies coupled with conformational and electronic characterization (by the ab initio molecular orbital method) performed on toloxatone [82,83] showed that the drug is a planar molecule within the presence of an electron delocalization of both the oxazolidinone and phenyl rings. Such structural and electronic properties account for the existence of a charge-transfer complex between toloxatone and riboflavine and establish the mechanism of MAO-A reversible inhibition exerted by toloxatone. Starting from these data, was performed conformational analysis on pyrrole analogue of toloxatone ,70a, to verify if such co-planarity between pyrrole and oxazolidinone rings could exist also in this molecule. Surprisingly, it was found that global minima conformations for toloxatone and 70a (from MOPAC93 calculations) resulted quite different, being optimal torsion angles = -1.28° (toloxatone) and +70.1° (70a) (Figs. M and N).

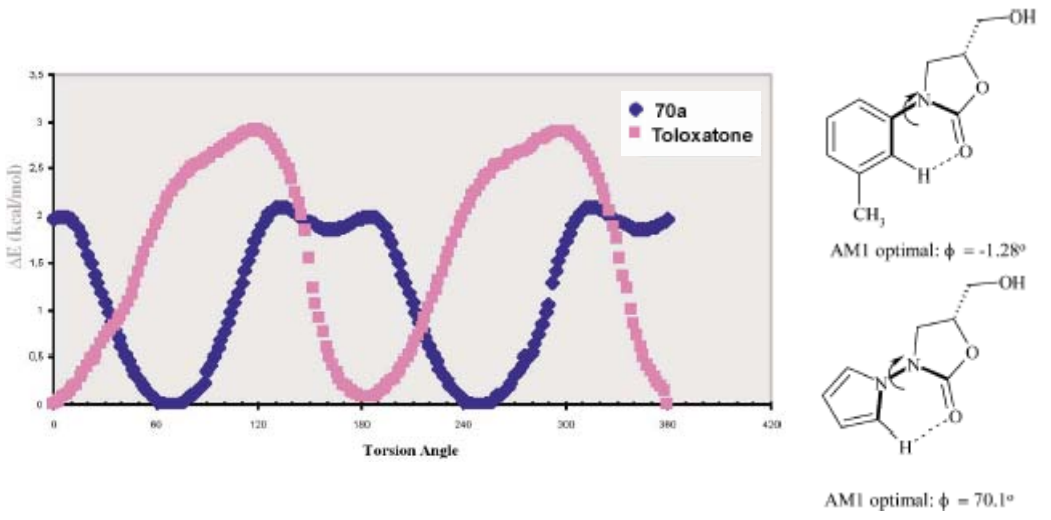


Fig.M. AM1 calculations of optimal torsion angles of toloxatone (left) and 70a (right).

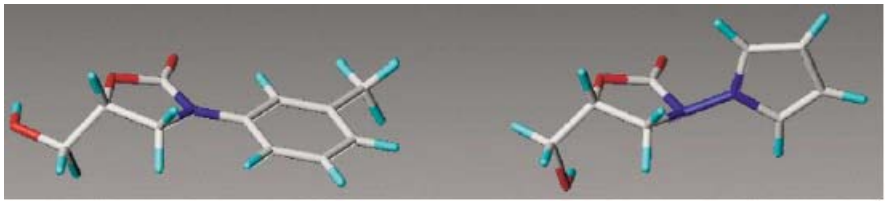


Fig. N. Global minima conformations for toloxatone (left) and 70a (right).

❖Antitubercular activities

The frequent appearance of multidrug-resistant strains of *Mycobacterium tuberculosis* and the growing importance of nontuberculosis mycobacterial (NTM) strains in infections of immunosuppressed patients have accentuated the need to search for new antimycobacterial drugs [84-90]. Previously, among the various active compounds already discovered, some azole derivatives have been shown to possess strong inhibitory activities *in vitro* and *in vivo* against *M. tuberculosis* strains[91]. In addition, metronidazole was found to be able to kill dormant cells of *M. tuberculosis*[92].

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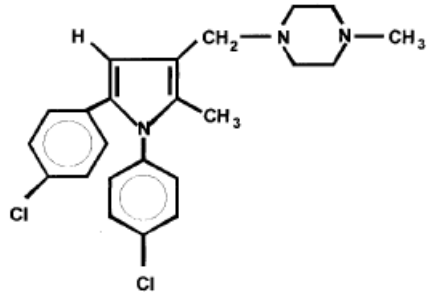
Delia Deidda, et al[93] have described that the pyrrole derivative BM212 [1,5-diaryl-2-methyl-3-(4-methylpiperazin-1-yl)methyl-pyrrole] was shown to possess strong inhibitory activity against both *Mycobacterium tuberculosis* and some nontuberculosis mycobacteria. It was suggested that BM212 appeared to be endowed with particularly potent and selective antimycobacterial properties, and consequently, some experiments were devised in order to characterize its activity against both drug resistant and intramacrophagic mycobacteria. Isoniazid (INH) and streptomycin (SM) were used as controls. The results suggested that BM212 showed potent antimycobacterial activities against several strains of *M. tuberculosis.* The MICs were between 0.7 and 1.5 ìg/ml for both collection and clinical strains; for only one strain was the MIC as high as 6.2 ìg/ml. These values were a little higher than those of INH (0.05 to 0.2) for most strains but were generally comparable with those of SM (from 0.4 to 6.2 ìg/ml). Also, some NTM strains appeared to be quite susceptible to the action of BM212. In fact, the MIC ranges were 3.1 to 12.5 ìg/ml for *M. fortuitum*, 3.1 to 25 ìg/ml for *M. smegmatis*, and 3.1 to 6.2 for *M. kansasii*, while for *M. avium*, it was between 0.4 and 3.1 ìg/ml. *M. marinum* (a single strain) and *M. gordonae* appeared less susceptible to the inhibiting activity of BM212.

The activity of BM212 against various drug-resistant mycobacteria was tested. Two strains were only resistant to ethambutol (EMB), three were resistant to amikacin (AMK), two were resistant to SM, two were resistant to INH, and two were resistant to both rifampin (RIF) and rifabutin (RIB). Two strains were resistant to both INH and RIF, and strain MSS3 was highly resistant to four drugs (INH, EMB, RIF, and RIB). BM212 had inhibitory activity against all strains tested, with MICs between 0.7 and 1.5 ìg/ml. The BM212 MIC for one strain of AMK-resistant mycobacterium was as high as 6.2 ìg/ml.

BM212 was inhibitory to drug-resistant mycobacteria and also exerted bactericidal activity against intracellular bacilli residing in the U937 human histiocytic lymphoma cell line. The activity study suggested that after 7days of

contact, BM212 completely inhibited the intracellular mycobacteria. The effect was dose dependent, and the MIC was found to be 0.5 ìg/ml. From a concentration of 1 mg/ml onwards the inhibition was 100%. Similar results were obtained with Rifampin at 3 ìg/ml. No relevant macrophage loss was detected after 10 days of incubation, both in the control and in the compound-treated cultures. Furthermore, BM212 exerted no inhibition on U937 cell culture replication up to a concentration of 12.5 ìg/ml.

The pyrrole derivative BM212 shows some interesting antimicrobial properties: (i) it is strongly inhibitory against both *M. tuberculosis* and *M. avium*, which are the two most common mycobacteria causing infection in immunosuppressed patients; and (ii) it also has marked activity against several species of yeasts, including *Candida albicans* and *Cryptococcus neoformans*. Considering the increased incidence of opportunistic infections caused by candidae and mycobacteria in immunocompromised patients, the development and use of new compounds, which would be active against both these types of microorganisms, is very attractive. Furthermore, BM212 is also highly efficacious against mycobacteria which show resistance to the most common traditional drugs, displaying no cross resistance with them, and it exerts bactericidal activity on intracellular mycobacteria. This fact is very important because mycobacteria can reside for years inside lymphoid cells and macrophages, where they are difficult to get rid of.



Chemical structure of BM212 -1,5-diaryl-2-methyl-3-(4-methylpiperazin-1-yl)-methyl-pyrrole.

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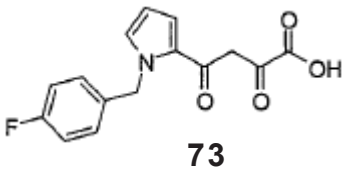
❖As HIV-1 intigrase Inhibitors

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunedeficiency syndrome (AIDS). The unique nature of the replicative cycle of HIV-1 provides many potential targets for chemotherapeutic intervention. One of these, the viral integrase, catalyzes the insertion of the proviral DNA into the genome of the host cell. Integration is a multistep process which includes three different biochemical processes: assembly of proviral DNA on integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA to host cell DNA[94-96]. Recently, diketo acid derivative 73 was reported to be a selective inhibitor of the strand-transfer process. This compound effectively prevents proviral DNA integration and inhibits HIV-1 replication in cell culture[95].



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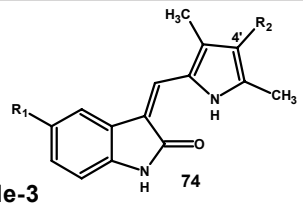
Strand Transfer IC_{50} 0.17ìM(n=37)[94-96]

Antiviral Activity CIC_{95} 9.6ìM(n=166)[97]

❖As Tyrosine Kinase Inhibitors

Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors have been well validated as targets for the treatment of cancers because of their critical roles in tumor growth and suvival via autocrine and paracrine loops[98-101]. In this regard, both receptor tyrosine kinases (RTKs) have been found to be expressed on the tumor cells and to directly affect tumor cell proliferation (e.g.,VEGF receptor in melanoma and PDGF receptor ingliomas)[98]. In addition, both RTKs have been found to play prominent roles in tumor angiogenesis by participating in the transmission of proliferation, migration, differentiation and survival signals between tumor cells and endothelial cells[99-101]. Thus, simultaneous inhibition of both endothelial growth factor receptor-2 (VEGF-R2) and platelet-derived growth factor receptor-â, (PDGFR,) might be expected to show better antitumor activity than by inhibiting only one of these RTKs.

To improve the antitumor properties and optimize the pharmaceutical properties including solubility and protein binding of indolin-2-ones, a number of different basic and weakly basic analogues were designed and synthesized by Li Sun, et al[102]. 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (75b or SU11248) has been found to show the best overall profile in terms of potency for the VEGF-R2 and PDGF-Rβ, tyrosine kinase at biochemical and cellular levels, solubility, protein binding, and bioavailability. 75b was in phase-I clinical trials for the treatment of cancers.



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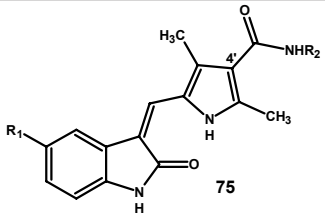

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Table-3

compd	R1	R2	biochemical activity against kinases IC_{50},[a] μM: VEGF-R2	PDGF-Rβ	FGF-R1	EGF-R	cellular kinase activity in 3T3 cell IC_{50},[a] μM: VEGF	PDGF	PDGF-induced BrdU incorporation IC_{50},[a] μM	cytotoxicity LD_{50},[a] μM	solubility,[b] μg/mL: pH 2	pH 6
74a	H	H	1.23	22.9	>100	>100	1.04	20.3	4.05	>50	<1	<1
74b	H	$(CH_2)_2COOH$	2.4	0.060	3.00	>20	1–2	0.1–1.0	16	>50	<5	18
74c	H	$(CH_2)_3N(CH_2CH_2)_2NCH_3$	0.3	0.060	4.20	>20	*d*	*d*	0.20	>50	*d*	*d*
75a	H	$(CH_2)_2N(C_2H_5)_2$	0.050	0.017	0.88	>20	0.05–0.5	0.1–1.0	<0.07	>50	3022	511
75b	F	$(CH_2)_2N(C_2H_5)_2$	0.080	0.0020	2.90	>20	0.005–0.05	0.01	0.008	48.9	2582	364
75c	Cl	$(CH_2)_2N(C_2H_5)_2$	0.027	0.0030	0.17	>20	0.005–0.05	0.01–0.1	<0.07	15.6	3259	186
75d	Br	$(CH_2)_2N(C_2H_5)_2$	0.032	0.0050	0.73	10	0.005–0.05	0.01–0.1	<0.07	16.3	1299	101
75e	F	$(CH_2)_2N(CH_3)_2$	0.080	0.0005	1.60	>20	0.005–0.05	0.01–0.1	0.015	48.5	3012	75
75f	F	$(CH_2)_2$-pyrrolidin-1-yl	0.060	0.0010	3.90	>20	0.005–0.05	0.01–0.1	<0.07	49	3319	9
75g	F	$CH_3CH(CH_2CH_2)_2N-CH_3$	0.025	0.0030	0.20	*d*	0.04	0.02	0.030	38	>250	>250
75h	F	$(CH_2)_2$-morpholin-4-yl	0.090	0.0020	2.60	>20	0.05	*d*	0.037	>50	2945	0.3
75i	F	CH_2-pyridin-4-yl	<0.16	0.0010	3.10	>20	0.005–0.05	0.01–0.1	0.080	>50	486	<LD[c]
75j	F	$(CH_2)_2$-triazol-1-yl	0.085	0.010	17.1	>20	0.05–0.5	0.1–1.0	0.19	>50	2	6

[a] IC_{50} and LD_{50} values were determined by at least two separate tests and reported as mean values. [b] Solubility of the compounds was determined in 20 mM buffered solutions (pH 2, KCl/HCl; pH 6, phosphate) after shaking for 24 h at 22 °C. Data presented are from a single determination or an average of two determinations. [c] LD- limit for detection. [d] Not tested.

The screening results(Table-3) suggested that converting the carboxylate group of 74b into an amino group (74c) enhances the inhibitory activity for VEGF-R2 (8-fold) while retaining the potency against PDGF-R, kinase. The difference in potency observed between inhibition of PDGF-induced BrdU incorporation and inhibition of cellular kinase activity for 74b might be related to its poor solubility. 75a-d exhibited potent inhibitory activity against VEGF-R2 and PDGF-Rβ, in biochemical and cellular assays. Specifically, compared to 74b, 75b was about 30-fold more potent against VEGF-R2 and PDGF-R, in biochemical assays,over 10-fold more potent in cellular kinase assays, and significantly more soluble under neutral (20-fold) and acidic (>500-fold) conditions.

Compared to 74b, the cellular potency of 75b against PDGF-induced proliferation was less likely to be affected by the presence of serum protein. In this regard, 75b was still a very potent inhibitor of PDGF-R, while 74b became inactive in the presence of a high level of serum.

❖Antiproliferative activity

Antiproliferative and cytotoxic drugs play a major role in cancer therapy, whether used alone or in concert with other treatment modalities such as surgery, radiation and biological therapy. Recently, aryl pyrroles have been reported as potent inhibitors of Ras farnesyltransferase with regression of tumors grown in nude mouse xenograft models[103].

For developing new antitumoral agents, Jose Pardon, et.al[104] have synthesized a number of 1,2,3,4-tetrasubstituted pyrrole derivatives (76a-t) (Table-4) and evaluated for their *in vitro* antiproliferative activities using the human promyelocytic leucamia cell line HL60. The activity of the compound was measured in terms of Growth inhibition of 50% (GI_{50}), which is the drug concentration resulting in a 50% reduction of cellular net growth when compared with values of untreated control cells, the drug concentration resulting in total growth inhibition (TGI), and the net loss in 50% of cells following treatment (LC_{50}) denoting cell kill.

The group of active compounds exhibited GI_{50} values in the range of 4-45ìM. From this series compounds 76h and 76n were the most active against HL60 cells, with GI_{50} values of 4.8 and 5.1ìM, respectively. The remaining active derivatives showed modest activity with GI_{50} values in the range 15–45ìM. Only five products proved to be inactive at the maximum test concentration, that is, 100ìM. Interestingly, compounds 76a, 76c, 76j–l, and 76n were the only products from the active group series that reached a TGI value. The TGI values for those products were in the range 80–95ìM. None of the evaluated pyrroles was able to show a LC_{50} value.

98.J.Cherrington, L.Strawn, L.Shawver, In *Advances in Cancer Research*, 1st ed.; G.Klein, G.Woude, Eds.; Academic Press: San Diego, CA, **70**, 1(2000).
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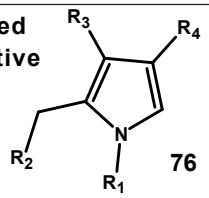
100.N.Ferrara, *Curr. Opin. Biotechnol.*,**11**, 617(2000).
101.S.Rosenkranz, A.Kazlauskas, *Growth Factors*,**16**,201(1999).

When considering GI_{50} data the following structure– activity relationship was obtained: (a) the aliphatic substituent on the nitrogen atom of compound 76t decreases activity when compared to compounds 76f and 76s, which posses aromatic side chains; (b) the absence of a substituent in position 3 of the pyrrole ring led to inactive compound 76d; (c) ethyl ester derivatives 76g, 76j, and 76n are more active than their corresponding methyl esters 76f, 76i, and 76m respectively; (d) hydrolysis of the aliphatic ester of 76m led to a less potent derivative 76l; (e) conversion of the aliphatic methyl ester in 76f to benzyl amide 76h led to the most potent compound of the series; (f) substituents other than methyl, ethyl, i-propyl, cyclopropyl, and n-hexyl at R3 produces decrease in activity as shown with products 76o, 76p, and 76r; and (g) the absence of an alkoxy carbonyl moiety at R2 position in 76b makes the compound more active than 76f, but such a difference was not observed for compounds 76a and 76c, when compared to 76e and 76m.

Table-4. Structures of substituted pyrroles and their antiproliferative activity



76

Compds	R^1	R^2	R^3	R^4	GI_{50}, μM[a]	TGI, μM[a]
76a	Bn	H	Me	CO_2Et	35.4 (±13.9)	80.9 (±22.1)
76b	Bn	H	Et	CO_2Me	31.4 (±1.9)	na
76c	Bn	H	*n*Hex	CO_2Me	26.6 (±8.7)	87.2 (±25.6)
76d	Bn	CO_2Et	H	CO_2Me	na	na
76e	Bn	CO_2Et	Me	CO_2Et	35.8 (±12.3)	na
76f	Bn	CO_2Me	Et	CO_2Me	43.1 (±7.6)	na
76g	Bn	CO_2Et	Et	CO_2Et	34.3 (±8.4)	na
76h	Bn	CONHBn	Et	CO_2Me	4.8 (±3.0)	na
76i	Bn	CO_2Me	*i*Pr	CO_2Me	34.7 (±12.9)	na
76j	Bn	CO_2Et	*i*Pr	CO_2Et	17.9 (±7.6)	88.4 (±16.5)
76k	Bn	CO_2Me	*i*Pr	CO_2Et	33.0 (±15.6)	88.3 (±18.8)
76l	Bn	CO_2H	*n*Hex	CO_2Me	40.4 (±8.9)	92.4 (±13.1)
76m	Bn	CO_2Me	*n*Hex	CO_2Me	17.7 (±9.1)	na
76n	Bn	CO_2Et	*n*Hex	CO_2Et	5.1 (±1.6)	94.5 (±11.1)
76o	Bn	CO_2Et	3-Butenyl	CO_2Et	na	na
76p	Bn	CO_2Et	$BnOCH_2$	CO_2Et	na	na
76q	Bn	CO_2Et	*c*Pr	CO_2Et	34.2 (±12.5)	na
76r	Bn	CO_2Et	(*S*)-$(Me)_2CCH(CH_2)_2CH(Me)CH_2$	CO_2Et	na	na
76s	(*S*)-PhCHMe	CO_2Me	Et	CO_2Me	39.1 (±10.6)	na
76t	(*R,S*)-$MeCHCH_2CO_2Et$	CO_2Me	Et	CO_2Me	na	na

102.L.Sun, C.Liang, S.Shirazian, Y.Zhou, T.Miller, J.Cui, J.Fukuda, J.Chu, A. Nematalla, X.Wang, H.Chen, A.Sistla, T.Luu, F.Tang, J.Wei, C.Tang, *J. Med. Chem.*,**46**,1116(2003)
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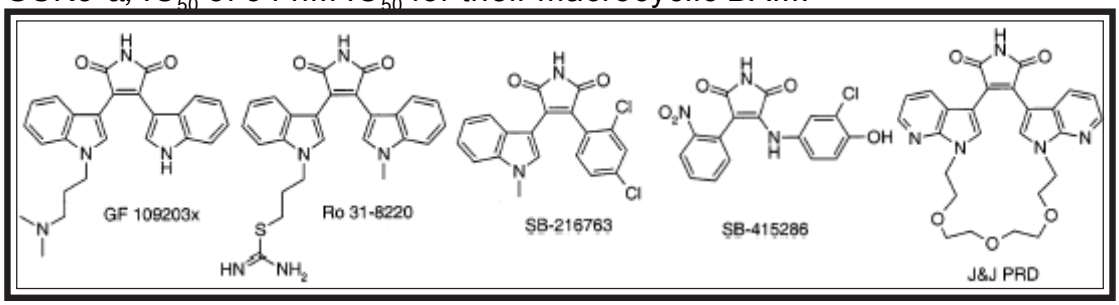
❖As Glycogen Synthase Kinase-3(GSK-3) Inhibitors

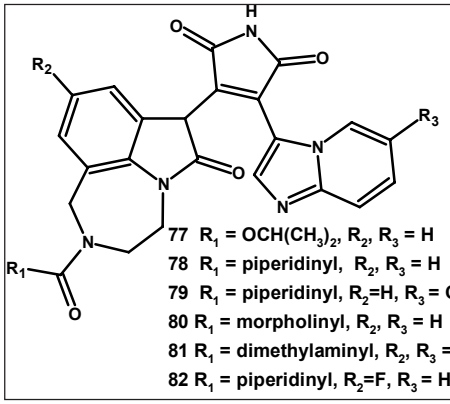
Glycogen synthase kinase-3(GSK3) is a serine-threonine kinase encoded by two independent genes, GSK3-á and â[105]. Purified GSK3-á and â show similar biochemical and substrate properties and display high sequence homology, 85% overall and 95% in the catalytic domain[106]. GSK3 was first identified as one of several kinases that phosphorylates glycogen synthase (GS), the enzyme that catalyzes the last step in glycogen synthesis. This phosphorylation step, in contrast to most signaling pathways, inhibits the action of GS[107]. It was later shown that signaling from the insulin receptor inactivates GSK3 via AKT-catalyzed phosphorylation of serine #9 on the amino terminus of GSK3[108]. Thus, inhibitors of GSK3 would be expected to have some of the same effects as insulin, such as its ability to activate glycogen synthase and stimulate the conversion of glucose to glycogen, thereby lowering plasma glucose. Abnormal regulation of GSK3 has also been demonstrated in insulin resistant rodent and human muscle tissue[109] Because of these facts, GSK3 has become an attractive target for the potential treatment of noninsulin-dependent diabetes mellitus(NIDDM)[110].

Recently, several groups have reported a variety of bis-aryl maleimide (BAM) inhibitors of GSK3 (As shown in figure below). BAMs GF 109203x and Ro 31-8220 were shown to inhibit GSK3 with IC_{50} values of 170-360 nM and 3-7nM, respectively, depending on the source of GSK3[111]. SmithKline Beecham reported SB-216763 and SB-415286 to inhibit GSK3-á with an IC_{50} of 34 and 77 nM, respectively,[112] while J&J PRD reports a GSK3-â, IC_{50} of 34 nM IC_{50} for their macrocyclic BAM.[113]



105.A.Ali, K.Hoeflich, J.Woodgett, *Chem. Rev.*,**101**, 2527(2001).
106.J.Woodgett, *EMBO J.*,**9**, 2431(1990).
107.P.Cohen, *The Enzymes*; Academic Press: New York, **17**, 461(1986).

T. Engler, et.al[114] reported the results of a new class of orally available, highly selective and potent GSK-3 inhibitors, the 3-imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-7-yl)pyrrole-2,5-diones (77-82).



77 R_1 = $OCH(CH_3)_2$, R_2, R_3 = H
78 R_1 = piperidinyl, R_2, R_3 = H
79 R_1 = piperidinyl, R_2=H, R_3 = CH$_3$
80 R_1 = morpholinyl, R_2, R_3 = H
81 R_1 = dimethylaminyl, R_2, R_3 = H
82 R_1 = piperidinyl, R_2=F, R_3 = H

Biochemical and Cellular Assay Data

no.	GSK3-β IC_{50} (nM)	P-Tau IC_{50} (nM)
staurosportine	56 ± 6.9	NT
77	1.3 ± 0.5	15[c]
78	2.0 ± 0.4[b]	1.6[c]
79	5.2 ± 0.8	41 ± 26[b]
80	1.3 ± 0.1[b]	2.6 ± 0.22
81	1.6 ± 0.3	12 ± 2.6
82	1.1 ± 0.3	0.7 ± 0.26

[a] Data represent mean values of at least three independent determinations (±SE) unless otherwise noted. [b] $N = 2$. [c] $N = 1$.

Inhibition of GSK3-â by compounds 77-72 was determined in both biochemical and cellular assays. As shown in Biochemical and Cellular Assay Data, all compounds are very potent inhibitors of GSK3-â in the biochemical assay IC_{50}s ranging from 1 to 5 nM. In the P-Tau assay a larger differentiation in activities ranging from 0.7 to 41 nM. Compound 79, bearing a 6-methyl group on the imidazopyridine, was the least potent inhibitor in both assays and was 25-fold less active in the P-Tau assay than the parent 78. The piperidine (78, 82) and morpholine (80) ureas were found to be favored substituents on the aliphatic ring nitrogen over other groups such as N,N-dimethylurea (81) and the isopropyl carbamate (77). Further, fluorine substitution on the 5-position of the indole in 82 resulted in a modest activity enhancement over the parent compound 78.

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111.I.Hers,J.Tavare, R.Denton, *FEBS Lett.*,**460**, 433(1999).
112.M.Coghlan, A.Culbert, D.Cross, S.Corcoran, J.Yates, N.Pearce, O.Rausch, G.Murphy, P.Carter, L.Cox, D.Mills, M.Brown, D.Haigh, R.Ward, D.Smith, K.Murray, A.Reith, J.Holder, *Chem.Biol.* ,**7**, 793(2000).
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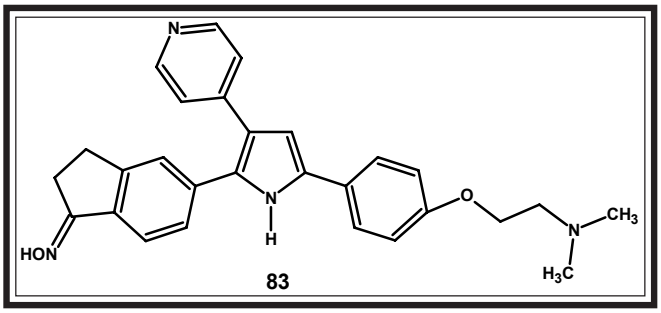
❖Analgesic and Antiinflammatory activity

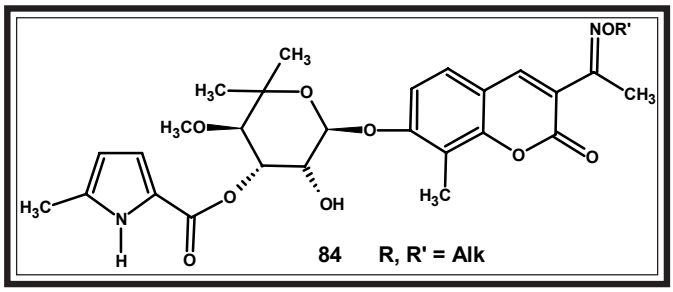
Oxime derivatives containing pyrrole and pyridine fragments were studied as inhibitors of Raf kinase[115]. All these compounds can be used as analgesics and agents againts migraine and the oxime (83) was one of the most active.The O-lauroyl and O-nicotinoyloximes of 1-methyl-2-acetylpyrrole possessed antiinflammatory activity[116].



83

❖ Bactericidal activities

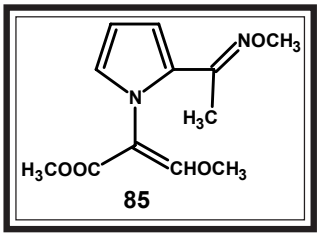
Derivatives of 2-pyrrolecarbaldehyde oximes have exhibited high bactericidal activity[117-119]. The pyrrole oxime fragment also enters into the structure of certain penicillin antibiotics[120]. It was shown that ethers of pyrrole oximes (8) have high bactericidal activity against resistant strains of bacteria[121].



84 R, R' = Alk

❖ As Fungicides and Plant Growth Regulators

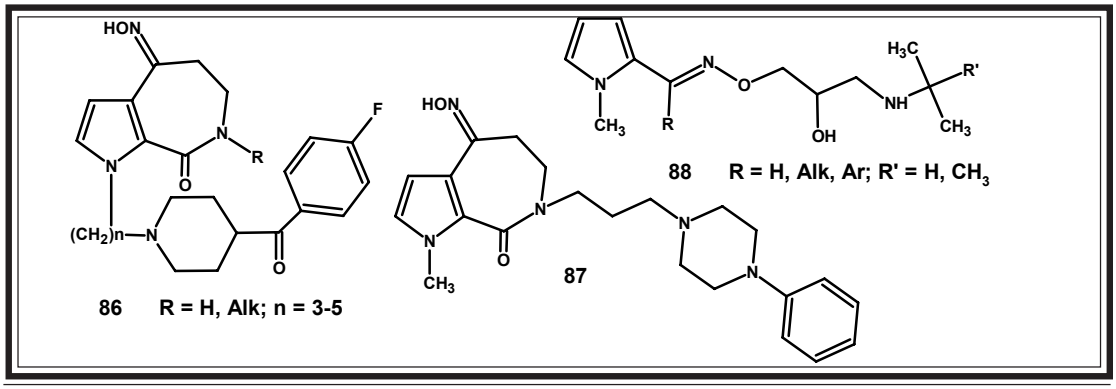
The ethers of pyrrole oximes exhibited high fungicidal, insecticidal and acaricidal activity[122]. Among these compounds, in particular, the ether(85) should be mentioned[123]. The ethers of pyrrole amidoximes [(pyrrolyl)C(NH_2HCl)=NOCHR'CO_2R", where R', R" = alkyl] exhibited good bactericidal activity[124].



85

❖Action on the Cardiovascular System

A.Mizuno, et al[125, 126] have described that pyrrole oximes(86), which exhibited anti-serotonin 5-HT_2-receptor activity were proposed as antihypertensive and anticoagulation drugs.The oxime derivatives of pyrroloazepines also exhibited vasodilation activity. Among these compounds the oxime (87) was mentioned as one of the most active[127,128]. The blocking action of pyrrole O-(2-alkylamino-2-hydroxypropyl)oximes (88) on â-adrenoceptors has also been investigated[129].



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129a.A.Baxter, J.Dixon, F.Ince, C.Manners, S.Teague, *J.Med.Chem.*, **36**(19), 2739(1993)
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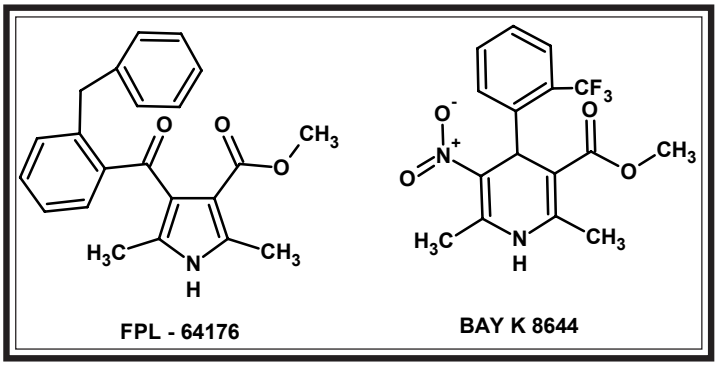
129c.T.Hoogland, P.Saggau, *J.Neuroscience*, **24**(39), 8416(2004)

A.Baxter et.al[129a] have suggested that Methyl 2,5-dimethyl-4-[2-(phenylmethyl)benzoyl]-1H-pyrrole-3-carboxylate, FPL 64176 is the first example of a new class of calcium channel activator (CCA) that does not act on any of the well-defined calcium channel modulator receptor sites, as typified by verapamil, diltiazem, and the dihydropyridines. The potent activity of FPL 64176, having the 2- (phenylmethyl)benzoyl substituent, was predicted using QSAR on an initial set of less potent benzoylpyrroles. When compared to the CCA Bay K 8644, FPL 64176 has similar potency on calcium uptake into GH3 cells (both have EC_{50} ~ 0.015 ìM) but is appreciably more potent functionally at increasing contractility in a guinea pig atria preparation (FPL 64176 has EC_{50} = 0.049 ìM vs Bay K 8644 EC_{50} = 1.95 ìM). FPL 64176 is an achiral, pharmacologically clean agonist with no demonstrable partial agonist properties and possesses appreciably higher efficacy than Bay K 8644. It should therefore become a useful biochemical and pharmacological tool for the study of calcium channels in many cell types.



❖As Neuronal L-type Calcium channel activator

T.Ginap, and co-workers[129b] tested FPL 64176 (methyl-2,5-dimethyl-4-(2-(phenylmethyl)benzoyl-[1 H]pyrrole-3-carboxylate) for an interaction with neuronal L-type voltage-sensitive calcium channels (L-VSCCs) by using a [^{3}H]isradipine ([^{3}H]ISR) binding assay, and for its ability to enhance K^+-evoked [^{3}H]norepinephrine ([^{3}H]NE) release from rat neocortical slices. The classical L-VSCC activator, the dihydropyridine (DHP) BAY K 8644, was also used for comparative purposes. FPL 64176 and BAY K 8644 both produced a similar concentration-dependent enhancement of 15 mM K^+-

evoked[^{3}H]NE release which could be completely blocked by the L-VSCC blocker ISR (0.1ìM). FPL 64176, in contrast to BAY K 8644, was a very weak inhibitor of [^{3}H]ISR binding to L-VSCCs. These findings indicate that FPL 64176 is a novel non-dihydropyridine L-VSCC activator, most probably by acting on a site different from the DHP binding site.

More over, T.M.Hoogland, et. al[129c] studied the contribution of L-type Ca^{2+} channels to action potential-evoked Ca^{2+} influx in dendritic spines of CA1 pyramidal neurons and the modulation of these channels by the $^2_2$ adrenergic receptor. L-type Ca^{2+} channel activators FPL64176 and Bay K8644 (both 10 ìM) significantly enhanced the spine Ca^{2+} transients by 40-50%, which revealed that L-type Ca^{2+} channels are functionally present in dendritic spines of CA1 pyramidal neurons, contribute to spine Ca^{2+} influx, and can be modulated by the â- adrenergic receptor through protein kinase-A(PKA) in a highly compartmentalized manner.

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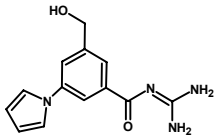
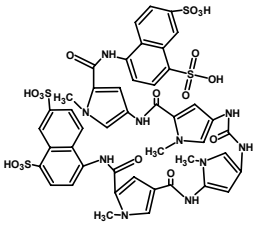
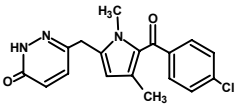
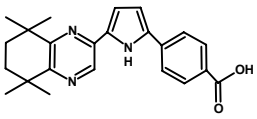
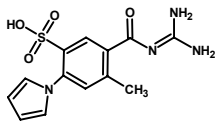
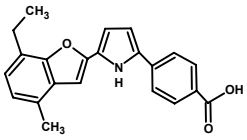
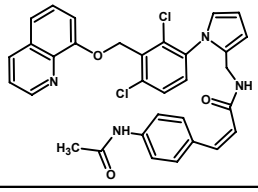
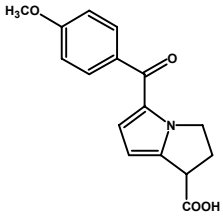
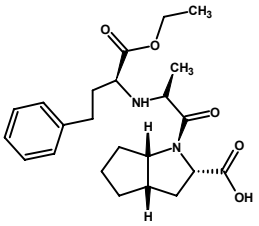
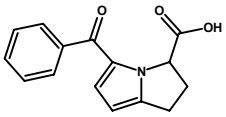
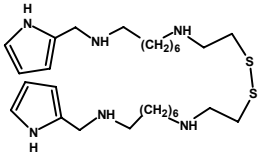
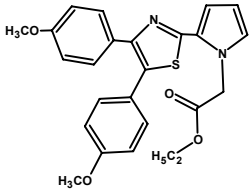
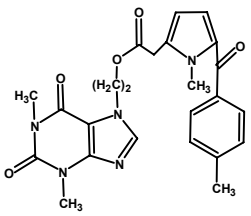
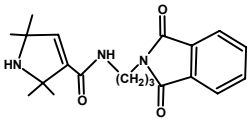
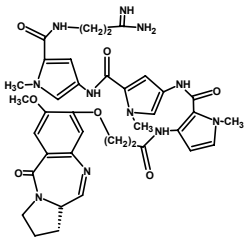
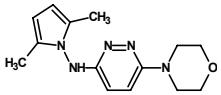
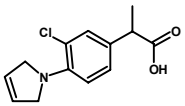
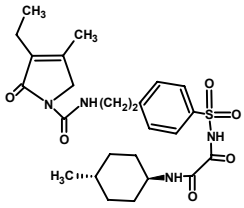
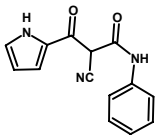
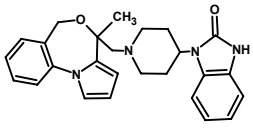
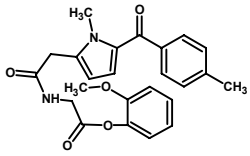
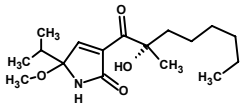
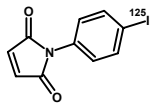
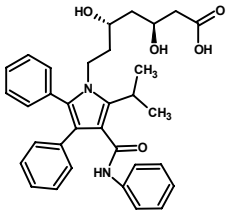
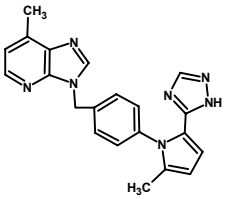
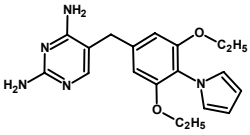
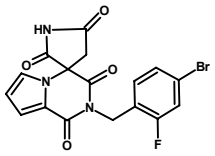
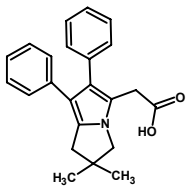
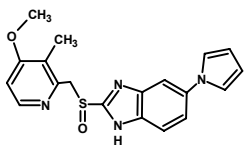
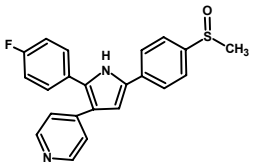
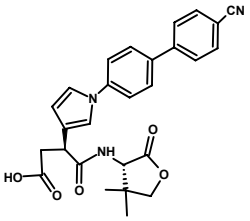
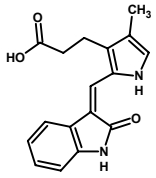
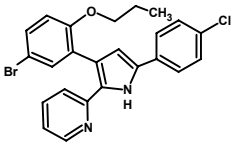
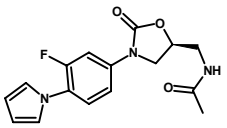
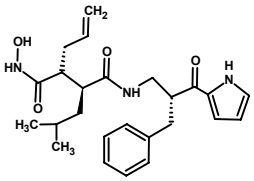
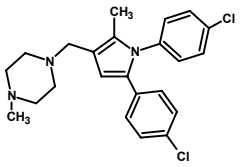
Some Pyrrole drugs & derivatives under Preclinical/Phase clinical trials.

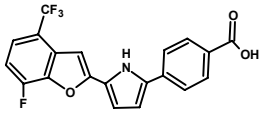
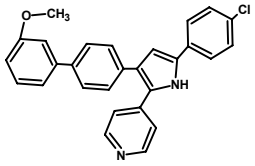
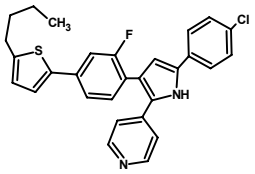
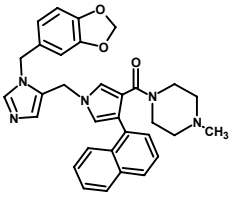
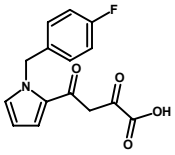
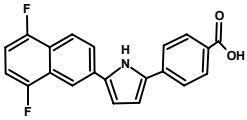
Drug Name/Code	Chemical Structure	Activity	Phase	Originator
FR-168888		Antianginal, Antiarrhythmic, Na^+/H^+ exchange inhibitor	Preclinical	Fugisawa
NSC-651016		Anti-HIV, Chemokine CCR_5, CCR_1 & CCR_2 antagonist	Preclinical	Uni. of vermont
RS-57067000		Cycloxygenase-2 inhibitor, NSAID	Preclinical	Roche
ER-34617		Oncolytic, Retinoid RARalpha agonist	Biological testing	Eisai
Eniporide		Acute myocardial infarction, Na^+/H^+ exchange inhibitor	Phase-II	Merck
ER-65250		Dermitologic, Immunomodulator, Oncolytic, Retinoid RARalpha agonist	Biological testing	Eisai
FR-193517		Bronchodilator, Bradykinin antagonist, Bradykinin B_2 antagonist	Preclinical	Fugisawa

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
Anirolac		NSAID	Phase-II	Roche Bioscience
Ramipril		Antihypertensive, ACE Inhibitor	Launched-1989	Aventis
Ketorolac		Non-opioid analgesic, NSAID	Launched-1990	Roche Bioscience
Pyrextramine		Alpha adrenoceptor antagonist	Biological testing	Universita degli study di
Pamicogrel		Antiplatelet therapy, Cyclooxygenase inhibitor	Preregistered	Kanebo
Medosan-27		Antiplatelet therapy, Bronchodilator, Thromboxane A2 antagonist, NSAID, Thromboxane synthetase Inhib.	Phase-I	Medosan
A-2545		Antiarrhythmic, Sodium channel blocker, Calcium channel antagonist	Preclinical	Alkaloida

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
-		DNA damaging drug, Oncolytic drug	Biological testing	Pharmacia
Mopidralazine		Antihypertensive, Vasodilator	Biological testing	Lepetit
Pirprofen, Rengasil		NSAID	Launched-1982	Novartis
Glimepiride		Antidiabatic	Launched-1995	Aventis
Prinomide		Non-opioid analgesic, NSAID	Phase-III	Novartis
Zalderide		Antidiarrheal agent, Calmodulin antagonist	Phase-III	Novartis
Amtolmetin guacil		Antiarthritic, NSAID	Launched-1997	Sigma-Ta

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
PI-091		Antiplatelet Therapy	Preclinical	Taisho
IPM-125I		Oncolytic, Monoclonal antibodies	Preclinical	University of Alabama
Atorvastatin		Lipoprotein disorder, HMG-CoA reductase inhibitor	Launched-1997	Pfizer
FR-143187		Antihypertensive, Angiotensine AT_1 Antagonist	Preclinical	Fugisawa
Epiroprim		Antibacterial, Protozoal disease, Dihydrofolate reductase Inhib , Trimethoprim analogue	Phase-I	Roche
SX-3202((+)-(-s)-isomer)		Symptomatic antidiabatic, Aldose reductase inhibitor	Phase-II	Dainippon
ML-3000		Antiarthritic, Cyclooxygenase inhibitor, Lipoxygenase inhibitor, NSAID	Phase-II	Merck
ly-81149		Gastroesophageal reflux disease, Agents for, Anti-Helicobacter pylori agent , Gastric antisecretory drug, H^+/K^+ ATPase inhibitor	Phase-II	Il-Yang

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
L-167307		Antiarthritic, P38 protein kinase inhibitor, TNF-alpha release inhibitor	Preclinical	Merck
AG-3433		Oncolytic, Angiogenesis Inhibitor, Matrix metalloproteinase inh	Preclinical	Agouron
SU-5402		Oncolytic, Inhibitor of signal trnansduction pathways, Tyrosine kinase inhibitor, Matrix metalloproteinase inhib.	Preclinical	Sugen
L-168049		Antidiabatic, Glucagon antagonist	Preclinical	Merck
PNU-107922		Antibacterial	Preclinical	Pharmacia
-		Treatment of Osteoporosis, Antiarthritic, Matrix metalloproteinase inh, TNF-alpha release inhibitor	Biological testing	Abbott
BM-212		Antimycobacterial agent	Biological testing	Universita degli study "La Sapienza"

Drug Name/Code	Chemical Structure	Activity	Phase	Originator
-		Dermatologic drug, Immunomodulator, Oncolytic, Retinoid RARalpha agonist	Biological testing	Eisai
-		Antidiabatic, Glucagon antagonist	Biological testing	Merck
-		Antidiabatic, Glucagon antagonist	Biological testing	Merck
LB-42908		Oncolytic, Inhibitor of signal transduction pathways, Farnesyl transferase inhibitor	Preclinical	LG Chem.
L-731988		Anti-HIV, HIV-integrase inhibitor	Biological testing	Merck
ER-35368		Oncolytic, Dermatologic drug, Immunomodulator, Retinoid RARalpha agonist	Biological testing	Eisai

Synthetic Approaches

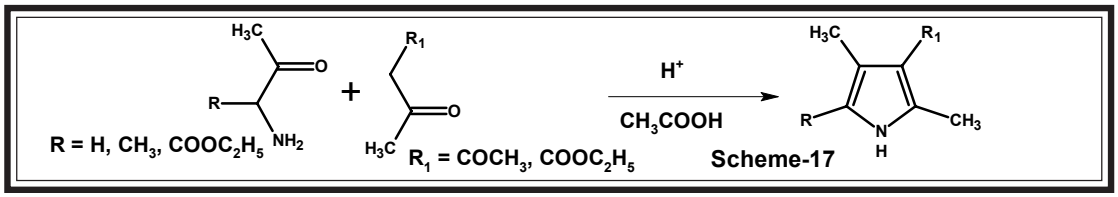
❖ Cyclization Reactions

Pyrroles are generally synthesized by the cyclization reactions involving nucleophilic-electrophilic interactions.

A. (3+2)Cyclization Reactions

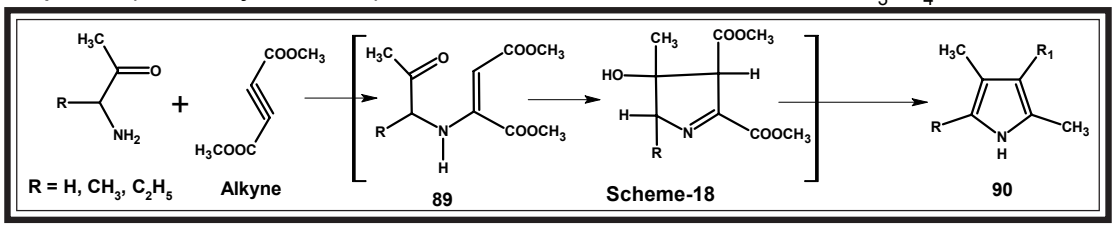
A-1. Reaction of á-aminoketones or á-amino-â-keto esters with â-diketones or â-ketoesters (Knorr Pyrrole Synthesis)

This is the most widely used method and involves the cyclizative condensation of á-aminoketones or á-amino-â-keto esters(three atom fragment with nucleophilic nitrogen and electrophilic carbonyl carbon) with â-diketones or â-ketoesters(two atom fragment with electrophilic carbonyl carbon and a nucleophilic carbon) with the formation of N-C_2 and C_3-C_4 bonds. (Scheme-17).



A-2. Reaction of á-aminoketones with alkynes

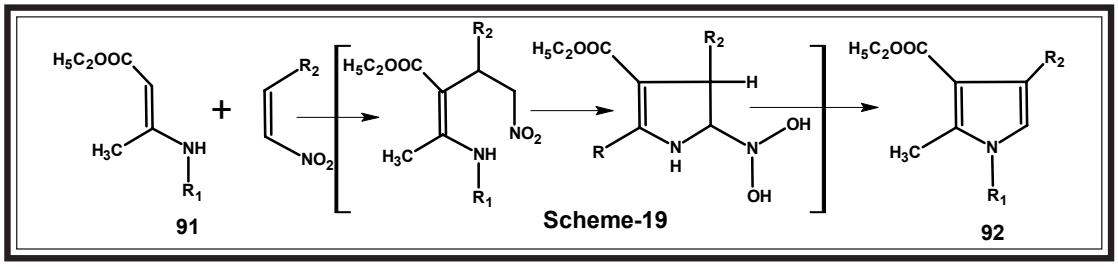
The reaction of á-aminoketones with alkynes proceeds with the nucleophilic addition of amino group to the electrophilic carbon of alkyne with the formation of enamine intermediate (89) which on intramolecular cyclization provides pyrroles (90) involving nucleophilc(â-carbon of the enamine intermediate)-electrophilic (carbonyl carbon) interaction with the formation of C_3-C_4 bond[130].



A-3. Reaction of â-amino-á,â-unsaturated esters with nitroalkenes

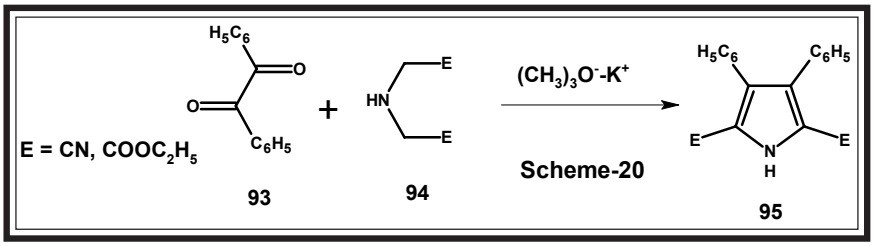
The reaction of â-amino-á,â-unsaturated esters 91(three atom fragment) with nitroalkenes(two atom fragment) provides substituted pyrrole 92 involving (3+2) cyclization with N-C_2 and C_3-C_4 bonds[131].(Scheme-19).

132.M.Friedman, *J.Org.Chem.*, **30**, 859(1965)
133.(a)H.Posvic, R.Dombro, H.Ito, T.Telinski, *J.Org.Chem.*, **39**, 2575(1974)
(b)H.Fritz, P.Uhrhan, *Leibigs Ann.Chem.*, **744**, 81(1971)



A-4. Reaction of á-diketones with amines

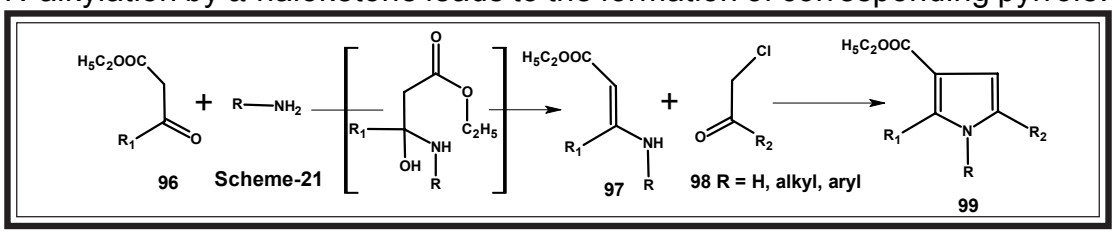
Base catalysed (3+2) cyclizative condensation of á-diketones(93) with amines(94) substituted with electron withdrawing substituents at á , á′-positions results in the corresponding pyrroles (95) with the formation of C_2-C_3 and C_4-C_5 bonds[132]. (Scheme-20).



B. (2+2+1) Cyclization Reactions

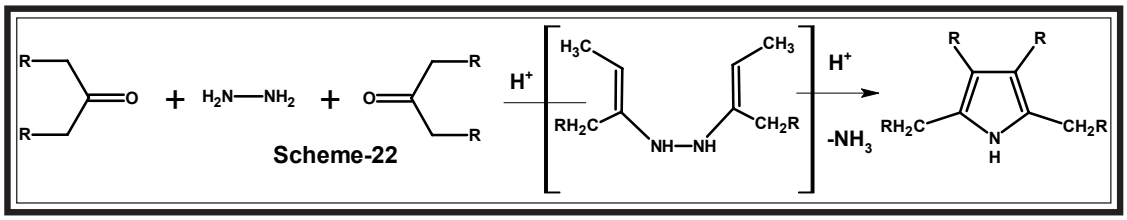
B-1. Reaction of â-ketoesters with á-haloketones (Hantzsch Synthesis)

The reaction of â-ketoesters(96) with á-haloketones or aldehydes(98) in the presence of ammonia or primary amine affords pyrroles(99) involving (2+2+1) cyclization with the formation of N-C_2, C_3-C_4 and N-C_5 bonds. The reaction proceeds via stabilized enamine intermediate (97) which on C-alkylation and N-alkylation by á-haloketone leads to the formation of corresponding pyrrole.



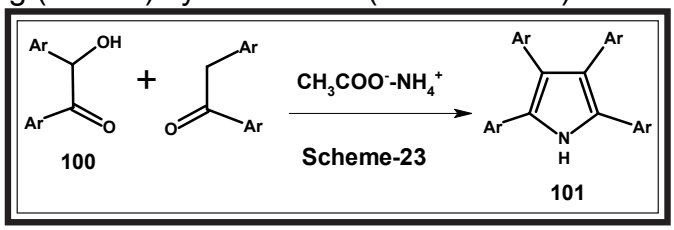
B-2. Reaction of aldehydes or ketones with hydrazine (Piloty-Robinson Synthesis)

The condensation of aliphatic aldehydes or ketones with hydrazine under strongly acidic conditions affords pyrroles involving (3+3) sigmatropic rearrangement and cyclization. This reaction can also be applied for the preparation of N-substituted pyrroles by condensing ketones with methyl hydrazine or N,N'-dimethyl hydrazine[133].(Scheme-22)



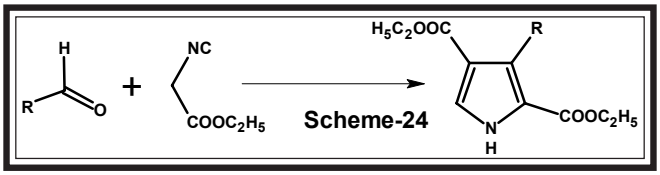
B-3. Reaction of Benzoin with Benzyl aryl ketones

The reaction of benzoin(100) with benzyl aryl ketones in the presence of ammonium acetate results in the formation of polyaryl substituted pyrroles (101) involving (2+2+1) cyclization[134].(Scheme-23)



B-4. Reaction of aldehydes with alkyl isocyanoacetates

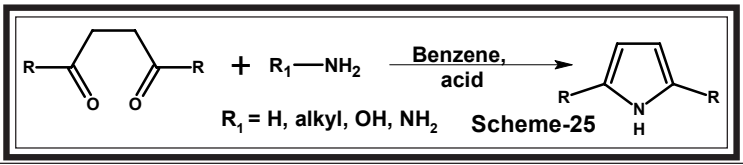
The condensation of alkyl isocyanoacetates with aldehydes in 2:1 ratio proceeds to involve an initial conjugated addition followed by an anionic cyclization with the formation of C_2-C_3, C_3-C_4 and C_4-C_5 bonds[135].(Scheme-24)



C. (4+1) Cyclization Reaction

C-1. Reaction of 1,4-diketones with ammonia or ammonium derivatives (Paal-KnorrSynthesis)

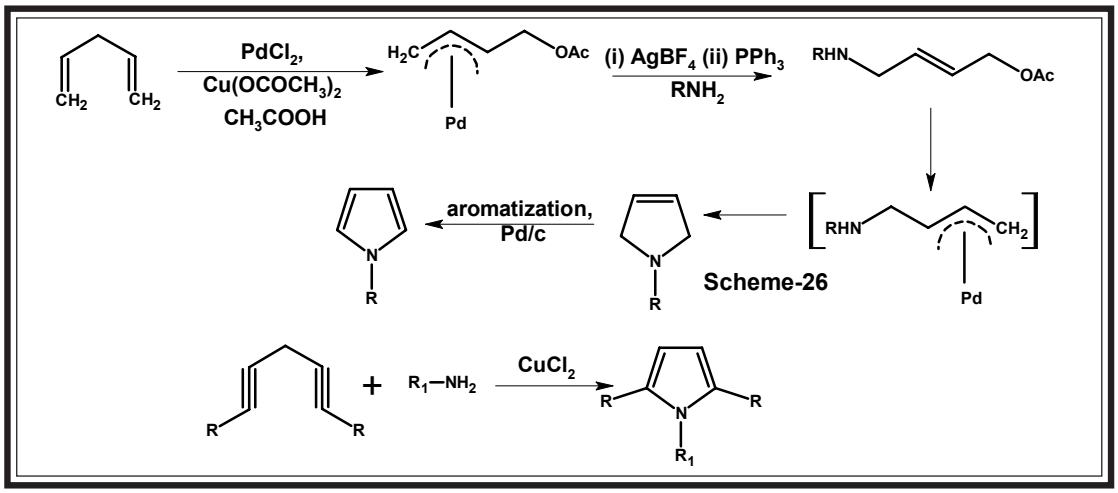
It is the most general method and involves (4+1)cyclizative condensation of 1,4-dikitones(enolizable) with ammonia or its derivatives with the formation of N-C_2 and N-C_5 bonds[59-64,136-137]. The reaction is considered to proceed with the successive attacks of nucleophilic nitrogen on the carbonyl carbon and finally followed by dehydration(aromatization) for which driving force is provided by the stability of the resulting pyrrole.(Scheme-25)



134. B.Tanaseichuk, S.Vlasova, E.Morozov, *Zh.Org.Khim.*, **7**, 1264(1971)
135.M.Suzuki, M.Miyoshi, K.Matsumoto, *J.Org.Chem.*, **39**, 1980(1974)

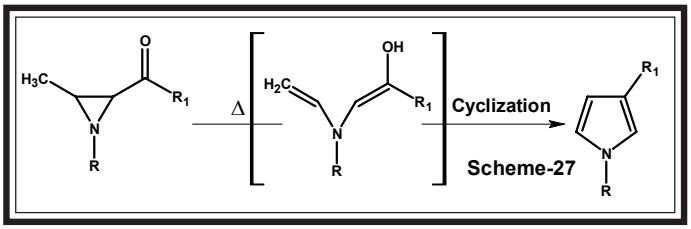
C-2. Reaction of 1,4-dienes or diynes with amines

Metal ion assisted amination of 1,4-dienes or diynes with primary amines followed by cyclization provides N-substituted pyrroles[138-140], Scheme-26.

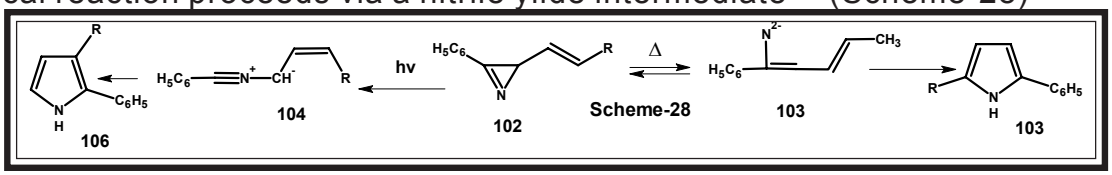


❖ Ring Expansion Reaction

2-Acylaziridines undergo ring expansion reactions to provide pyrroles(99) involving ring-opened dipolar intermediates[141].(Scheme-27)



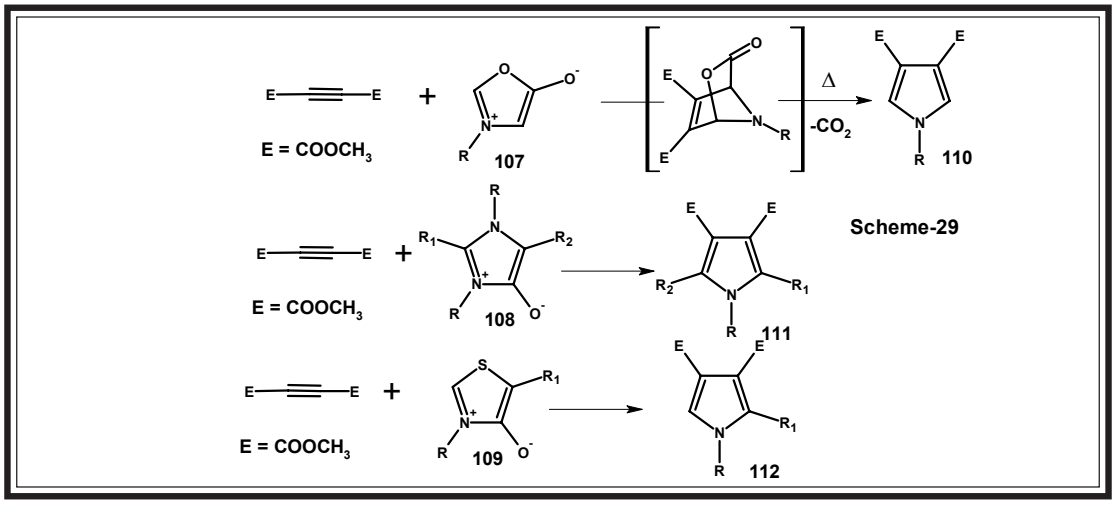
Vinylazirines 102 also undergo ring expansion reactions, but two isomeric pyrroles 105 and 106 are obtained depending on the reaction conditions. Thermal reactions involves a nitrene intermediate 103, while photochemical reaction proceeds via a nitrile ylide intermediate[142] (Scheme-28)



❖Extrusion Reaction

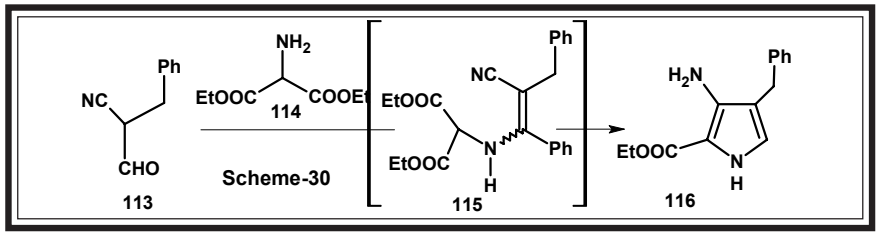
The complex ring systems, formed by (3+2) cycloaddition of activated alkynes substituted with electron-withdrawing substituents, with mesoionic heterocycles 107-109, undergo extrusion reactions with the cleavage of larger ring leaving the pyrrole ring system intact[143-144].(Scheme-29)

136.(a) A.Kartritzky, J.Suwinski, *Tetrahedron*,**31**, 1549(1975)
(b) P.Chiu, K.Lui, P.Maini, M.Sammes, *J.Chem.Soc. Chem.Commun.*, 109(1987)
(c)V.Amarnath, D.Anthony, K. Amarnath, W.Valentine, L.Wetterau, D.Graham, *J.Org.Chem.*, **56**, 6924(1991)



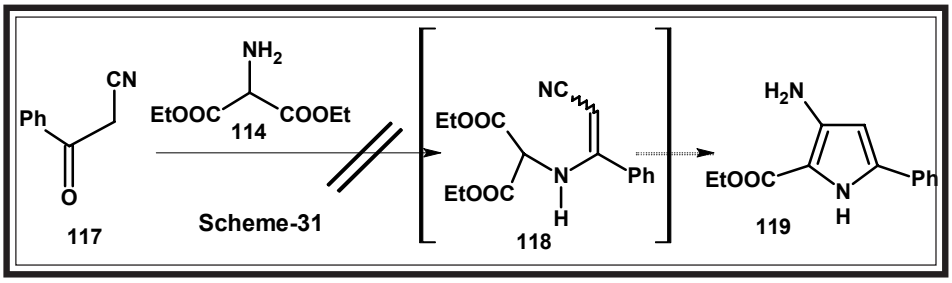
❖Miscellaneous Approaches

Ethyl-3-amino-5-phenyl-1H-pyrrol-2-carboxylate found to be a useful intermediate in the sysnthesis of other nitrogen heterocycles[145]. How ever Elliott and co-workers[146,147] have described that condensation of aldehyde(113) with aminomalonate(114) gave 3-substituted enamine(115), which was cyclized in the presence of sodium methoxide to give 3-amino-4-benzyl-1H-pyrrol-2-carboxylate (116)(Scheme-30)

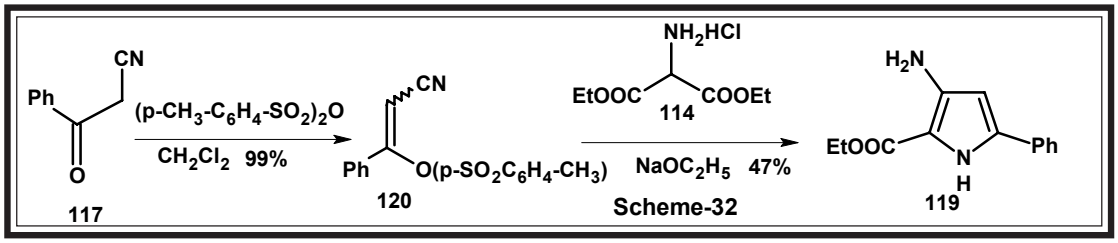


Using a similar approach toward the synthesis of pyrrole (119), Ning Chen, et. al[148] were failed to synthesize 2-substituted enamine intermediate(118) from benzoyl acetonitrile(117) and diethyl aminomalonate(114) using standard conditions such as azeotropic removal of water[149], dehydration with molecular sieves or Lawis acid catalysis($TiCl_4$[150], $BF_3{\cdot}OEt_2$)(Scheme-31)

137.(a) N.Kozlov, L.Moiseenok, S.Kozintsev, *Khim. Geterosikl. Soedin.*, 1483(1979)
(b) R.Ramanasseul, A.Rassat, *Bull.Soc.Chim.France,* 4330(1970)
138. J.Backvall, J.Nystrom, *J.Chem.Soc.Chem.Commun.*, 59(1981)
139.A.Makhsumov, A.Safaev, N.Madikhanov,*Khim. Geterosikl. Soedin.*,125(1970)
140.A.Chalk, *Tetrahedron.Lett.*,3487(1972)
141.A.Padwa, D.Dean, T.Oine, *J.Am.Chem.Soc.*, **97**, 2822(1975)
142.A.Padwa, J.Smolanoff, A.Tremper, *J.Am.Chem.Soc.*, **97**, 4682(1975)
143.(a) R.Huisgen, H.Gotthardt, H.Bayer, F.Schaefer, *Chem.Ber.*, **103**,2611(1970)
(b) I.Benages, S.Albonico, *J.Org.Chem.*, **43**, 4273(1978)
144. K.Potts, S.Chen, *J.Org.Chem.*, **42**,1639(1977)
145.(a) S.Niitsuma,K.Kato, T.Takita, H.Umezawa, *Tetrahedron Lett.*,**26**, 5785(1985).
(b) A.Geies, A.El-Dean, O.Moustafa, *Monatsh. Chem.*,**127**,1263(1996).
(c) A.Monge, J.Palop, P.Parrado, C.Perez, E.Fernandez, *J. Heterocycl. Chem.*,**24**, 437(1987).
(d) A.Monge, J.Palop, T.Goni, F.Martinez-Crespo, I.Recalde, E.Fernandez-Alvarez, *J. Heterocycl. Chem.*,**23**, 647(1986).



Considering the lower electrophilicity of the aryl ketone (117) compared to the aldehyde (113), an alternative strategy for preparing enamine (118) was examined. Since amines are known to undergo substitution reactions with chloro- and alkylthio-substituted olefins to yield enamines[151], a similar approach was considered and the reaction of *p*-toluenesulfonyl enol ester of á-cyano ketone (120) with diethyl aminomalonate (114) was investigated. Tosylate (120) was prepared by reacting benzoyl acetonitrile with *p*-toluenesulfonic anhydride (1.2 equiv) in dichloromethane and triethylamine (1.5 equiv) in 99% yield. When a mixture of tosylate (120) and diethyl aminomalonate hydrochloride (1.2 equiv) were treated with an ethanolic solution of sodium ethoxide (0.2 M, 3.5 equiv)[152], the pyrrole (119) was directly obtained without isolation of enamine (118). Addition of the amine, cyclization and decarboxylation occurred in one pot at room temperature to give a 46% overall yield of (119) from benzoyl acetonitrile.(Scheme-32)



(e)A.Monge, I.Aldana, M.Font, J.Arraras, E.Santiago, M.Lopez-Unzu, J.Martinez-de-Irujo, E.Alberdi, E.Fernandez-Alvarez, *Arch. Pharm. (Weinheim, Ger.)*,**325**, 439(1992).
146.A.Elliott, P.Morris, S.Petty, C.Williams, *J.Org. Chem.*,**62**, 8071(1997).
147.(a) M.Lim, R.Klein, J,Fox, *J. Org. Chem.*,**44**, 3826(1979).
(b) M.Lim, W.Ren, B.Otter, R.Klein, *J. Org. Chem.*,**48**, 780(1983).
148.N.Chen,Y. Lu, K.Gadamasetti, C.Hurt, M.Norman,C.Fotsch,*J. Org. Chem.*,**65**, 2603(2000)
149.(a) M.Furukawa, T.Okawara, Y.Noguchi, Y.Terawaki, *Chem. Pharm. Bull.*, **27**, 2223(1979).
(b) G.Birnberg, W.Fanshawe, G.Francisco, J.Epstein,*J.Heterocycl.Chem.*,**32**, 1293(1995).
150.(a)R.Carlson, A.Nilsson, M.Stroemqvist, *Acta Chem.Scand.*,**B37**, 7(1983).
(b) M.Selva, P.Tundo, C.Marques, *Synth. Commun.*, **25**, 369(1995).
151(a).B.Erdmann, A.Knoll, J.Liebscher, J. *J. Prakt. Chem.*,**330**, 1015(1988).
(b)K.Gewald, H.Schaefer, P.Bellmann, U.Hain, *J. Prakt. Chem.*,**334**, 491(1992).
152.A.Elliott, J.Montgomery, A.Walsh, *Tetrahedron Lett.*,**37**, 4339(1996).
153.K.Raval, *Ph.D. Thesis,* Saurashtra University (2004)
154.

Work Done at Our Laboratory

The literature servey described in the present chapter reveals that 1,3,4-oxadiazole shows potent biological activities as antimicrobial, analgesic, antiinflammatory, antiproteolytic, anticonvulsant, local anaesthetic, antiasthmatic, phenyl-glycine dipeptidomimetics, tyrosine kinase inhibitors, TNF-α-inhibitors and cyclic nucleotide inhibitors, monoamine oxidase type-A inhibitors and antidepressent, while pyrrole derivatives are showing promissing pharmacological activities like monoamine oxidase type-B inhibitors, antitubercular, tyrosine kinase inhibitors, antitubercular, HIV-1 integrase inhibitors, antiproliferative, glycogen synthase kinase-3 inhibitors, analgesic and anti-inflammatory, bactericidal, fungicidal and plant growth regulators, cardiovascular and neuronal L-type calcium channel activators.

With an innovative approach to study the biological activity of the 1,3,4-oxadiazoles and 1H-pyrrole derivatives clubed with each other, from this laboratory, K. Raval[153] has initiated to sythesize 3,5-bis-1,3,4-oxadiazolyl-2,4-dimethyl-1H-pyrrole derivatives, which includes synthetic stretagy starting from acid catalysed cyclocondensation of ethyl ester derivative of 3-amino-2-butenoic acid (generated insitu from ethylacetoacetate upon oximenation followed by reduction)with ethylacetoacetate to afford diethyl-2,4-dimethyl-1H-pyrrole-3,5-dicarboxylate, which was derivatized into corresponding dihydrazide derivative on treatment with hydrazine hydrate. The dihydrazide derivative on reaction with different aromatic and heteroaromatic acid, in the presence of phosphorous oxychloride affords 3,5-bis-1,3,4-oxadiazolyl-2,4-dimethyl-1H-pyrrole derivatives.

Current Work Plan

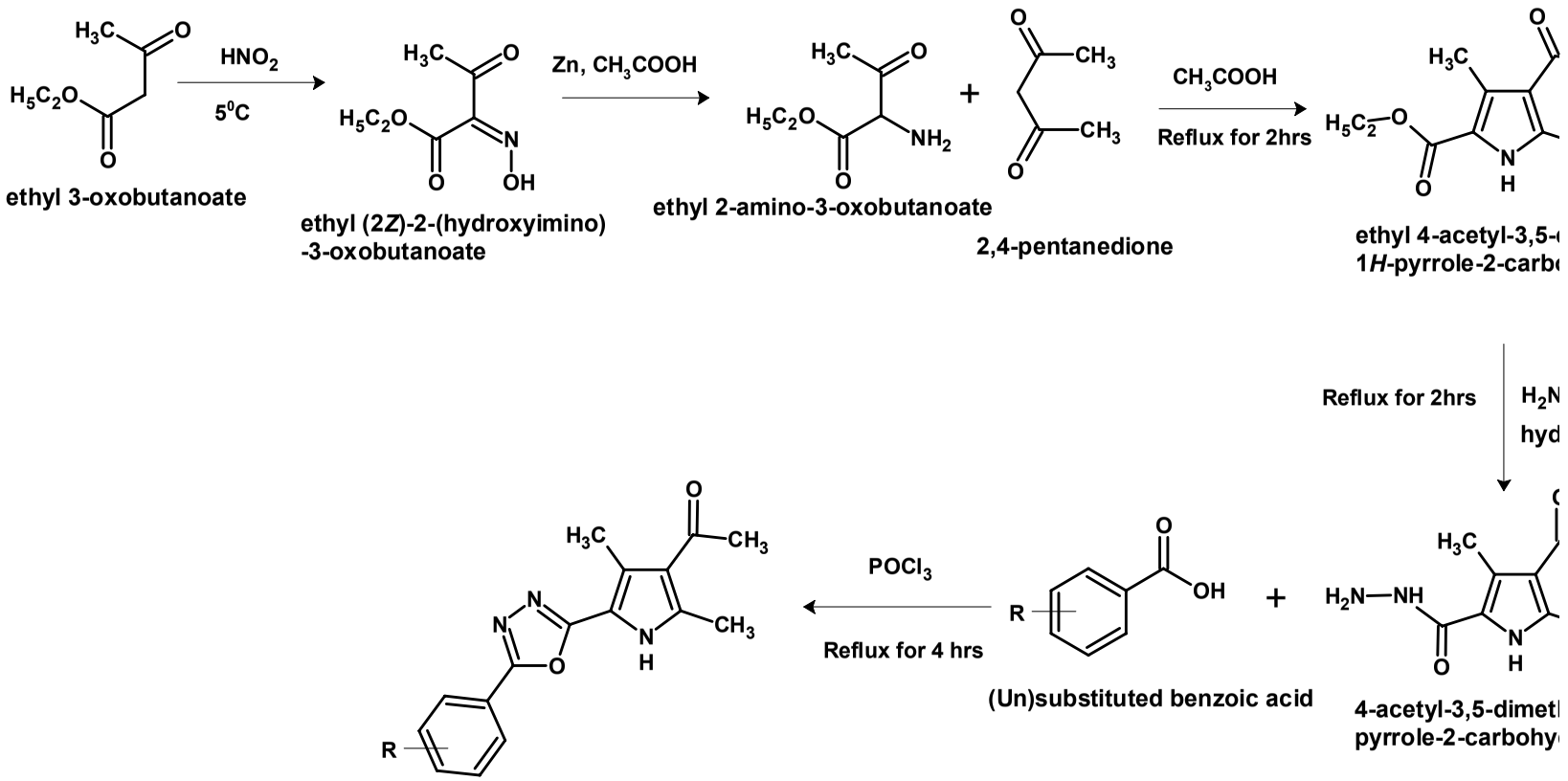
In continuation with our study on 1H-pyrrole derrivatives clubed with substituted-1,3,4-Oxadiazoles, in the present chapter 1-[2,4-dimethyl-5-(5-phenyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrrol-3-yl]ethanone derivatives were synthesized. The synthetic stretagy follows starting with acid catalysed cyclocondensation of ethyl ester derivative of 3-amino-2-butenoic acid (gen

erated insitu from ethylacetoacetate upon oximenation followed by reduction)with acetylacetone to afford ethyl-3-acetyl-2,4-dimethyl-1H-pyrrole-5-carboxylate, which was derivatized into corresponding hydrazide derivative on treatment with hydrazine hydrate. The hydrazide derivative on reaction with different aromatic and heteroaromatic acid, in the presence of phosphorous oxychloride affords 1-[2,4-dimethyl-5-(5-phenyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrrol-3-yl]ethanone derivatives.

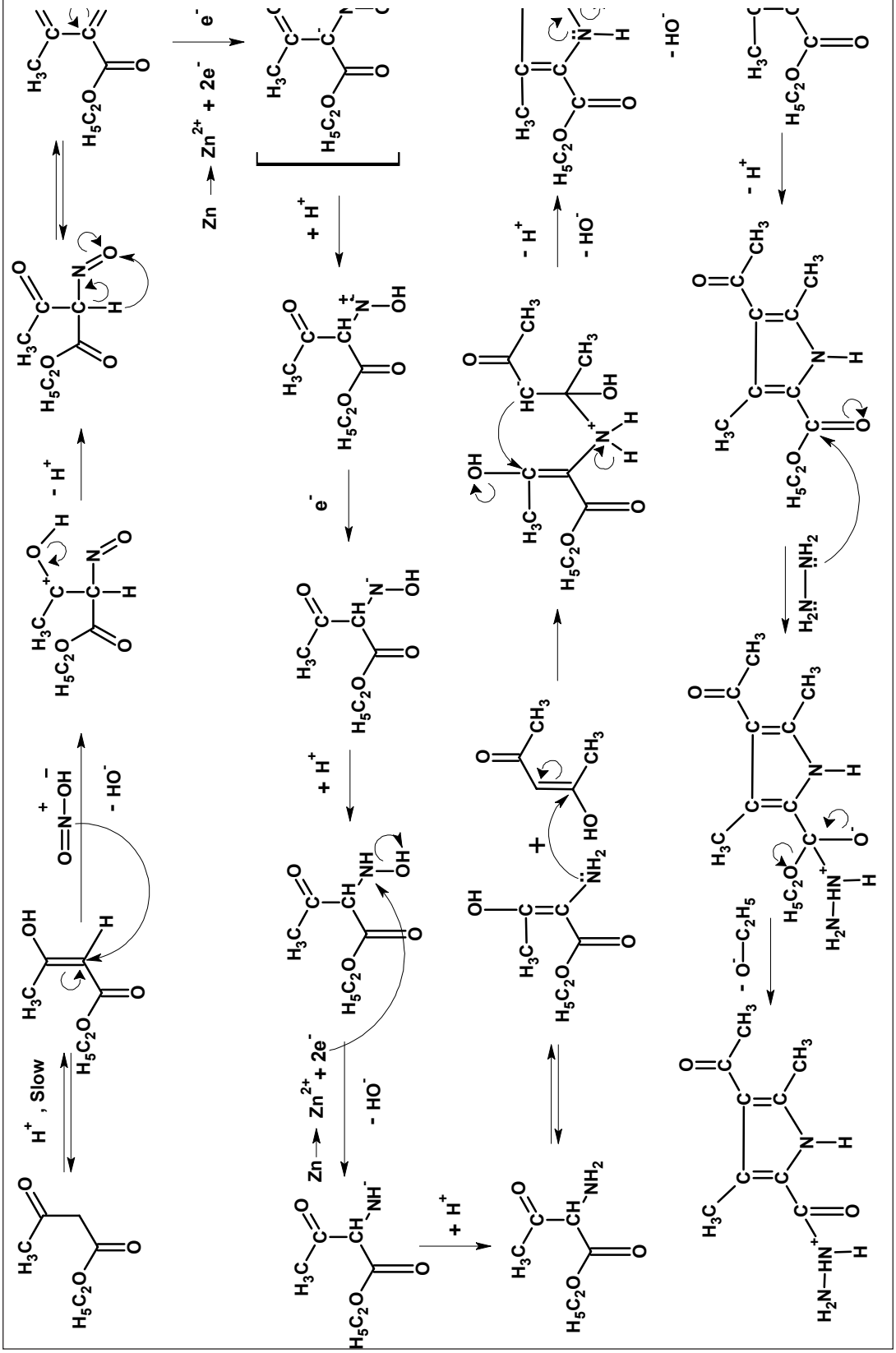
The structures of all the synthesized compounds were confirmed by IR, ^{1}HNMR and Mass spectral data and elemental analysis.

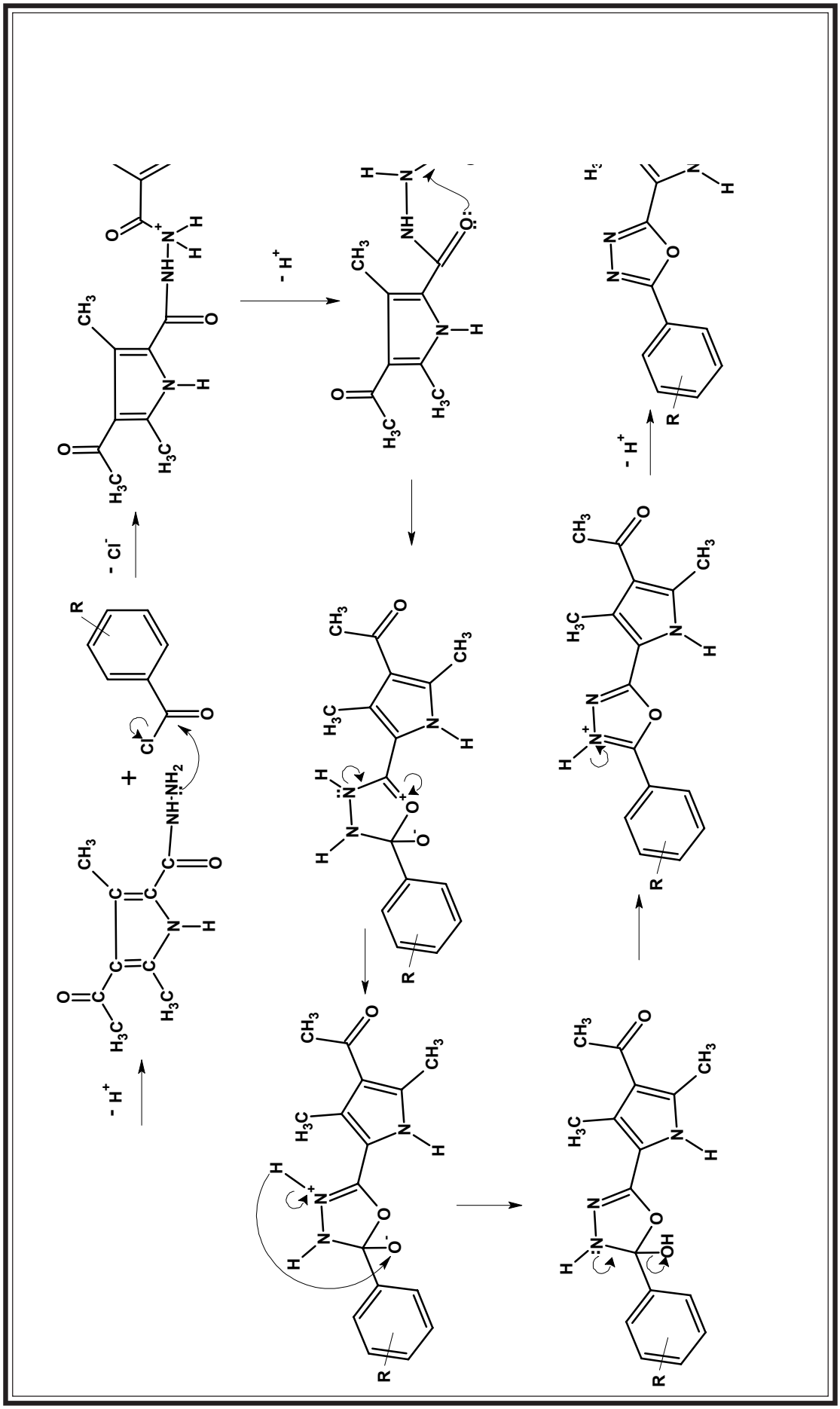
The compounds of the present invention were screened for antitubercular and antibacterial activity.

Reaction Scheme



Reaction Mechanism





Experimental Protocols

All the starting materials, ethyl acetoacetate, sodium nitrite, gla. AcOH, Zinc dust, acetyl acetone, hydrazine hydrate, substituted aromatic acids and phosphorous oxychloride were obtained from the commercial sources i.e. Spectrochem Ltd., s.d.fine chem. ltd., Sisco Research Lab., Ranbaxy Lab., Allied Chem. Ltd. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature using standard Zeal's thermometer and are uncorrected. The commencement of the reaction and purity of the synthesized compounds were ensured using thin layer chromatography (TLC) silica gel-G, used as stationary phase, the TLC plates were purchased from Merck India Ltd. ethyl acetate: hexane was used as the mobile phase. However other solvent systems like acetone :benzene and methanol:chloroform was also employed but the best results observed with ethyl acetate:hexane system.

Experimental

Synthesis of Ethyl 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate:

In a 3-l. three-necked flask provided with a stirrer and surrounded by an ice bath are placed 402 g.(3.09 moles) of ethyl acetoacetate and 1.2 l. of glacial acetic acid. To this solution is then added dropwise with stirring a solution of 246 g. (3.55 moles) of sodium nitrite in 400 ml. of water. The rate of addition is controlled so that the temperature does not rise above 12°. After the sodium nitrite solution has been added, the mixture is stirred an additional 2–3 hours. It is then allowed to warm up to room temperature and stand about 12 hours, after which 348 g. (3.48 moles) of acetylacetone is added at one time. To the reaction mixture 450 g. of zinc dust is added in portions of about 10 g. with vigorous stirring. The rate of addition is regulated so that the emperature never rises above 60°. After the addition is complete, the mixture is refluxed for 2–3 hours on a hot plate until the unreacted zinc dust collects in balls. The hot solution is then poured through a fine copper sieve, with stirring, into 30 l. of ice water. The crude product which separates is contaminated with zinc. On recrystallization from 1.5 l. of 95% ethanol, pure 2,4-dimethyl-3-acetyl-5-carbethoxypyrrole is obtained. Yield 55–60%, m.p. 140-142^{o}C(Reported m.p.143^{o}C[154]).Purity was checked by TLC using ethyl acetate: hexane(3:7) as mobile phase.

Synthesis of 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbohydrazide :

A mixture of 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carboxylate(0.01 mole) and 99% hydrazine hydrate(0.32 mole) in 20ml methanol was refluxed in a waterbath for 12-14hrs. The reaction mixture was poured into crushed ice to afford crude 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbohydrazide. The solid product was filtered out and recrystallized from rectified spirit. Yield 71%, m.p.172^{o}C. Purity was checked by TLC using ethyl acetate: hexane(3:7) as mobile phase.

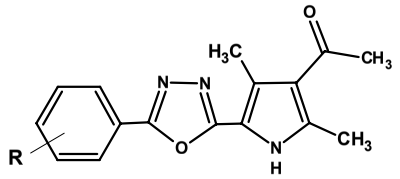
Synthesis of 1-{2,4-dimethyl-5-[(5-sub.phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanone (General method):

A mixture of 4-acetyl-3,5-dimethyl-1*H*-pyrrole-2-carbohydrazide(0.01mole), substituted aromatic acid(0.01mole) and 15ml $POCl_3$ was refluxed on water bath for 5 hrs. The reaction mixture was poured into crushed ice. The crude product was filtered and washed thoroughly with cold water till it become acid free. The product so obtained was recrystallized from methanol.The purity of each compound was checked by TLC using ethyl acetate:hexane (4:6) as mobile phase.

The elemental analysis of all the compounds performed by **PERKIN ELMER 470R** are in total agreement with the theoritical values.

The physical constants and elemental analysis of all compounds were shown in **Table 4.1**.

Table 4.1 : Physical constants of 1-{2,4-dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C ,H ,N %)		
						C	H	N
1	KUPO-1	Phenyl	$C_{16}H_{15}N_3O_2$	281.3	192-194	68.31	5.37	14.94
						68.29	5.36	14.96
2	KUPO-2	2-Cl Phenyl	$C_{16}H_{14}ClN_3O_2$	315.8	206-209	60.86	4.47	13.31
						60.83	4.45	13.32
3	KUPO-3	4-Cl Phenyl	$C_{16}H_{14}ClN_3O_2$	315.7	217-219	60.86	4.47	13.31
						60.83	4.45	13.32
4	KUPO-4	4-NO_2 Phenyl	$C_{16}H_{14}N_4O_4$	326.3	196-199	58.89	4.32	17.17
						58.87	4.34	17.15
5	KUPO-5	2-OH Phenyl	$C_{16}H_{15}N_3O_3$	297.3	198-201	64.64	5.09	14.13
						64.67	5.07	14.11
6	KUPO-7	4-OCH_3 Phenyl	$C_{17}H_{17}N_3O_3$	311.3	222-225	65.58	5.50	13.50
						65.55	5.48	13.47

Note:- The underline values denote the calculated percentage of composition

Table 4.1: Physical constants of 1-{2,4-dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C ,H ,N %)	
						C	H
8	KUPO-9	3-NO_2 Phenyl	$C_{16}H_{14}N_4O_4$	326.3	158-161	58.89	4.32
						58.87	4.34
9	KUPO-10	2-CH_3 Phenyl	$C_{17}H_{17}N_3O_2$	295.3	216-219	69.14	5.80
						69.12	5.78
10	KUPO-11	4-CH_3 Phenyl	$C_{17}H_{17}N_3O_2$	295.3	263-265	69.14	5.80
						69.12	5.78
11	KUPO-12	2-OH Napthyl	$C_{20}H_{17}N_3O_3$	347.3	223-225	69.15	4.93
						69.12	4.95
12	KUPO-13	2-Indolyl	$C_{18}H_{16}N_4O_2$	320.3	83-85	67.49	5.03
						67.46	5.05
13	KUPO-14	2-$OCOCH_3$ Phenyl	$C_{18}H_{17}N_3O_4$	339.3	149-152	63.71	5.05
						63.74	5.06
14	KUPO-15	4-Br Phenyl	$C_{16}H_{14}BrN_3O_2$	360.2	242-245	53.35	3.92
						53.32	3.94
15	KUPO-16	2 - C_6H_5NH Phenyl	$C_{22}H_{20}N_4O_2$	372.4	220-223	70.95	5.41
						70.93	5.43

Note:- The underline values denote the calculated percentage of composition

SPECTRAL CHARACTERIZATION

The constitution of newly synthesized compounds were supported by FT - IR, ^{1}H NMR and EI-Mass spectral study.

IR Spectral Study

Instrument: SHIMADZU FT-IR 8400 Spectrophotometer

Sample technique: KBr pellet

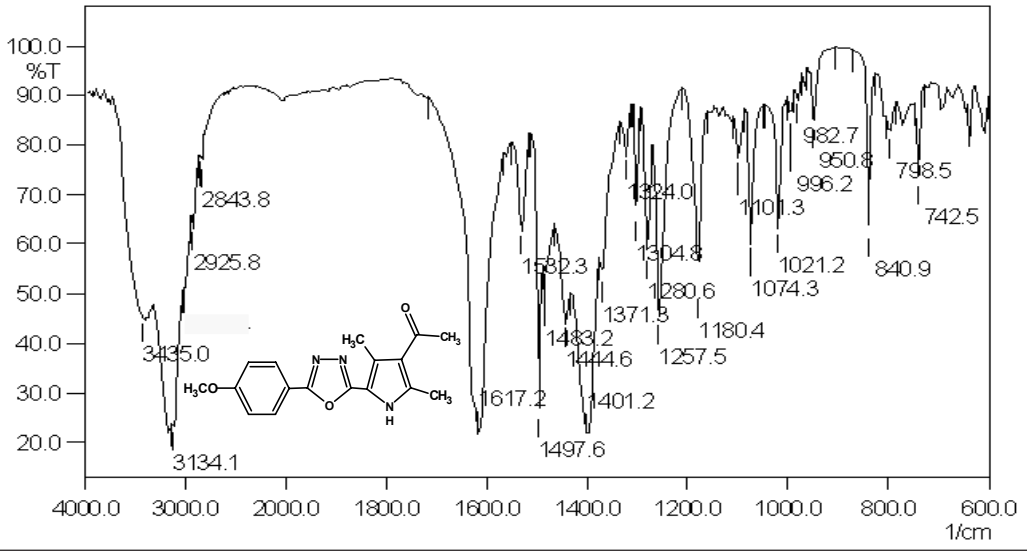
Frequency range: 400-4000 cm^{-1}

The 1-{2,4-dimethyl-5-[(5-sub.phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO Series) indicates the presence of very specific functional groups like secondary amine group of pyrrole skeleton, methyl group, carbonyl group, C=N and C-O-C vibration of 1,3,4-oxadiazole ring, aromatic ring skeleton, etc., which absorb IR radiations of specific frequency and show sharp, medium or weak intense signals and hence supports in identifiction and confirmation of the molecule.

The IR spectrums of The 1-{2,4-dimethyl-5-[(5-sub.phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones show common frequences as 3400-3450cm^{-1} for 2^{o}amino group, 3140-3110cm^{-1} for aromatic C-H str., 1660-1650cm^{-1} for C=Ostr.of carbonyl group,1620-1600cm^{-1} for C=Nstr., merge with aromatic ring skeleton, where as aromatic region was observed extended upto 1480cm^{-1}. Methyl groups situated at 2 and 4 position give band according to stretching vibrations between 2925-2920cm^{-1} and 2855-2850cm^{-1} respectively as well as a merge band of in plane deformation range between 1405-1400cm^{-1}, secondary N-Cstr. was observed range between 1360-1256cm^{-1}, while sharp intense band observed in range bet ween 1159-1060cm^{-1} for O-C str., Phenyl substitutions observed between 970-730cm^{-1}. While characteristic vibration corresponds to substitution at phenyl residue was clearly observed in each case.

A comparative chart of IR spectral data of all compounds of KUPO series are shown in **Table 4.2**

IR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-methoxy)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanone (KUPO-7)



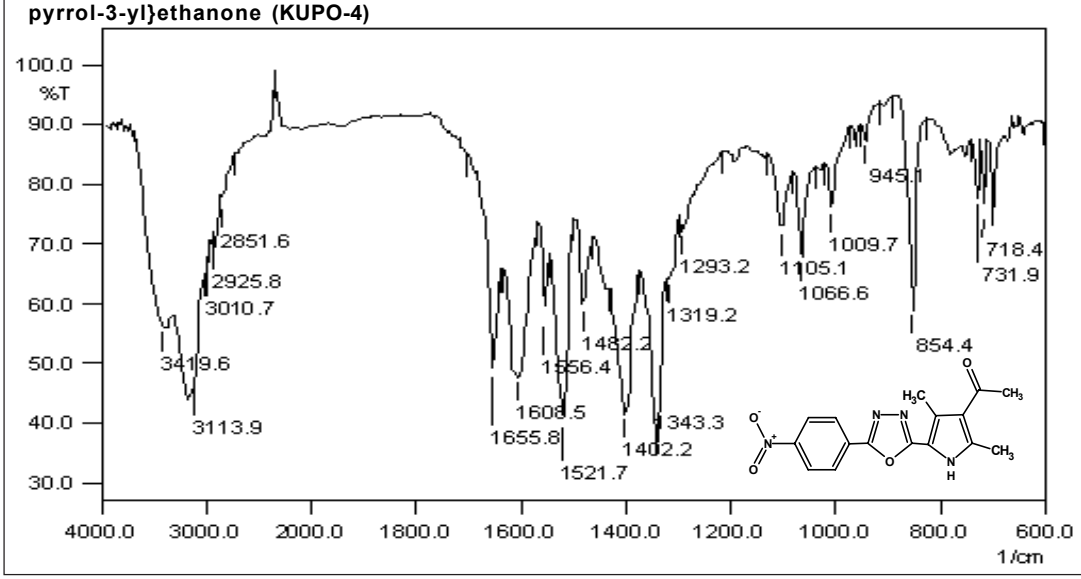
KUPO-7

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3435
-CH Aromatic	-C-H str.	3132
$-CH_3$	-C-H str.	2925,2843
Carbonyl	-C=O str.	1657
-C=N	C=N str.	1617
Aromatic Skeleton	-C=C- str.	1532,1497,1483
	1:4 di substitution	840
2°N-C	-N-C str.	1280
C-O-C	-O-C str.	1074
$-OCH_3$	-C-O str.	1257

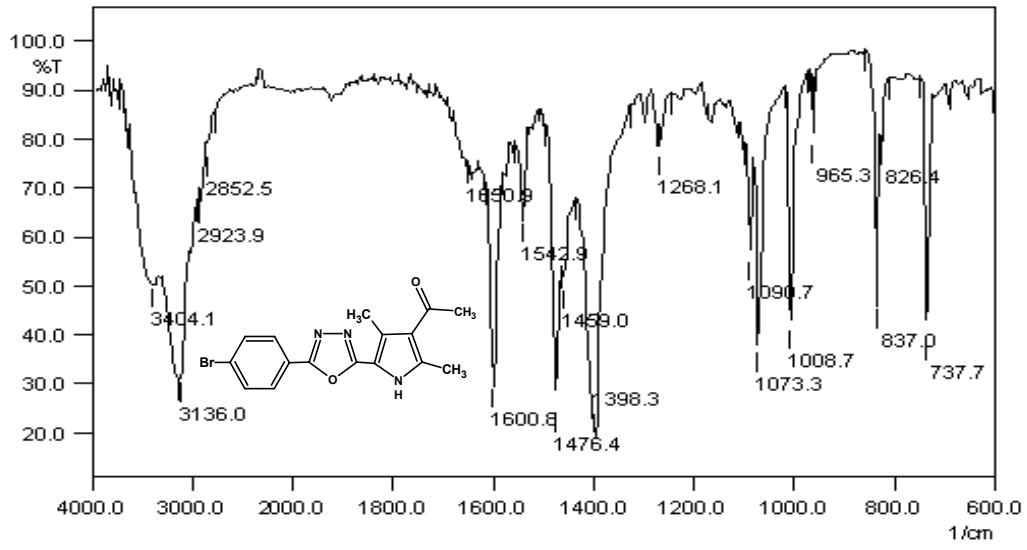
KUPO-4

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3419
-CH Aromatic	-C-H str.	3113
$-CH_3$	-C-H str.	2925,2851
Carbonyl	-C=O str.	1655
-C=N	C=N str.	1608
Aromatic Skeleton	-C=C- str.	1556,1521,1482
	1:4 di substitution	854
2°N-C	-N-C str.	1293
C-O-C	-O-C str.	1066
NO_2	-N-O str.	1343

IR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-nitro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanone (KUPO-4)



IR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-bromo)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanone (KUPO-15)



KUPO-15

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3404
-CH Aromatic	-C-H str.	3136
$-CH_3$	-C-H str.	2923,2852
Carbonyl	-C=O str.	1650
-C=N	C=N str.	1600
Aromatic Skeleton	-C=C- str.	1542,1476,1459
	1:4 di substitution	837
2°N-C	-N-C str.	1268
C-O-C	-O-C str.	1073
Halogen	-C-Br str.	737

KUPO-4

Functional Group	Vibration Mode	Frequency (cm^{-1})
-NH	N-H str.	3419
-CH Aromatic	-C-H str.	3126
$-CH_3$	-C-H str.	2923,2853
Carbonyl	-C=O str.	1650
-C=N	C=N str.	1620
Aromatic Skeleton	-C=C- str.	1604,1581,1530
	1:4 di substitution	839
2°N-C	-N-C str.	1320
C-O-C	-O-C str.	1091
Halogen	-C-Cl str.	737

IR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-chloro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanone (KUPO-3)

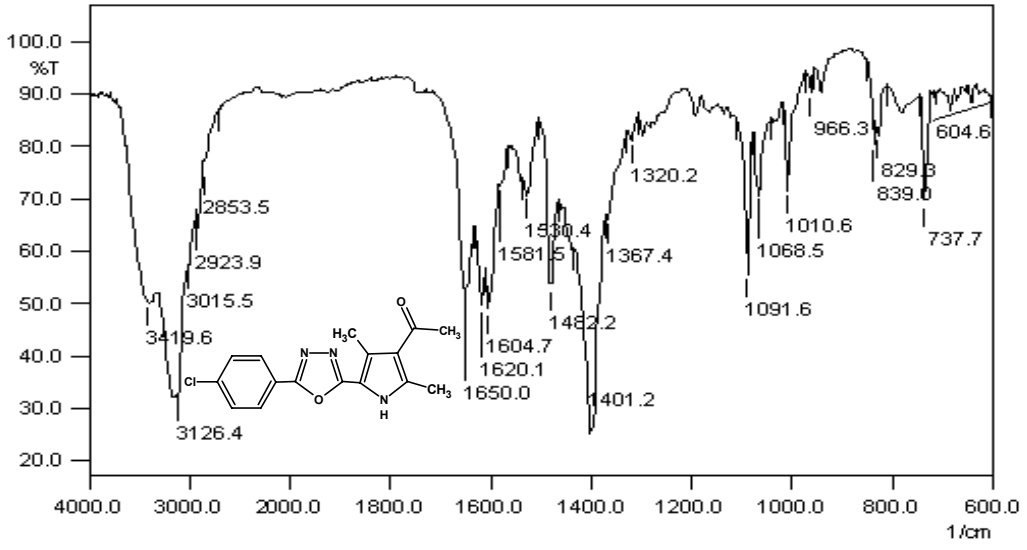


Table 4.2 : IR Spectral data of 1-{2,4-dimethyl-5-[(5-sub.phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones

Code	Substitution (R)	-NH Str.	Ar-H Str.	$-CH_3$ Str.	>CO Str.	>C=N Str. / Aromatic Region (>C=C< str)			$-CH_3$ i.p. deformation	>C-NH< Str. (2^o amine)	C-O-C Str.	Phenyl Substitution	
KUPO-1	H	3435	3130	3023 2925	1655	1615	1588	1555	1400	1367	1057	731 (mono sub)	
KUPO-2	2-Cl	3435	3129	3016 2924	1654	1604	1534	1475	1401	1297	1089	758 (1:2 di sub.)	
KUPO-5	2-OH	3404	3128	3008 2924	1654	1619	1530	1479	1401	1295	1064	752 (1:2 di sub.)	(O-
KUPO-9	3-NO_2	3419	3129	3014 2924	1652	1603	1527	1477	1401	1262	1095	900 (1:3 di sub)	
KUPO-10	2-CH_3	3419	3118	3008 2925	1650	1620	1528	1475	1401	1322	1073	733 (1:2 di sub)	
KUPO-11	4-CH_3	3450	3118	3015 2922	1654	1622	1583	1529	1401	1293	1061	822 (1:4 di sub)	
KUPO-12	2-$OHC_{10}H_6$	3402	3137	2924 2852	1660	1602	1587	1559	1400	1249	1091	755 (1:2 di sub.)	(O-
KUPO-13	2-Indolyl	3402	3130	2923 2854	1655	1603	1515	1480	1401	1261	1190	744 (mono sub.)	(N
KUPO-14	2-$OCOCH_3$	3419	3132	2924 2853	1652	1606	1542	1509	1400	1292	1093	752 (1:2 di sub.)	(C
KUPO-16	2-NHC_6H_5	3419	3129	2924 2852	1637	1594	1561	1553s	1400	1256	1159	754 (1:2 di sub.)	(N-

^{1}H NMR Spectral Study

^{1}H NMR Spectrum of the 1-{2,4-dimethyl-5-[(5-sub.phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones show signals relevant to the number of protons and their electronic environment known as chemical shift. Chemical shift may move to either upfield (Shielding) or down field(deshielding), according to the electronic environment of the corresponding proton. In addition to this, each ^{1}H NMR signal further splitted into number of subpeaks according to the number of neighbouring protons present in the skeleton of molecule. The splitting of the NMR signal further provides signification about the degree of interaction between neighbouring protons by means of spin-spin coupling constant J.

Instrument : BRUKER AC 300MHz FT-NMR

Internal Reference : TMS Solvent : $CDCl_3$ or DMSO d_6

1-{2,4-Dimethyl-5-[(5-(4-methyl)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-3)

^{1}H NMR Spectrum of KUPO-1 shows two singlets due to methyl protons occupied at 4-position of phenyl residue and C-4 and C-2 substitutions of pyrrole ring, relevant to six and three protons appears at δ 2.38 ppm and δ2.57 respectively. Which reveals that the methyl protons of the C-4 substitution of the phenyl residue and that of C-3 of the pyrrole ring experience almost same electronic environment while methyl protons of the C-2 substitution of the pyrrole ring were slightly more deshielded. A singlet relevant to C-3 substituted acetyl protons of the pyrrole ring appears at δ 2.51. While an amine proton of the pyrrole ring resonates at 12.17δ ppm. Phenyl residue substituted at 1,3,4-oxadiazole ring possess two pair of chemically equivalent but magnetically non-equivalent protons viz. $H_{(e)}$ & $H_{(d)}$ appear as two doublet at δ 7.405 with J value of 3Hz and δ7.93 with J value of 6Hz respectively.

1-{2,4-Dimethyl-5-[(5-(4-nitro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-4)

^{1}H NMR Spectrum of KUPO-4 shows two singlets appears due to C-4 and C-2 methyl protons of pyrrole ring at chemical shift value of δ2.39 and δ2.60 ppm respectively. A singlet due to C-3 substituted acetyl protons of pyrrole ring appears at δ 2.53. While amine proton of the pyrrole ring resonates at δ12.28 ppm. Phenyl residue substituted at 1,3,4-oxadiazole ring possess two pair of chemically equivalent but magnetically non-euivalent protons viz. $H_{(d)}$ & $H_{(e)}$ appear as two doublet at δ 8.275 with J value of 9Hz and δ 8.435 with J value of 9Hz respectively.

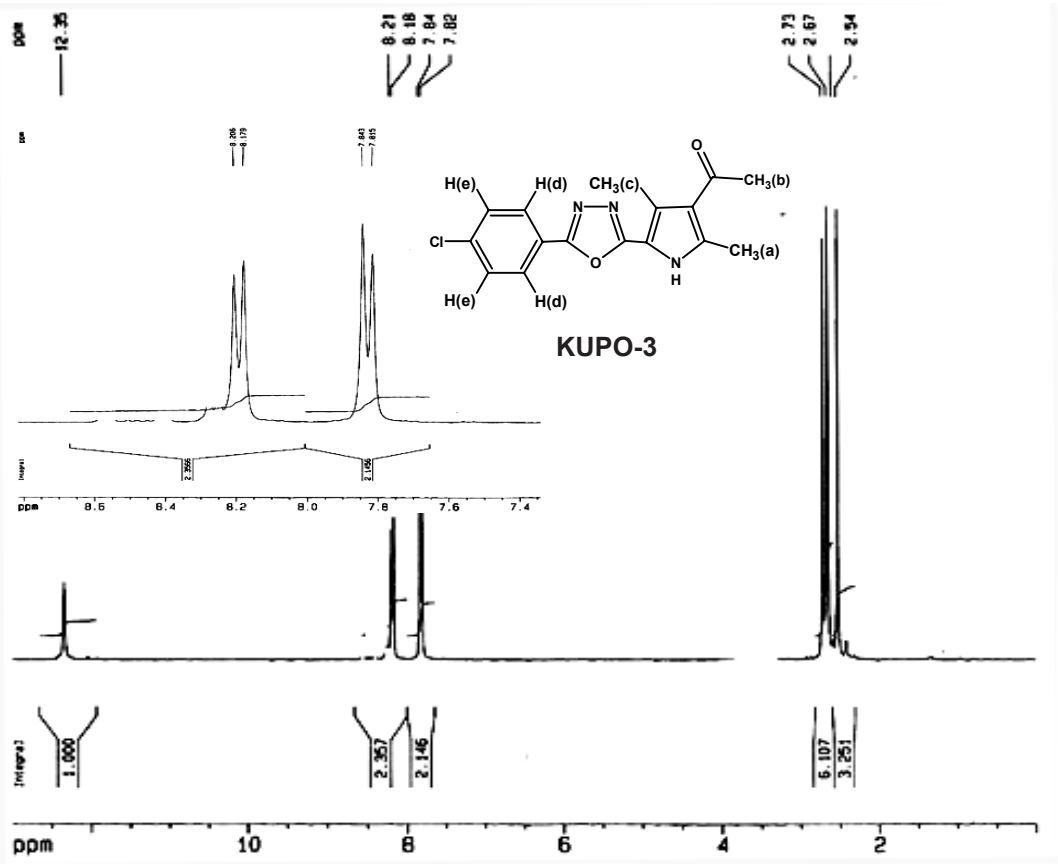
1-{2,4-Dimethyl-5-[(5-(4-methoxy)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-7)

In the ^{1}H NMR Spectrum of KUPO-7 shows two singlets due to C-4 and C-2 substituted methyl protons of pyrrole ring appears at δ 2.38 and δ2.57 respectively. A singlet due to acetyl protons substituted at C-3 position of pyrrole ring appears at chemical shift value of δ 2.51 ppm. An amine proton of the pyrrole ring resonates at δ12.14. Phenyl residue substituted at 1,3,4-oxadiazole ring possess two pair of chemically equivalent but magnetically non-euivalent protons viz. $H_{(e)}$ & $H_{(d)}$ appear as two doublet at δ7.14 with J value of 6Hz and δ 7.975 with J value of 9Hz respectively.

1-{2,4-Dimethyl-5-[(5-(4-chloro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-3)

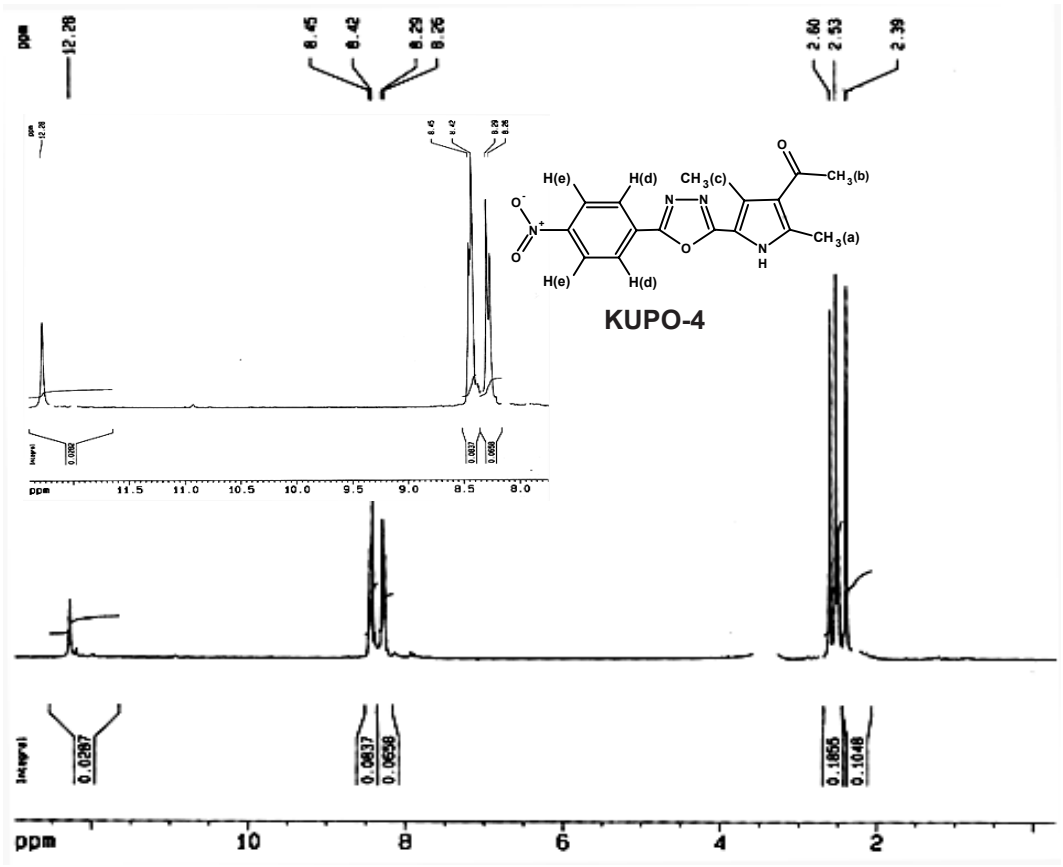
In the ^{1}H NMR Spectrum of KUPO-3 the C-2 and C-4 methyl protons of the pyrrole ring shows two singlets at δ2.73 and δ 2.54 respectively. The difference between chemical shift indicates the difference in their relevant electronic envirment. A singlet due to acetyl proton occupied at C-3 position of pyrrole ring, appears at δ 2.67. An amine proton of the pyrrole ring resonates at δ12.35 ppm. Phenyl residue substituted at 1,3,4-oxadiazole ring possess two pair of chemically equivalent but magnetically non-equivalent protons viz. $H_{(e)}$ & $H_{(d)}$ appear as two doublet at δ 7.83 with J value of 6Hz and δ 8.195 with J value of 9Hz respectively.

^{1}H NMR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-chloro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-3)



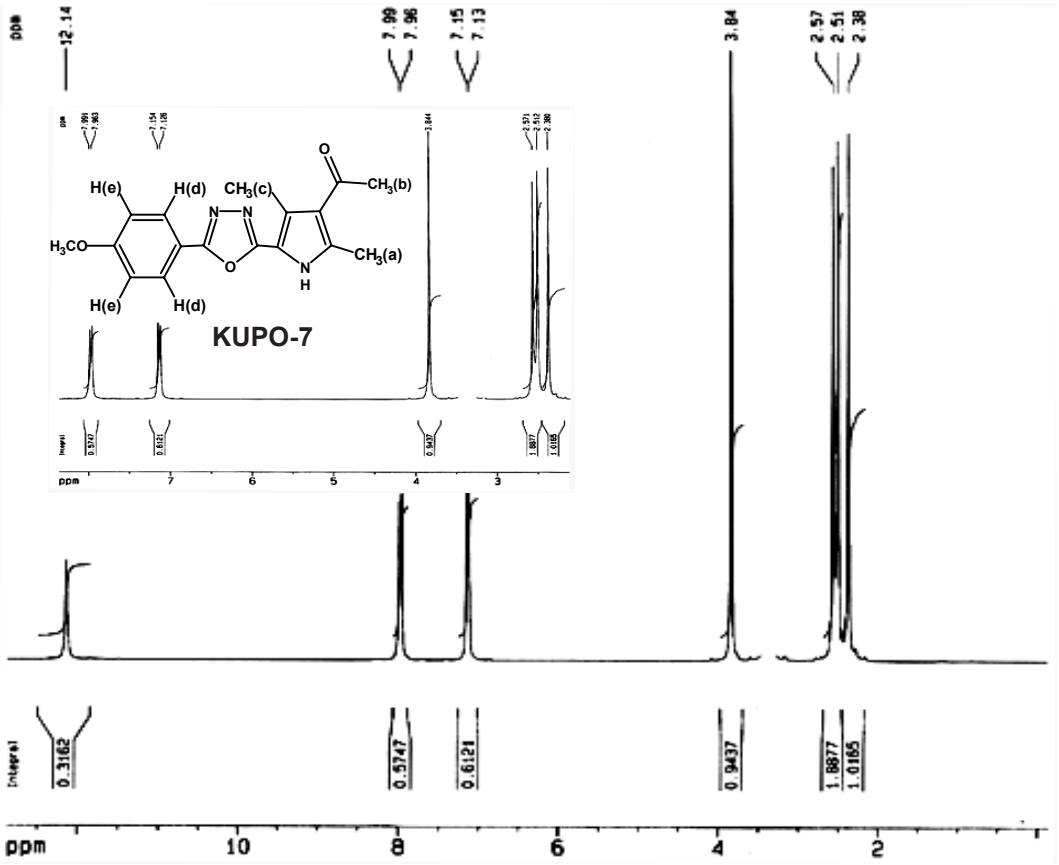
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{3(c)}$	2.54	3	s	-
-$COCH_{3(b)}$	2.67	3	s	-
-$CH_{3(a)}$	2.73	3	s	-
Ar-$H_{(e)}$	7.83	2	d	6
Ar-$H_{(d)}$	8.195	2	d	9
-NH	12.35	1	s	-

^{1}H NMR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-nitro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-4)



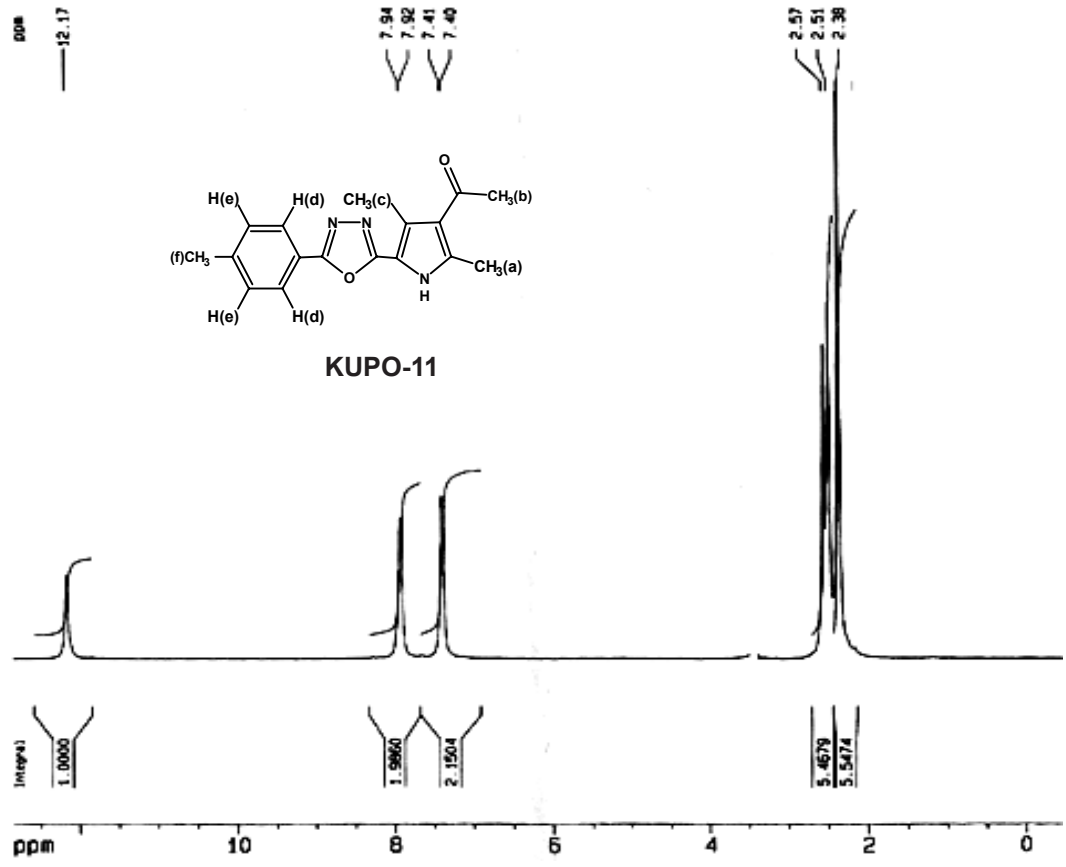
Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{3(c)}$	2.39	3	s	-
-$COCH_{3(b)}$	2.53	3	s	-
-$CH_{3(a)}$	2.60	3	s	-
Ar-$H_{(d)}$	8.275	2	d	9
Ar-$H_{(e)}$	8.435	2	d	9
-NH	12.28	1	s	-

^{1}H NMR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-methoxy)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-7)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
- $CH_{3(c)}$	2.38	3	s	-
-$COCH_{3(b)}$	2.51	3	s	-
-$CH_{3(a)}$	2.57	3	s	-
-OCH_3	3.84	3	s	-
Ar-$H_{(e)}$	7.14	2	d	6
Ar-$H_{(d)}$	7.975	2	d	9
-NH	12.14	1	s	-

^{1}H NMR Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-methyl)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones (KUPO-11)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value
$-CH_{3(c,f)}$	2.38	6	s	-
$-COCH_{3(b)}$	2.51	3	s	-
$-CH_{3(a)}$	2.57	3	s	-
$Ar-H_{(e)}$	7.405	2	d	3
$Ar-H_{(d)}$	7.93	2	d	6
-NH	12.17	1	s	-

Mass Spectral Study

The FAB mass analysis of the 1-{2,4-Dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanones molecular ion peak(M+) of the compounds are in total agreement with it's molecular weight.

Instrument: JEOL SX 102/ DA-6000 Spectrograph for FAB

FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-1)

The FAB Mass spectrum of the KUPO-1 shows the Molecular Ion peak at m/z value of 281, the base peak of the fragmentation is M+1 peak at m/z282, while other ions generates are m/z 266, m/z 240, m/z 223, m/z 210, m/z 154, m/z 136 etc.

FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(2-chloro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-2)

The FAB Mass spectrum of the KUPO-2 shows the Molecular Ion peak at m/z value of 315, the base peak of the fragmentation is M+1 peak at m/z316, while other ions generates are m/z 300, m/z 274, m/z 164, m/z 154, m/z 135,m/z 120 etc. In addition to this a characterestic ionization patern of (m+1), (m+2), (m-1) peaks of chloro substitution is also observed.

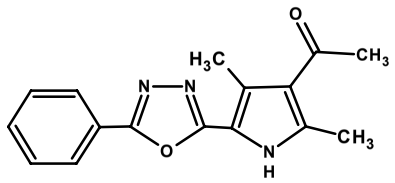
FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-nitro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-4)

The FAB Mass spectrum of the KUPO-4 shows the Molecular Ion peak at m/z value of 326, the M+1 peak obsrved at m/z327, while other ions generates are m/z 307, m/z 240, m/z 289, m/z 273, m/z258, m/z 242, m/z 136 etc.

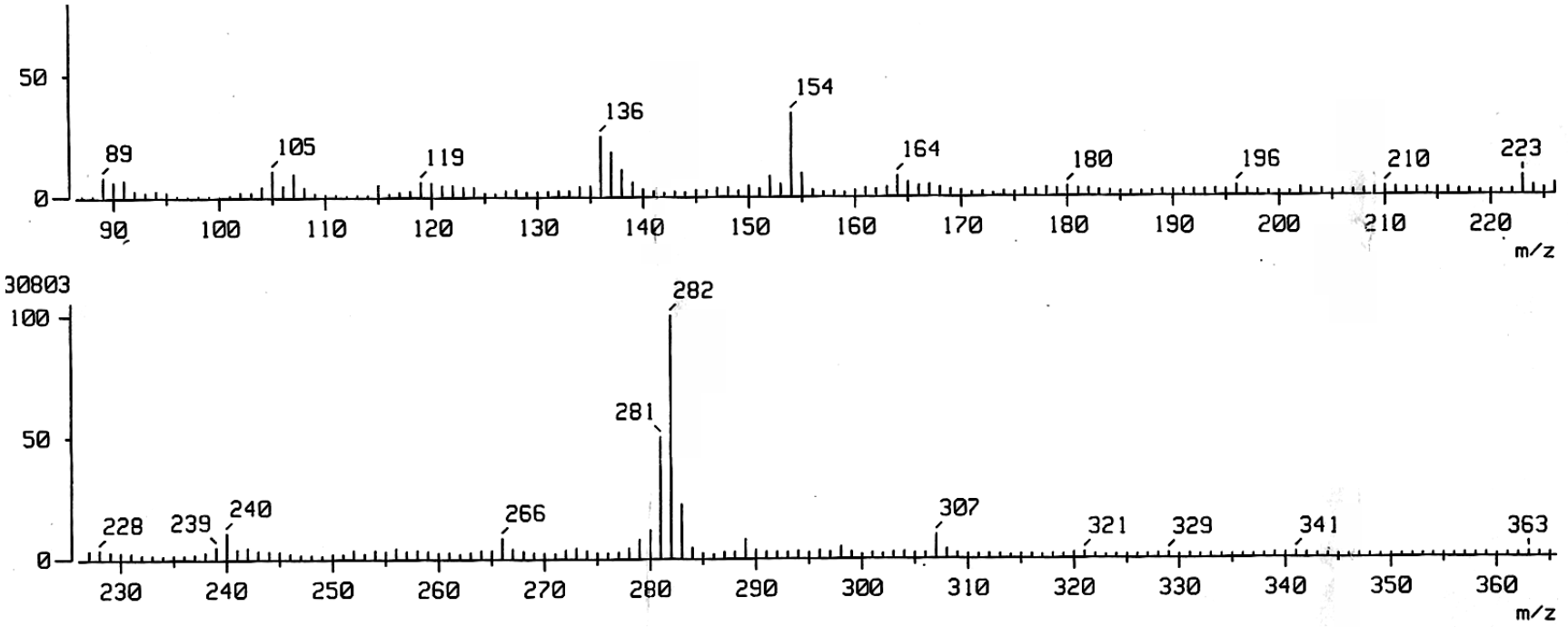
FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-methoxy)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-7)

The FAB Mass spectrum of the KUPO-4 shows the Molecular Ion peak at m/z value of 312, M+1 peak obsrved at m/z 313, while other ions generates are m/z 289, m/z 282, m/z 266, m/z 211, m/z164, m/z 149, m/z 135etc.

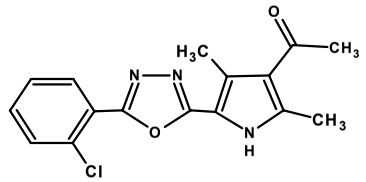
FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-1)



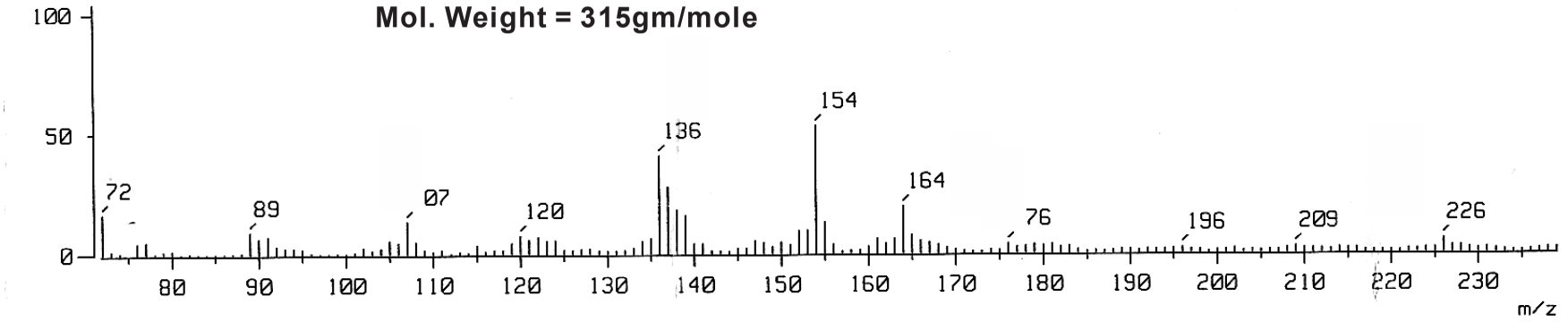
Mol. Weight = 281gm/mole

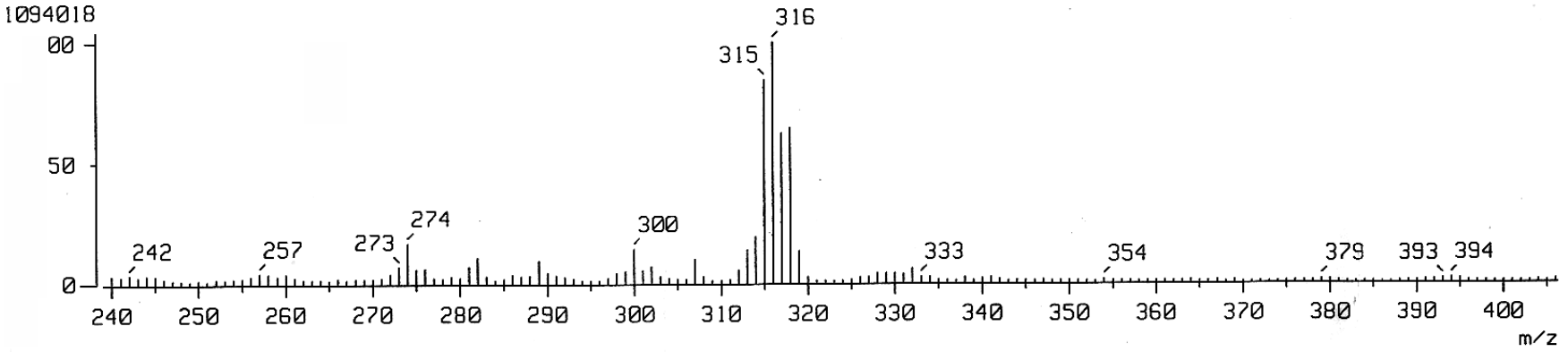


FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(2-chloro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-2)

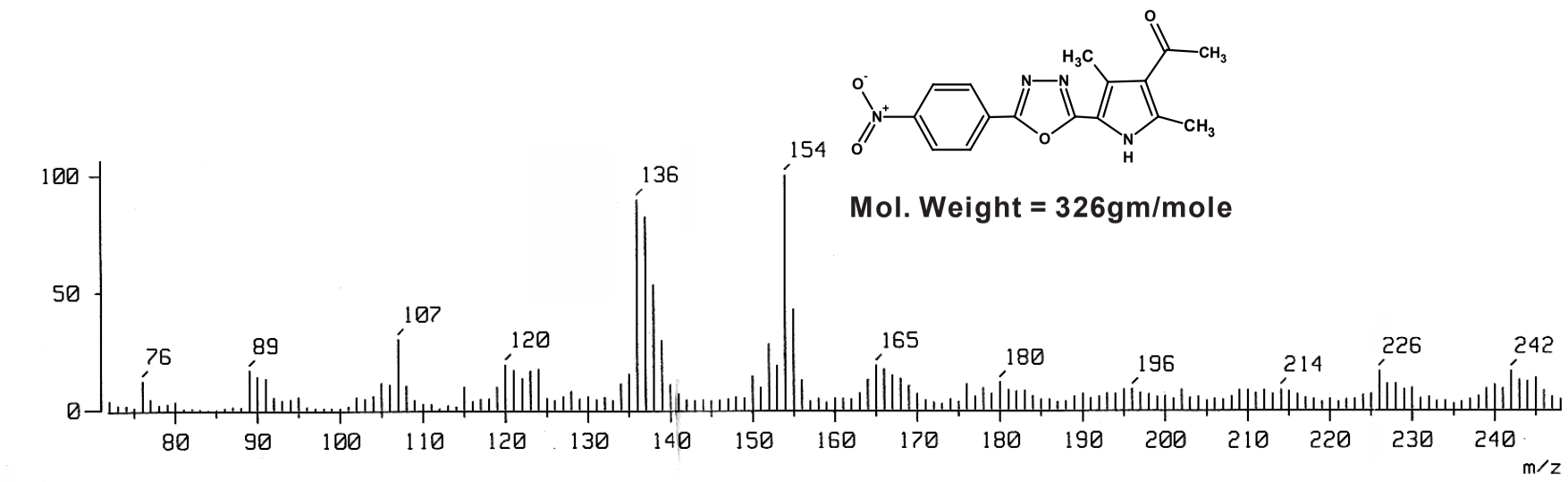


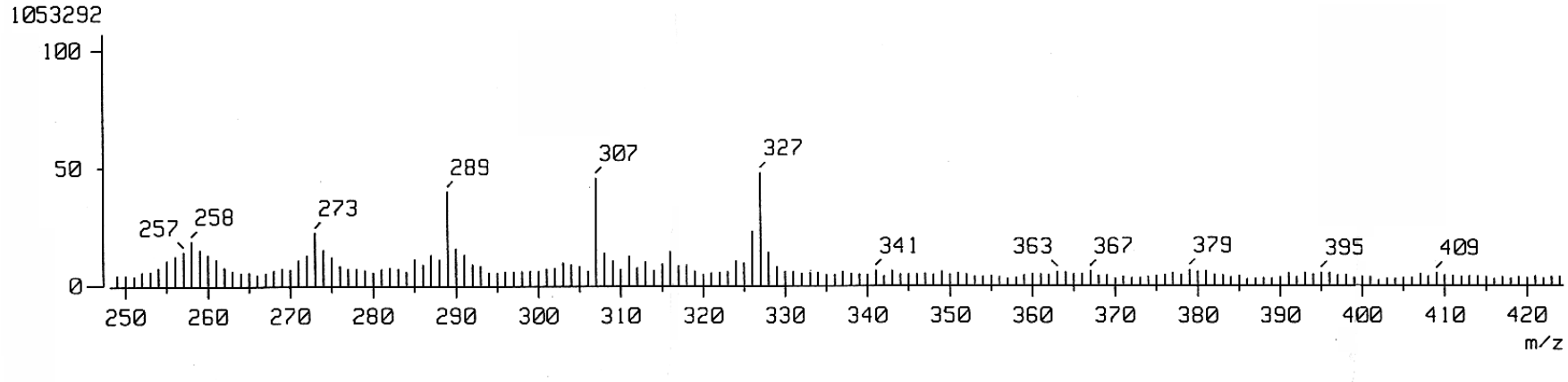
Mol. Weight = 315gm/mole



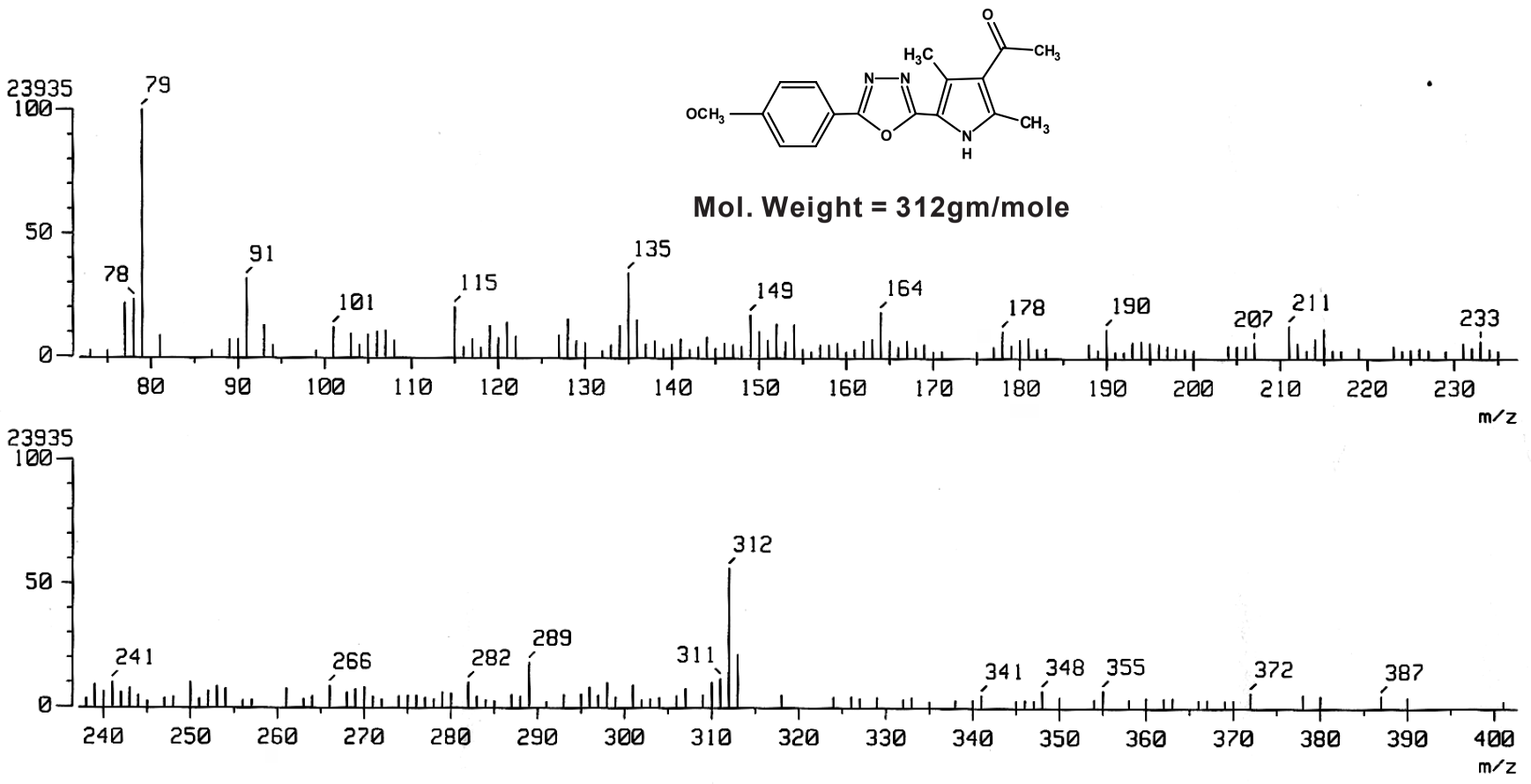


FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-nitro)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-4)



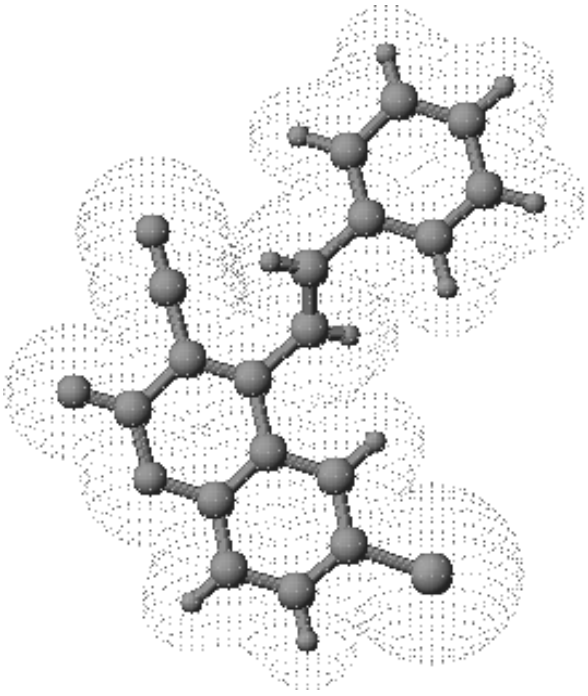


FAB MASS Spectrum of 1-{2,4-Dimethyl-5-[(5-(4-methoxy)phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1*H*-pyrrol-3-yl}ethanon (KUPO-7)



Mol. Weight = 312gm/mole

CHAPTER-5



Synthesis and Characterization of Some 6-Chloro-2oxo-4-[(E)-2-phenylethyl]-2H-chromene-3-carbonitriles

Introduction

Coumarins comprise a group of natural compounds found in a variety of plant sources. The very long association of plant coumarins with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems. The coumarins that were studied have diversed biological properties and various effects on the different cellular systems. A lot of biological parameters should be evaluated to increase our understanding of mechanisms by which these coumarins act. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection. The coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. The hydroxycoumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The coumarins display a remarkable array of biochemical and pharmacological actions, some of which suggest that certain members of this group of compounds may significantly affect the function of various mammalian cellular systems. The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which can influence their biological activity. Vast majority of coumarins, completely innocuous, may be beneficial in a variety of human disorders, in spite of some ongoing controversy.

There has been, in recent years, a major rekindling of interest in pharmacognosy. Coumarins turn out to be present in many natural therapeutically utilized products. They hold a place apart in view of their cytotoxic activity. It was suggested that alterations in the chemical structure of coumarins could change their cytotoxic properties[1].

Coumarin and its hydroxy derivatives have been prominently accepted as natural pharmaceuticals[2] world wide, has revealed new biological activities with interesting therapeutic applications, besides their traditional employment as anticoagulants(anti-vitamin K activity)[3], antibiotics(novobiocin and analogues[4]) and anti AID[5]. Apart from this, they also possess anti-cancerous[1], antibacterial[6], neurotropic[7], immunosupressive[8], anti inflammatory[9], antiulcerous[10], anti PAF(anti platelet activating factor)[11] and antimutagenic[12] effects.

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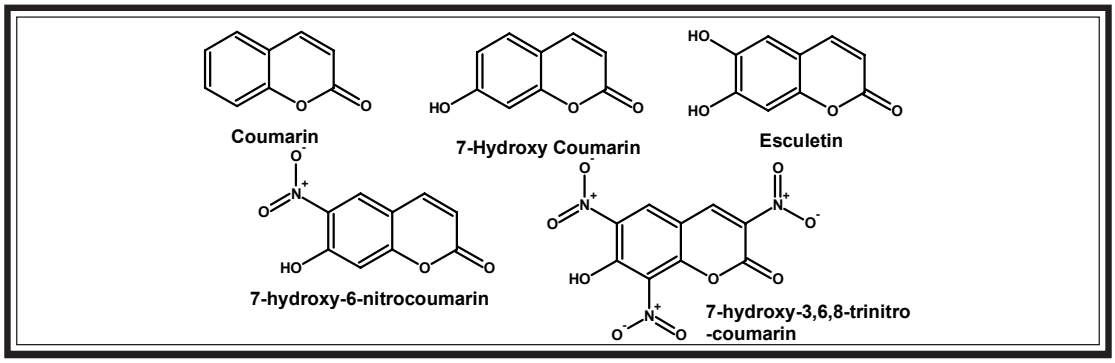
Pharmacology

❖Anti Cancer activity Profile of Benzopyran Derivatives

Analysis of scientific literature revealed numerous reports on the antiproliferative and antitumor activities of a variety of coumarin compounds, e.g., both coumarin itself and 7-hydroxycoumarin have been reported to inhibit the proliferation of a number of human malignant cell lines *in vitro* [13-16] and have demonstrated activity against several types of animal tumors [17-21]. These compounds have also been reported in clinical trials to demonstrate activity against prostate cancer, malignant melanoma, and metastatic renal cell carcinoma [22-24].



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For coumarins, generally the *in vitro* structure-activity relationship studies have shown that cytotoxicity is found with derivatives containing ortho-dihydroxy substituents[25]. Also, the chemical-structure/ biological activity study of the coumarins showed that the addition of a cathecolic group to the basic structure induces increased cytotoxic activity in tumor cell lines [25]. The different cytotoxic values found for the coumarins could be related to presence and the positions of the hydroxyls in their structures.

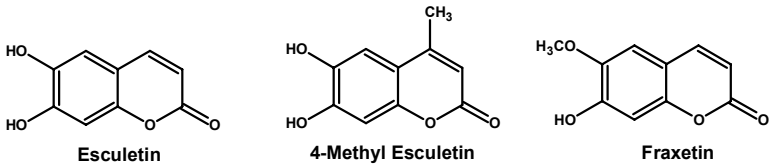
The cytotoxicity of 22 natural and semi-synthetic simple coumarins was evaluated in GLC4, a human small cell lung carcinoma cell line, and in COLO 320, a human colorectal cancer cell line[25]. From the structure-cytotoxicity relationship, it is conspicuous that all the potentially active natural compounds possess at least two phenolic groups in either the 6, 7- or 6, 8-positions. In addition, the 5-formyl-6-hydroxy substituted semi-synthetic analogue was found to be potent, reflecting the importance of at least two polar functions for high cytotoxicity.

Several hydroxylated and/or methoxylated coumarin derivatives were tested for their relative cytotoxicity on four human tumor cell lines (oral squamous cell carcinoma HSC-2, HSC-3, melanoma A-375 and promyelocytic HL-60) and three normal human cells (gingival fibroblast HGF, periodontal ligament fibroblast HPLF and pulp cell HPC) [26]. Tumor cell-specific cytotoxicity was detected in all 6, 7-dihydroxy-substituted coumarins only. The observations indicated that the tumor-specific cytotoxicity of the naturally occurring coumarin esculetin (6, 7-dihydroxycoumarin) could be further enhanced by proper substitutions at 3- and/or 4-position(s) of the molecule. Agarose gel electrophoresis revealed that esculetin and its derivatives with tumor-specific cytotoxicity induce internucleosomal DNA fragmentation in HL-60 cells.

A selected group of natural and synthetic coumarin compounds, including the hydroxylated and nitrated derivatives, were assessed for their cytotoxic potential for cellular viability[27]. This study utilized both human skin malignant

melanocytes (SK-MEL-31) and normal human skin fibroblastic cells (HS613.SK), allowing identification of those coumarin derivatives that are selectively toxic. Novel synthetic nitrated coumarins, 6-nitro-7-hydroxycoumarin and 3, 6, 8-nitro-7-hydroxycoumarin, were shown to be significantly more toxic to SK-MEL-31 than HS613.SK cells. In the malignant melanocyte skin cell line (SK-MEL-31), the cytotoxic effects of these nitro-derivatives were shown to be dose and time dependent. Therefore, the cytotoxic potential of coumarins appears to be highly dependent on the nature and position of the functional group. In addition, nitration of 7-hydroxycoumarin produced compounds that were cytotoxic to malignant melanocytes, suggesting that these nitro-derivatives may have a chemotherapeutic role in future.

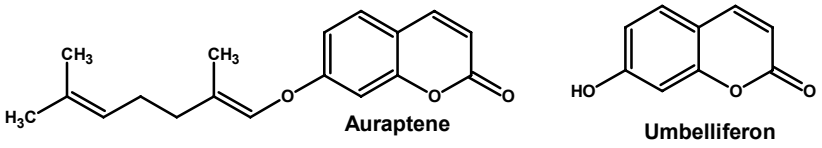
Protective effects of coumarins against cytotoxicity induced by linoleic acid hydroperoxide were examined in cultured human umbilical vein endothelial cells[28]. When the cells were incubated in medium supplemented with linoleic acid hydroperoxide and coumarins, esculetin (6, 7-dihydroxycoumarin) and 4-methylesculetin protected cells from injury by linoleic acid hydroperoxide.



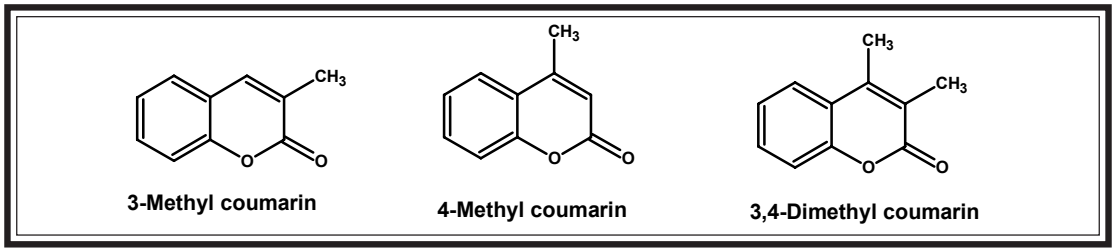
Esculetin and 4-methylesculetin provided synergistic protection against cytotoxicity induced by linoleic acid hydroperoxide with alpha-tocopherol. Furthermore, the radical-scavenging ability of coumarins was examined in electron spin resonance spectrometry. Esucletin, 4-methylesculetin, fraxetin, and caffeic acid showed the quenching effect on the 1, 1-diphenyl-2-picrylhydrazyl radical. These results indicate that the presence of an ortho catechol moiety in the coumarin molecules plays an important role in the protective activities against linoleic acid hydroperoixde-induced cytotoxicity[28].

An antioxidant auraptene (7-geranyloxycoumarin) isolated from the peel of citrus fruit (*Citrus natsudaidai Hayata*) has been reported to have chemopreventive effects on chemically induced carcinogenesis. Dietary administration of auraptene significantly increased the activities of detoxification (phase II) enzymes, such as quinone reductase and glutathione *S*-transferase, in the liver and colon of rats. In addition, expression of cell proliferation biomarkers, such as ornithine decarboxylase activity and polyamine biosynthesis, in the colonic mucosal epithelium was significantly inhibited by dietary feeding of auraptene. These biological functions of auraptene may contribute to its anti-tumorigenic effect[29].

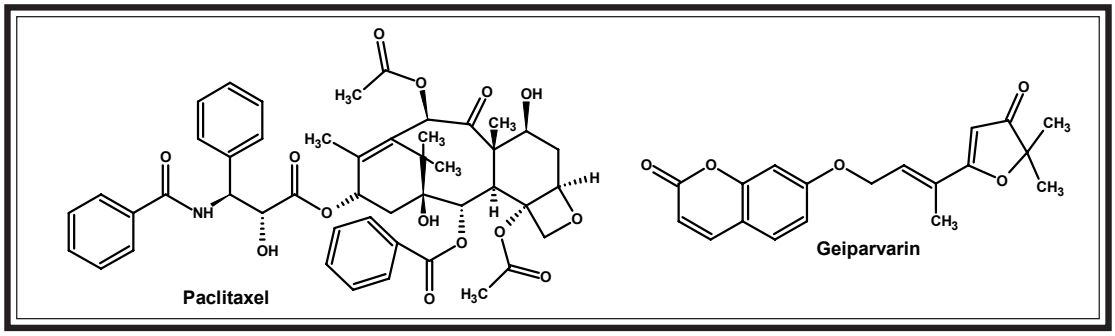
In addition to this, auraptene have been demonstrated its anti-tumor promoting effect in mouse skin and anti-carcinogenesis activities in rat tongue, esophagus and colon[30]. Murakami A. *et al*.[30] reported that Auraptene suppresses superoxide anion (O_2^-)generation from inflammatory leukocytes in *in vitro* experiments. In the study, they investigated the anti-inflammatory activities of Auraptene and compared them with those of Umbelliferone (7-hydroxycoumarin), a structural analog of Auraptene that is virtually inactive toward O_2^- generation inhibition. Double pre-treatments of mouse skin with Auraptene, but not Umbelliferone, markedly suppressed edema formation, hydrogen peroxide production, leukocyte infiltration, and the rate of proliferating cell nuclear antigen-stained cells. These inhibitory effects by Auraptene are attributable to its selective blockade of the activation stage. Umbelliferone did not show any inhibitory effect. This contrasting activity profile between Auraptene and Umbelliferone was rationalized to be a result of their distinct differences in cellular uptake efficiencies, i.e. the geranyloxyl group in Auraptene was found to play an essential role in incorporation.



The rat hepatic toxicity of coumarin and methyl analogues (3-methylcoumarin, 4-methylcoumarin and 3, 4-dimethylcoumarin) has been determined *in vivo* and *in vitro*[31]. Coumarin at a dose of approximately 1 mmol/kg produced clear histological evidence of centrilobular necrosis, while the methyl analogues at an equivalent dose were much less toxic. By use of a systematic random sampling protocol and quantitative morphometry it was determined that there was a lobar variation in the extent of hepatic damage but that this exhibited random inter-animal variation. The order of cytotoxicity *in vitro* was identical to that observed *in vivo*.

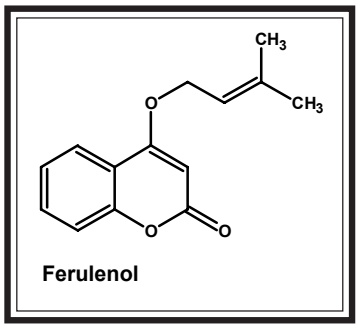


Geiparvarin, containing coumarin moiety, is an antiproliferative compound isolated from the leaves of *Geijera parviflora*, and may represent a new drug which targets tubulin. To better explore the potential use of this agent, A. Miglieta, et al[32] investigated the antimicrotubular and cytotoxic effects of new synthetic aromatic derivatives of geiparvarin. These drugs inhibited polymerization of microtubular protein, particularly when the assembly was induced by paclitaxel.



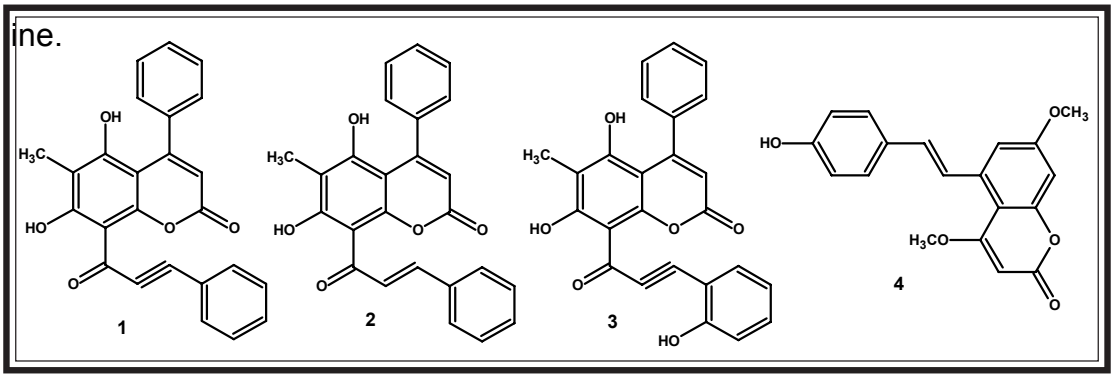
Bocca C. *et al*. [33] investigated biological activity of ferulenol, a prenylated 4-hydroxycoumarin from *Ferula communis*. Ferulenol stimulates tubulin polymerization *in vitro*, and inhibits the binding of radiolabeled colchicine to tubulin. It rearranges cellular microtubule network into short fibres, and alters nuclear morphology. Remarkably, ferulenol exerts a dose dependent

cytotoxic activity against various human tumor cell lines.



Ferulenol

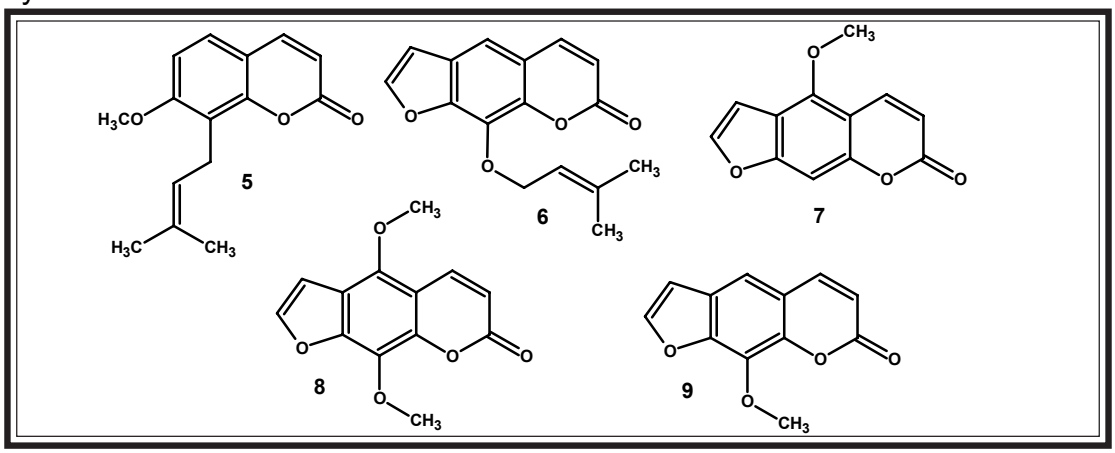
Three new coumarin derivatives along with furanocoumarins and a novel dioxocane derivative were isolated from the fern *Cyclosorus interruptus* (Willd.)H.Ito[34]. Based on spectrometric and spectroscopic analysis (FAB or EI mass spectrometry as well as 1D and 2D NMR experiments) their structures were characterised as 5,7- dihydroxy - 6 - methyl - 4 - phenyl - 8 - (3 - phenylpropionyl) -benzopyran-2-one (1), 5, 7-dihydroxy-6-methyl-4-phenyl-8-(3-phenyl-trans-acryloyl)-1-benzopyran-2-one (2), 5,7-dihydroxy - 8 - (2 - hydroxy - 3 - phenylpropionyl) - 6 - methyl - 4phenyl-1-benzopyran-2-one (3). Among which compounds 5,7- dihydroxy - 6 - methyl - 4 - phenyl - 8 - (3 - phenylpropionyl) - 1benzopyran-2-one and 5, 7-dihydroxy-6-methyl-4-phenyl-8-(3-phenyl-trans-acryloyl)-1-benzopyran-2-one, were cytotoxic to a KB cell line.



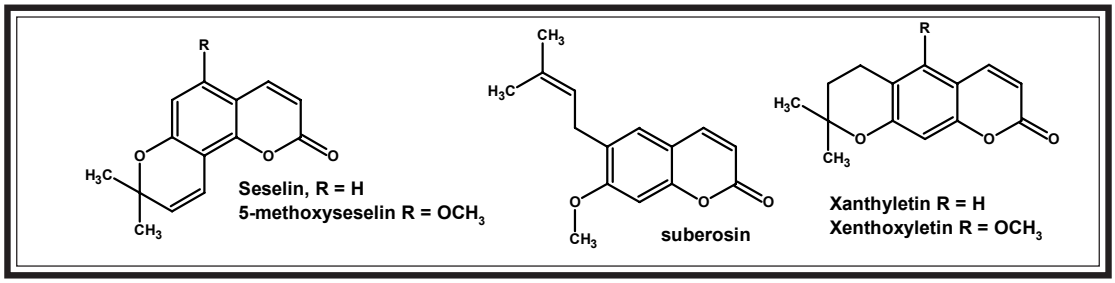
A new coumarin, 5-(4-hydroxyphenethenyl)-4, 7-dimethoxycoumarin (4) was isolated from the combined ethylacetate extracts of the root bark, root wood and stem bark of *Monotes engleri*, and found to be cytotoxic against two cell lines in a human tumor panel [35]. Its structure was determined on the basis of spectroscopic methods.

In a Chinese herb cytotoxicity screening test, the ethanol extract of *Cnidii monnieri Fructus* exhibited strong effects on human leukemia (HL-60),

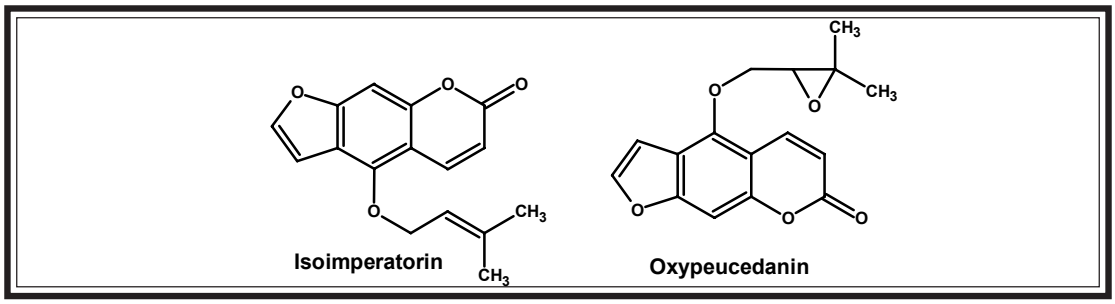
cervical carcinoma (HeLa) and colorectal carcinoma (CoLo 205) cells. Then, the *Cnidii monnieri Fructus* extract was subjected to silica gel column chromatography and recrystallization to give five coumarins:osthol (5), imperatorin (6), bergapten (7), isopimpinellin (8), and xanthotoxin (9). Among these compounds, osthol showed the strongest cytotoxic activity on tumor cell lines. The structure-activity relationship established from the results indicated that the prenyl group has an important role in the cytotoxic effects. However, imperatorin showed the highest sensitivity to HL-60 cells and the least cytotoxicity to normal PBMCs. Osthol and imperatorin both caused apoptotic bodies, DNA fragmentation, and enhanced PARP degradation in HL-60 cells by biochemical analysis. These results indicate that osthol and imperatorin can induce apoptosis in HL-60 cells. Therefore, osthol and imperatorin are cytotoxic marker substances in the fruits of *Cnidium monnieri*[36].



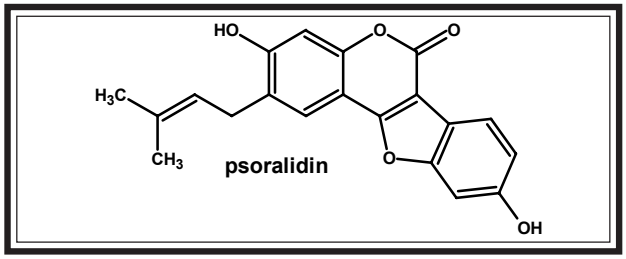
Five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) were isolated from the roots of *Plumbago zeylanica*[37]. All coumarins were not previously found in this plant. Cytotoxicity of these compounds to various tumor cells lines was evaluated, and they were significantly suppressed growth of Raji, Calu-1, HeLa, and Wish tumor cell lines.



Fractionation of the methanol extract of *Angelica dahurica Benth et Hook* resulted in the isolation of six furocoumarins, imperatorin, isoimperatorin, (+/-)-byakangelicol, (+)-oxypeucedanin, (+)-byakangelicin and (+)-aviprin [38]. Among these, compounds imperatorin and (+)-byakangelicin exhibited strong hepatoprotective activities, displaying EC_{50} values of 36.6 +/- 0.98 and 47.9 +/- 4.6 mM, respectively.



A coumestan derivative, psoralidin was found to be a cytotoxic principle of the seeds of *Psoralea corylifolia L.*(Leguminosae) with the IC_{50} values of 0.3 and 0.4 mg/mL against the HT-29 (colon) and MCF-7 (breast) human cancer cell lines, respectively[39].



25.H.Kolodziej, O.Kayser, H.Woerdenbag, W.van Uden, N.Pras, *Z. Naturforsch*,**52**, 240(1997).
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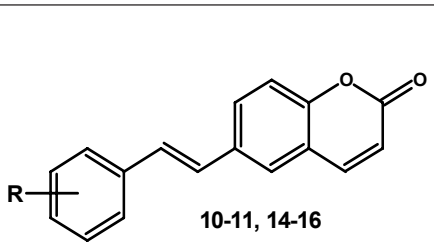
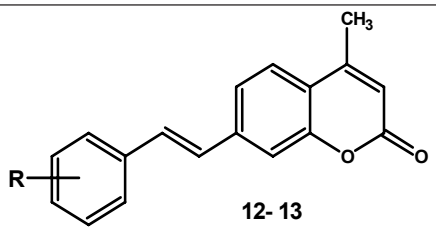
39.W.Mar, K.Je, E.Seo, *Arch. Pharm. Res. (Korea),***24**,211(2001).

A series of styrylcoumarin derivatives had been designed by Xu Song et al[40] in order to find compounds of antitumor activities by screening *in vitro*. The title compounds were synthesized by phase-transfer Wittig reaction and screened by several antitumor models *in vitro*.Thirty new compounds of 6- or 7-styrylcoumarin were synthesized and their configurations were determined. Seven compounds(10-16) showed different inhibitory effects on L-1210, HL-60, HCT-8, KB and Bel-7402 cell lines *in vitro*. The activity data representes as shown in Table-1. Some 6- or 7-styrylcoumarin derivatives showed antitumor activities and is worth further study.



10-11, 14-16



12- 13

Compd	R	Geoisomer
10	3-COOEt	Trans
11	H	Trans
12	3-COOEt	Trans
13	3-COOEt	Cis
14	3,4-OCH_2O-	Trans
15	2-Cl	Trans
16	3-NO_2	Trans

Table-1: *In vitro* antitumor activities of the analogues (IC_{50}) (ì g/ml)

Compd. No.	Bel-7402	L-1210	HL-60	KB	HCT-8
10	10	-	-	>10	>10
11	>10	-	-	8.1	>10
12	>10	-	-	8.8	>10
13	4.1	-	-	0.6	2.1
14	>10	>10	5.8	-	-
15	>10	>10	6.8	-	-
16	>10	>10	8.7	-	-

40. X.Song, X.Shiping, L.Lanmin, *Acta Pharmaceutica Sinica*, **35**(2), 103(2000)
41. S. Xu, S.Su, L.Li, *Yaoxue Xuebao*, **36**(4), 273(2001)
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43.D.Parks, D.Granger, *Acta Physiol. Scand.,* **548**, 87(1986).
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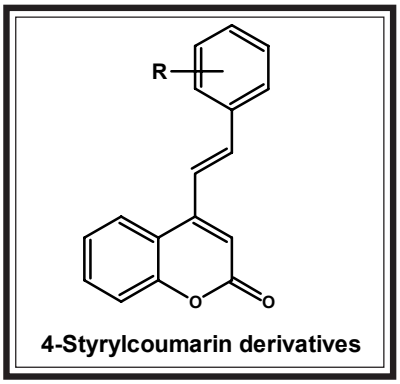
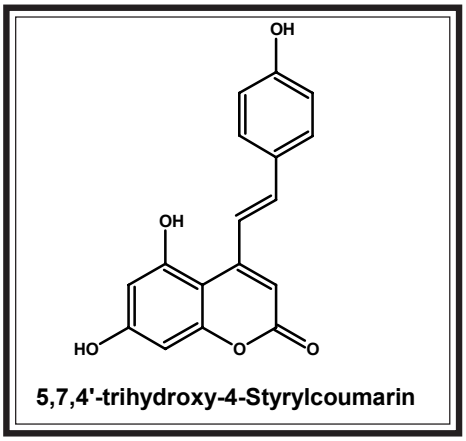
In continuation with this, a series of 4-styryl coumarin had been synthesized for *in vitro* antitumor activity study[41]. The titled compounds were synthesized by Phase transfer Wittig reaction or Wittig-Horner reaction and screened by several antitumor modeles *in vitro*. Among a series of 20 compounds, only one had effects on KB cell lines *in vitro* and possess certain antitumor activities and it was selected for further studies.



4-Styrylcoumarin derivatives

❖Antiviral activity

The ether soluble fraction of the roots of *Ononis vaginalis Vahl. Symb.* afforded three new compounds: 3-hydroxy-4, 9-dimethoxycoumestan, maginaldehyde [2-(4-hydroxy-2-methoxyphenyl)-5, 6-dimethoxy-3-benzofurancarboxaldehyde] and 5, 7, 4'-trihydroxy-4-styrylcoumarin[42]. The styrylcoumarin derivative showed significant antiviral activity against *Herpes simplex* type 1 and weak cytotoxicity.



5,7,4'-trihydroxy-4-Styrylcoumarin

45.F.Morpeth, *Biochim. Biophys. Acta*, **744**, 328(1983).
46.M.Xia, R.Dempski, R.Hille, *J. Biol. Chem.*, **274**,3323(1999).
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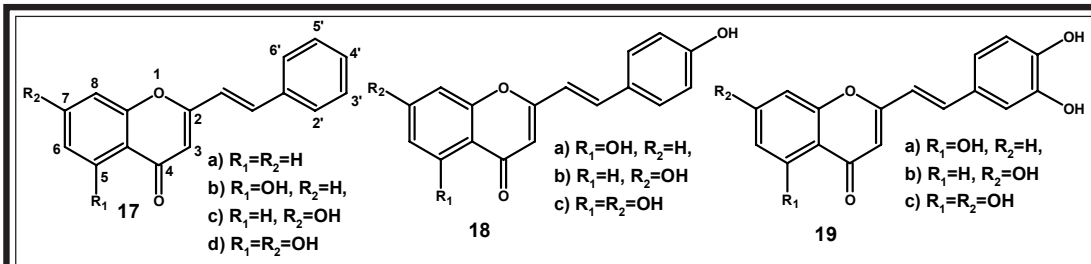
❖Benzopyran analogues as Xanthine Oxidase(XO) Inhibitors

XO is a highly versatile enzyme, which is widely distributed among species and within the various tissues of mammals[43]. XO exhibits a broad activity toward reducing substrates. It can hydroxylate a wide variety of purines (notably xanthine and hypoxanthine), pteridines, related aromatic heterocycles, and also a range of aliphatic and aromatic aldehydes, taking these to the corresponding carboxylic acids, with concomitant reduction of molecular oxygen[44 – 46]. In XO-catalysed reactions, oxygen is reduced by one or two electrons giving rise to superoxide radical (O_2^-) or hydrogen peroxide(H_2O_2)[47,48]. Consequently, xanthine oxidase is considered to be an important biological source of reactive oxygen species (ROS), which induce oxidative stress and are involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging[49]. It has also been shown that xanthine oxidase decomposes low molecular weight S-nitrosothiols (e.g. S-nitrosoglutathione and Snitrosocysteine) by superoxide-dependent and -independent mechanisms, and, according to the availability of oxygen in the system, secondarily leads to peroxynitrite formation[50]. This may alter the transport and storage of nitric oxide (NO) by Snitrosothiols and the activity of proteins that are regulated by S-nitrosylation. Thus, the superoxide radical destroys the endothelium-derived vascular relaxing factor (nitric oxide) causing vascular constriction[51]. It is of note that XO tissue levels are increased after ischaemia reperfusion[52] with serum levels increased in hepatitis[53] and brain tumours[54]. It is also known that an extensive metabolism of xanthine by XO will increase body uric acid levels. Due to the low solubility of uric acid, there is a tendency for urate crystals to be deposited in the urinary tract and in the synovial fluid of joints, a process associated with painful inflammation, designated gout[55]. Therefore, XO inhibitors are expected to be therapeutically useful for the treatment of the aforementioned pathological states.

2-Styrylchromones are a small group of natural heterocyclic compounds. Only two natural 2-styrylchromones are known and they were extracted from

the blue-green algae Chrysophaem taylori in the 1980s[56,57]. Natural derivatives have demonstrated cytotoxic activity against leukaemia cells, while those obtained by synthesis have exhibited anti-allergic, antitumour and anticancer properties[56,57,58,59].

2-Styrylchromones have a common structural feature with flavones in containing the benzopyrone moiety. Taking into account that flavones are known to be xanthine oxidase (XO) inhibitors,[60-63] the evaluation of this activity for 2- styrylchromones was thought to be of prime importanceby E. Fernandes and co-workers[64] and have studied the activity profile of some synthetic 2-styrylchromone derivatives of type shown in figure below as Xanthine Oxidase inhibitors.



Structure of the tested 2-Styrylchromones (17-19)

All the tested 2-styrylchromone derivatives 17–19 were found to be inhibitors of the XO-mediated oxidation of xanthine to uric acid in a concentration-dependent manner. Allopurinol, a known xanthine oxidase inhibitor clinically used in the treatment of gout[65] was also very effective in the present assay, giving an IC_{50} of 5.43 ± 0.8 μM. Four of the tested 2-styrylchromones were found to be more potent than this drug and the rank order of xanthine oxidase inhibition was 19c>19b>17d>19a>allopurinol>18c>17c>18b>17b>18a>17a. Kinetic studies were performed in order to determine the type of inhibition of these compounds indicated that the inhibition by the most potent compound 19c, as well as two other representative compounds 17d and 18c, was of a non-competitive inhibition type.

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54.E.Kokoglu, A.Belce, E.Ozyurt, Z.Tepeler, *Cancer Lett.*, **50**, 179 (1990).
55.E.Pascual, *Curr. Opin. Rheumatol.*, **12**, 213(2000).
56.W.Gerwick, A.Lopez, V.Duyne, J.Clardy, W.Ortiz, A.Baez, *Tetrahedron Lett.,* **27**, 1979(1986).

The structure activity relationship derived from the screening resultssuggested that the catechol group linked to the styryl moiety of the molecule (compounds 19a–c) strongly contributes to the inhibition of xanthine oxidase. In fact, the absence of hydroxyl groups in the aromatic ring linked to the styryl moiety (compounds 17a–d), or the presence of only one phenolic group (compounds 18a–c) substantially decreased the inhibition.

The low IC_{50} found for 3c was probably the result of a potentiation of effects by the catechol group linked to the styryl moiety of the 5,7-dihydroxylated benzopyrone. The hydroxylation pattern in the benzopyrone moeity was important for the potency of XO inhibition. This finding is in agreement with previous studies with flavones[62] and coumarins.[61] The presence of hydroxyl groups at the C-5 and C-7 positions of the benzopyrone lead to an observed increase in activity, when compared with the presence of only one substitution.It was found that the 7-hydroxylated derivatives were more potent than the respective 5-hydroxylated derivatives. Again, the importance of the C-7 hydroxylation has also been observed for the XO inhibitory activity of flavone derivatives[62] and coumarin derivatives.[67] Some other interesting structural features of flavones including the presence of the double bond between C-2 and C-3 (essential for planarity of the molecule) as well as the absence of a hydroxyl at C-3 enhances XO inhibitory activity.[66] Furthermore, the presence of an additional hydroxyl at C-6 has been shown to considerably increase the activity of some flavones and decrease it in others.[66]

The mode of inhibition of the studied compounds was of a mixed non-competitive inhibition type. This means that the binding site of these compounds to XO is not the molybdenum site but more probably the iron–sulfur group of the enzyme.

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59.G.Doria, V.Romeo, A.Forgione, P.Sberze, N.Tibolla, M.Corno, G.Cruzzola, G.Cadelli, *Eur. J. Med.Chem.—Chim. Ther.,* **27**, 347(1979).
60.W.Chang, Y.Lee, F.Lu, H.Chiang, *Anticancer Res.*, **13**, 2165(1993).
61.L.Costantino, G.Rastelli, A. Albasini, *Pharmazie*,**51**, 994(1996).
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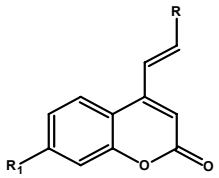
Antimicrobial activity

As an attempt to to preliminary exploration B.Varu, A.Shah, M. Kawase and et al[68] have synthesized a series of polyfunctionalized 3-cyano-4-styrylcoumarin(20-31) of the following general formula as novel antimicrobial agents to determine basic structural features required for the required biological activity.



3-Cyano-4-Styryl Coumarin Derivatives (20-31)

Compd.	R	R_1
20	2,4-diCl Phenyl	H
21	3-Phenoxyphenyl	H
22	2-Cl Phaenyl	H
23	4-Methylthiophenyl	H
24	1- Naphthyl	H
25	4-Hydroxyphenyl	H
26	2-Hydroxy Phenyl	H
27	3-Bromophenyl	H
28	3-OCH_3-4-OH Phenyl	H
29	2-NitroPhenyl	H
30	2-Methoxy Phenyl	H
31	1-Naphthyl	CH_3

The screening results suggested that compounds 20, 21, 29, 30 and 31 shows moderate activity as 20,29-31against *S.aureus NCTC 8530, S.aureus NCTC 8531, S.aureus ML 36,* 20 and 21 against *Sh. dysenteriae 1, E.coli Raw,* 21 and 29 against *V. cholerae 945, V. cholerae 946,* 29, 30 and 31 against *E.coli Raw,* 30 against *Shigella Sonnei* in terms of MIC value of 25 µg/ml.

63.G.Schmeda-Hirschmann, J.Zuniga, M.Dutra-Behrens, G.Habermehl, *Phytother. Res.,* **10**, 260(1996).
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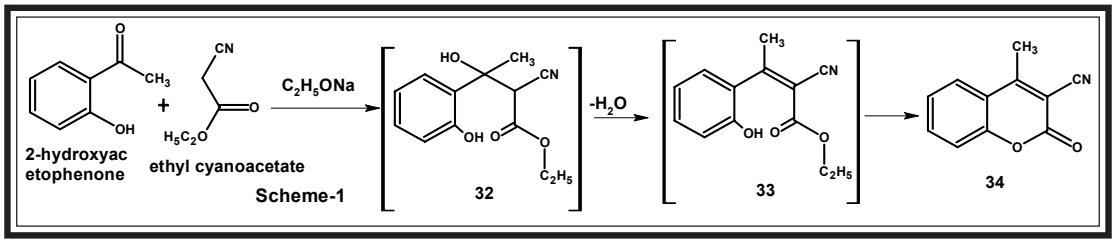
67.W.Chang, H.Chiang, *Anticancer Res.,* **15**,1969 (1995).
68.M.Kawase, B.Varu, A.Shah, N.Motohashi, S.Tani, S.Saito, S.Debnath, S.Mahapatra, S.Dastidar, A.Chakrabarty, *Arzneim.-forsch/ Drug Res.,* **51**(I), 67(2001)
69.C.Schroeder, K. Link, *J.Am.Chem.Soc.*, **75**, 1886(1953)
70.H.Madkour, *Heterocycles*, **36**(5), 947(1993), *Chem.Abstr.*, **120**, 8440k(1994)
71. J.Kendall, A.Axford, *Brit.Pat.*, 672741, *Chem.Abstr.*, **49**, 84g(1955)
72.E.Hafez, M.Elnagdi, A.Elgamey, M.El-Tawari, *Heterocycles*, **26**(4), 903(1987)
73.H.Junek, *Monatsh Chem.*, **95**(1), 234(1964), *Chem.Abstr.*, **60**,14461h(1964)
74. F.Aly, A.Bedair, M.Selim, *Afiniadad*, **44**(412),489(1987), *Chem.Abstr.*,**99**,170183y(1988)
75.M.Khodeir, *Indian J.Chem.*, **32(B)**, 783(1993)
76.Y.Voznyi, M.Dekaprilevich, D.Yufit, Y. Struchkov, A. Izu, *Ser.Khim.*, **6**,1371(1992), *Chem.Abstr.*, **118**, 124249v(1993)
77. Y.Anghelova, E.Dimitrova, *OPPI BRIEFS*, **21**(3), 341(1989)
78. E.Dimitrova, Y.Anghelova, *Syn.Commun.*, **16**(10), 1195(1986)
79.C.Inanov, Y.Anghelova, S.Spirova, *Synthesis*, 723(1979)
80.G.Wittig, *Chem.Ber.*, **57**,88(1924)
81(a).F.Chen, C.Chang, C.Lin, *J.Formosan Sci.*, **6**,81(1952)
(b). J.Corse, L.Ingraham, *J.Org.Chem.*, **16**, 1345(1951)
(c).G.Amin, N.Shah, *Org. Syn.Collect.Vol.***III**, 281
(d).C.Chang, F.Chen, *J.Chem.Soc.*, 3155(1961)
(e).U.Joshi, R.Kelkar, M.Paradkar, *Indian.J.Chem.*, **22**, 151(1983)

Synthetic approaches

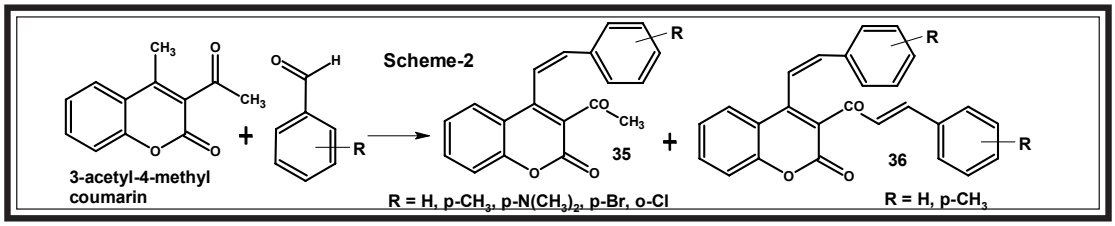
In 1953, Schroeder and Link[69] prepared 3-cyano coumarin(34) by reaction of 2-hydroxyacetophenone with ethylcyanoacetate in the presence of sodium ethoxide as catalyst. The reaction was conviniently carried out in one operation. It is suggested that it proceeds with the formation of an aldol condensate (32), which on dehydration forms intermediate(33). The intramolecular Claisen condensation let to yield 3-cyano-4-methyl coumarin(34) (Scheme-1). The catalyst such as pyridine and piperidine[70] were also employed in the preparation but comparatively lower yields were obtained. Other catalysts used were triethylamine[71] and ammonium acetate[70,72].



Scheme-1

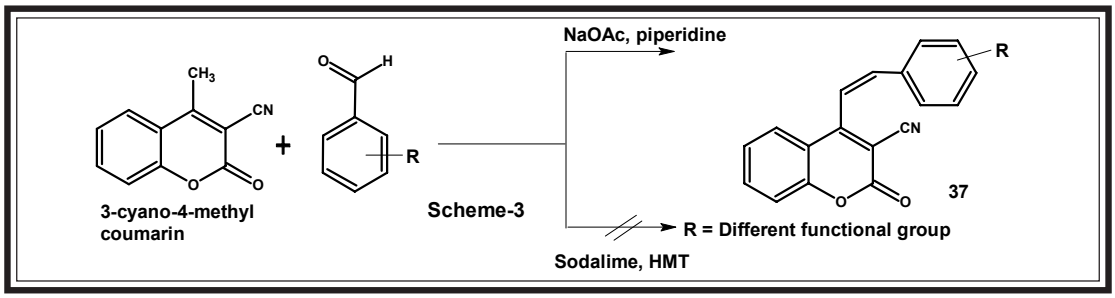
Junek[73] modified the route where 2-hydroxyacetophenone reacted with malanonitrile[72] or cyanoacetamide[72] or ethylcyanoacetate in the presence of ammonium acetate and acetic acid, using benzene as solvent medium. Further it appears that if any electron withdrawing groups like cyano[70,72-77], carboxylic acid[74], amide[74], trifluoroacetyl[76], acetyl[78] etc. if present at C_3 position of substituted 4-methylcoumarin, the methyl group becomes highly reactive towards electrophilic reagents.

Dimitrova et al[78] studied the reaction of 3-acetyl-4-methylcoumarin with different aromatic aldehydes in presence of piperidine afforded 4-(2-arylvinyl)-acetyl coumarins(35). The condensation process in the case of different aldehydes occurs at only one of the methyl group, which was confirmed by IR and NMR data. But in case of benzaldehyde and p-tolualdehyde over and above the product, another distyryl(36) derivative was also obtained in small yield.(Scheme-2)

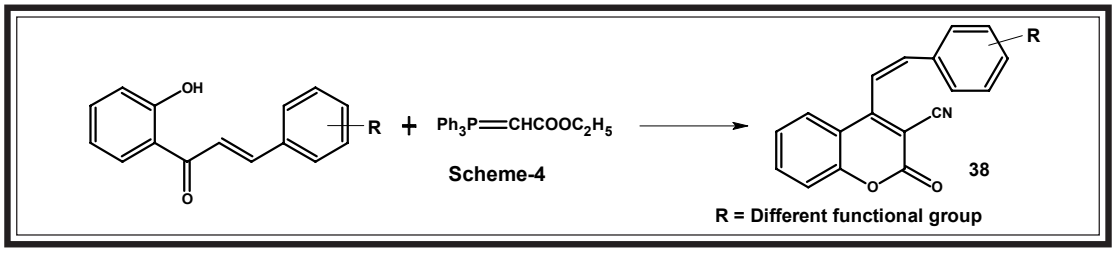


Scheme-2

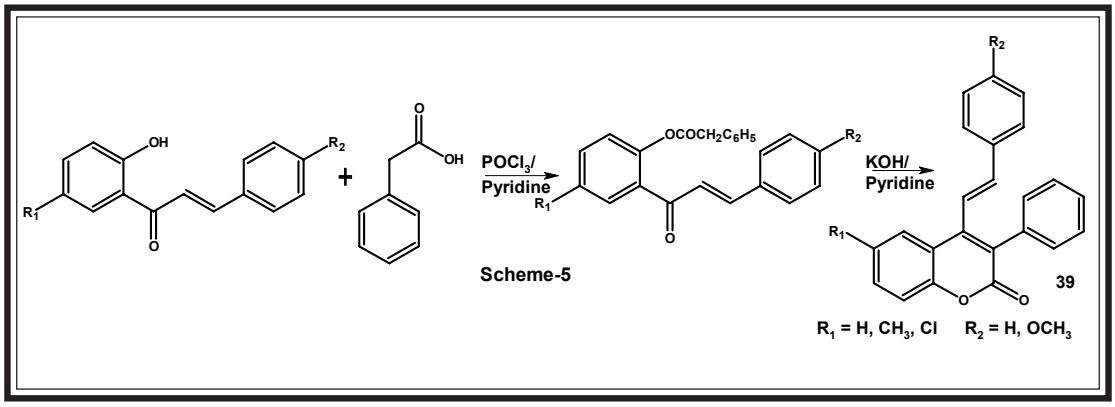
In a similar vein, 3-cyano-4-methylcoumarin was condensed with different aldehydes in the presence of sodium acetate[74] or piperidine[75,77] to yield various 3-cyano-4-(2-arylvinyl)coumarins[70,72](37). Anghelova and co-workers[79] have used sodalime in hexamethylenetetramine (HMT) but were unable to obtained the product(37).(Scheme-3)



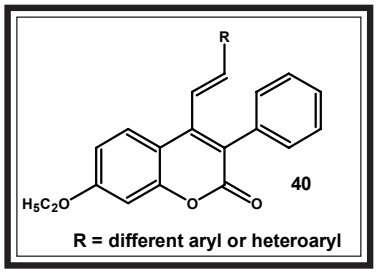
In 1924, Wittig[80] prepared alkenes by Wittig reaction of aldehydes or ketones with alkylidine triphenylphosphoranes. Thus 4-(2-aryl vinyl)coumarin was prepared(38)[81] by action of Wittig reagent (carbethoxy methylene) triphenylphosphorane [$Ph_3P{=}CHCOOC_2H_5$] on 2-hydroxy chalcone.(Scheme-4)



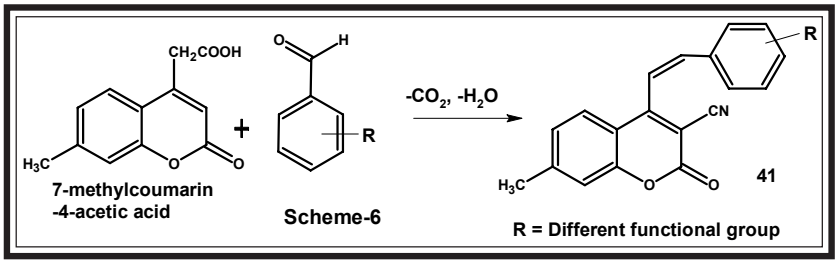
Ghiya and Lokhande[82] have carried out systematic work on different pathways for preparation of styryl coumarins. Substituted 3-phenyl-4-(2-aryl vinyl)coumarins of the type (39) were prepared by the condensation of appropriate chalcone of 2-hydroxyacetophenone with phenylacetic acid in presence of phosphorous oxychloride to form corresponding ester, which was further cyclized with pulverized potassium hydroxide in pyridine.(Scheme-5)



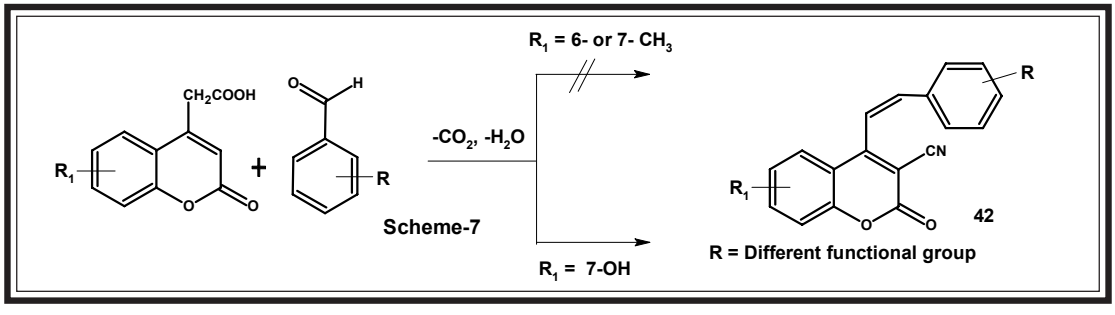
Barot[83] has prepared 7-ethoxy-3-phenyl-4-(2-aryl vinyl) coumarins(40) by a similar method. Mahal and Venkataraman[84] have prepared 3-phenyl-4-(2-arylvinyl) coumarin by condensation of 2-hydroxy acetophenone, sodium salt of phenyl acetic acid and acetic anhydride.



Dey and Row[85] and subsequently Soliman and co-workers[86] have studied the reactivity of -CH_2COOH group, where removal of carbondioxide under specific conditions paved the way for styryl derivative formation. Thus, 7-methyl coumarin acetic acid was reacted with aromatic aldehydes in the presence of piperidine to form 7-methyl-4-(2-arylvinyl) coumarin(41).(Scheme-6)

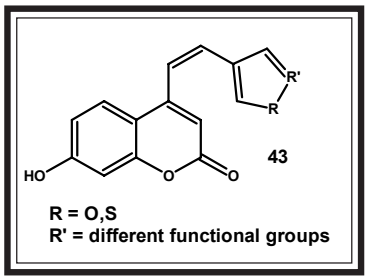


Contrary to this, Ghiya[87] reported that the presence of methyl group at 6 or 7position of coumarin-4-acetic acid, didn't allow reaction with aromatic aldehydes. The hydroxy group is necessary at 7 position for condensation with aldehydes to form substituted 4-(2-aryl vinyl)coumarin(42)[88].(Scheme-7)

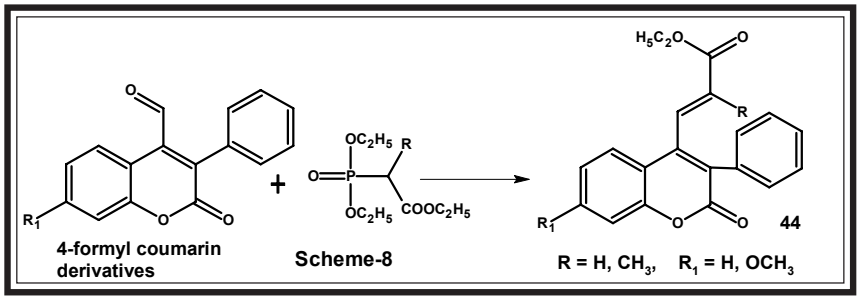


Shridhar and co-workers[89] prepared substituted 4-[(2-heteroaryl)vinyl]coumarins (43) as reaction product from substituted coumarin-4-acetic acid and formyl heterocycles.

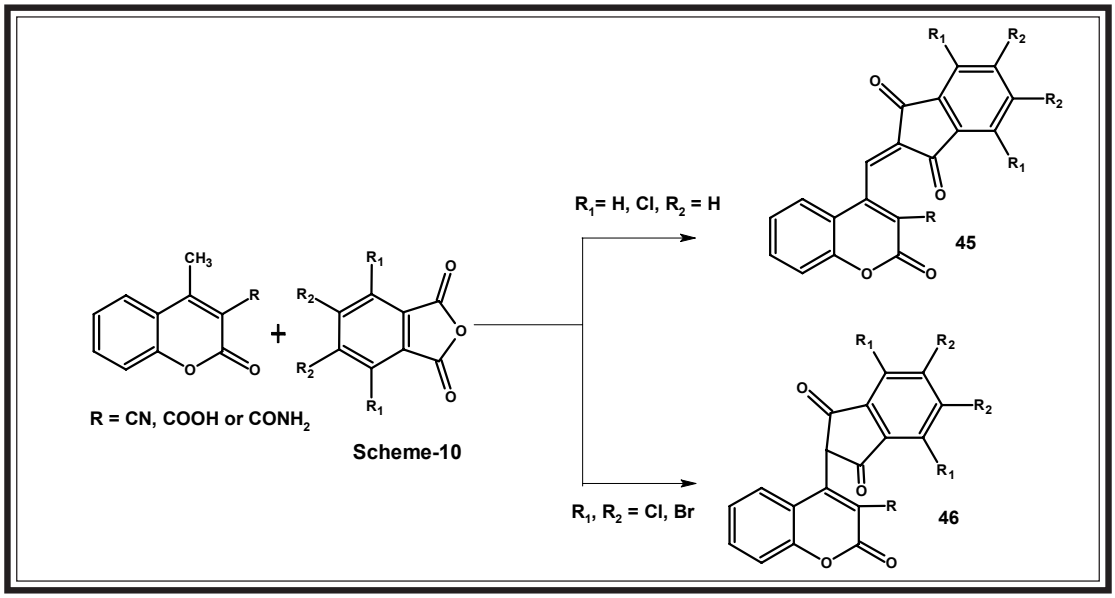
82.P.Lokhande, B.Ghiya, *J.Ind.Chem.Soc.*, **66**314(1989)



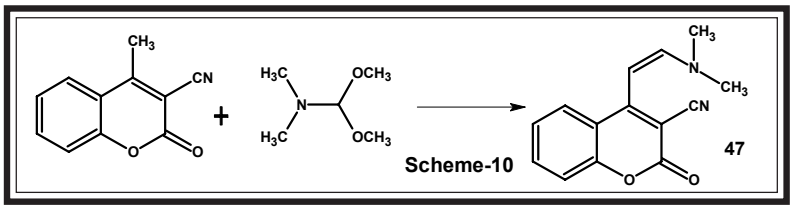
Dutta and Prabir Kumar[90] prepared β-coumarinyl acrylates(44) by reaction of 4-formyl coumarin with Wittig reagent $Ph_3P{=}CRCOOC_2H_5$ or $(H_5C_2O)_2P(O)CHRCOOC_2H_5$.(Scheme-8)



Aly and co-workers[74] reported the reaction of 4-methyl-3-cyano coumarin with phthalic anhydride and confirmed that with different substitution in phthalic anhydride, the condensation product were found to be different. Unsubstituted and dichloro phthalic anhydride gave the same product (i.e. phthalidylidine methyl)coumarins(45), while tetrahalogeno(dichloro, dibromo) phthalic anhydride gave exclusively dioxoindanyl coumarins(46). This reaction was carried out successfully both in 4-methylcoumarin-3-carboxylic acid and 3-aminocarbonyl-4-methyl coumarin.(Scheme-10)

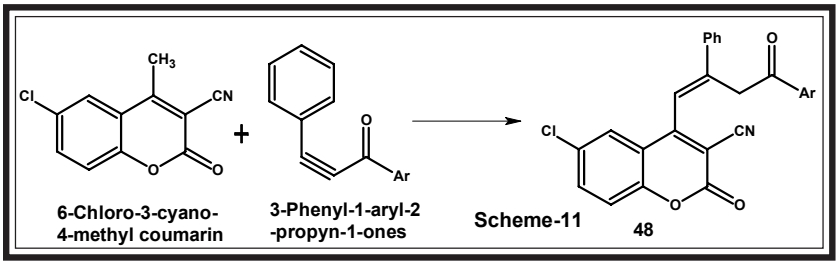


Walter et al[91] reported the reaction of 3-cyano-4-methyl coumarin with N,N-dimethylamino dimethyl orthoformate leading to the styryl coumarin of type(47).(Scheme-10)



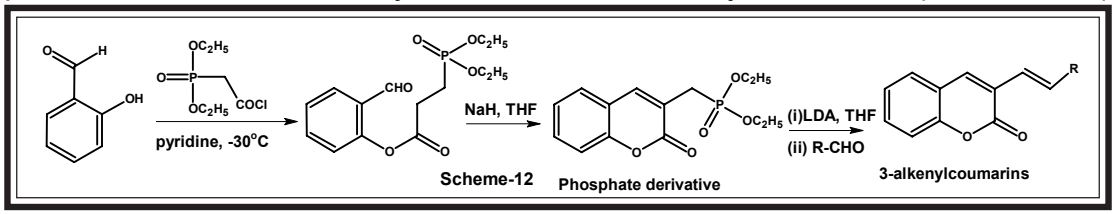
Scheme-10

Youssef et al[92] have described that 6-chloro-3-cyano-4-methyl coumarin reacted with 3-phenyl-1-aryl-2-propyn-1-ones to give the corresponding 3-amino-4-aroyl or carbomethoxy-8-chloro-5-arylbenzo[c]coumarins(48) as well as propenylcoumarins and benzo[c]coumarins by Michael addition.(Scheme-11)



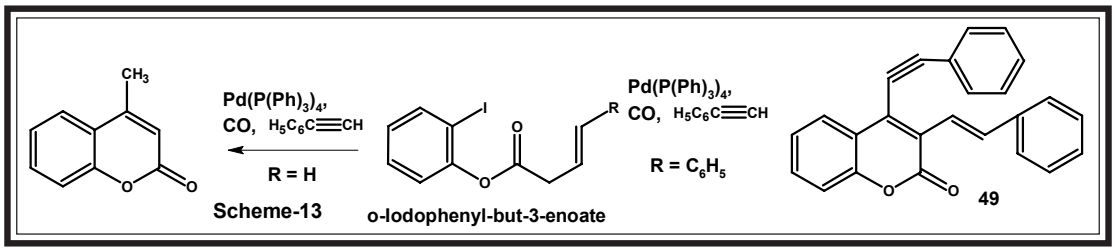
Scheme-11

An intramolecular aldol condensation enables the phosphate to be prepared, from which 3-alkenylcoumarins are readily obtained[93].(Scheme-12)



Scheme-12

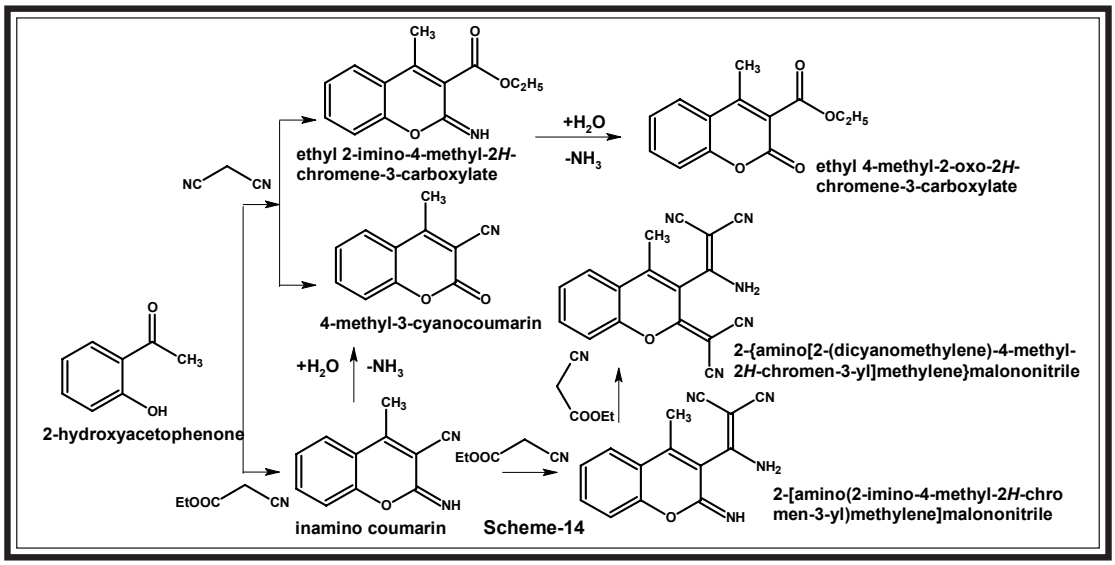
The intramolecular cyclization of cyclization of o-Iodophenyl-but-3-enoate(R=H) leads to 4-methyl coumarin only if the palladium catalysed process is carried out in the presence of carbon monoxide and a promoter such as phenyl acetylenewhich can coordinate to the catalyst[94]. However, in case where R=alkyl or aryl, the phenyl acetylene is incorporated into the product(49)[95].(Scheme-13)



Scheme-13

o-Iodophenyl-but-3-enoate

The condensation of 2-hydroxyacetophenone with ethylcyanoacetate runs nearly analogously to the reaction of salicylic aldehyde and gives rise to the formation of 3-cyano-4-methyl-coumarin, usually the reaction of 2-hydroxyacetophenone with malanonitrile gives, independent of the ratio of the educts used and the conditions applied, differently substituted products. Thus, by starting with equimolar amounts of 2-hydroxyacetophenone and malanonitrile, the iminocoumarin is formed in moderate yields only. Using an excess of malononitrile, the products 2-[amino(2-imino-4-methyl-2***H***-chromen-3-yl)methylene]malononitrile and 2-{amino[2-(dicyanomethylene)-4-methyl-2***H***-chromen-3-yl]methylene}malononitrile, together with some other products of unknown structure, are obtained [73]. The reason for the differences in the reactivity between salisaldehyde and 2-hydroxyacetophenone seems to be the lower carbonyl reactivity of 2-hydroxy acetophenone caused by the methyl group at C-4 [96].(Scheme-14)

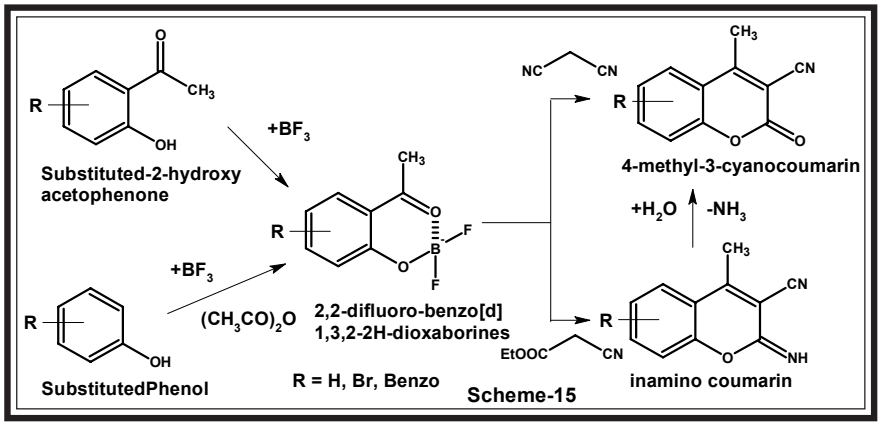


Scheme-14

A raising of the carbonyl reactivity in 2-hydroxy-acetophenone as well as in its ring substituted derivatives is expected if these compounds are transformed into 4-methyl-substituted 2,2-difluoro-benzo[d]-1,3,2-2H-dioxaborines by the reaction of a substituted- 2-hydroxy- acetophenone with boron trifluoride in acetic acid solution[97] or by condensation of a suitably substituted phenol with acetic acid anhydride in the presence of boron trifluoride [98].

83. V.Barot, *Asian J.Chem.*, **81**(4), 799(1996)
84.H.Mahal, K.Venkataraman, *J.Chem.Soc.*, 616(1933)

Boutome and Hartmann[99] have described that the 4-methyl-substituted 2,2-difluoro-benzo [d]-1,3,2-2H-dioxaborines condense with ethyl cyanoacetate or malononitrile under the influence of weak bases to give the corresponding 3-cyano-4-methyl-coumarins or their imino derivatives, respectively. The later compounds can be hydrolyzed to the parent 3-cyano-4-methyl-benzo[b]pyran-2-ones *(3-cyano-4-methyl-coumarins)* by reaction with mineral acid in aqueous solution.(Scheme-15)



Scheme-15

A characteristic property of the 3-cyano-4-methyl-coumarins is, *inter alia,* the acidity of the methyl group at C-4. Thus, these compounds are able to condense with aromatic aldehydes under the influence of bases. With 4-dimethylamino-benzaldehyde and catalytic amounts of piperidine, the 4-dimethyl-amino-sub-stituted styryl derivatives are available. These compounds are able, to react with amines, such as piperidine, to give pyrido[3,4-c]benzo[h]pyran-2-one derivatives. Thus, the *pyrido[3,4-c]benzo[h]pyran-2-one* derivative(Scheme-15) has been synthesized either by adding an excess of piperidine to the reaction mixture of 4-methyl-3-cyanocoumarin derivatives and 4-dimethylamino-benzaldehyde or by addition of piperidine to a solution of styryl derivatives in acetonitrile, the styryl coumarins are deeply coloured compounds which exhibit intense absorption bands in the visible region at about 500 nm. Their absoptions are strongly influenced by the polarity of the solvent. Therefore, the styryl coumarins are promising candidates for manufacturing materials with non-linear optical properties.

85.B.Dey, K.Row, *I.Ind.Chem.Soc.*, **1**, 277(1925)
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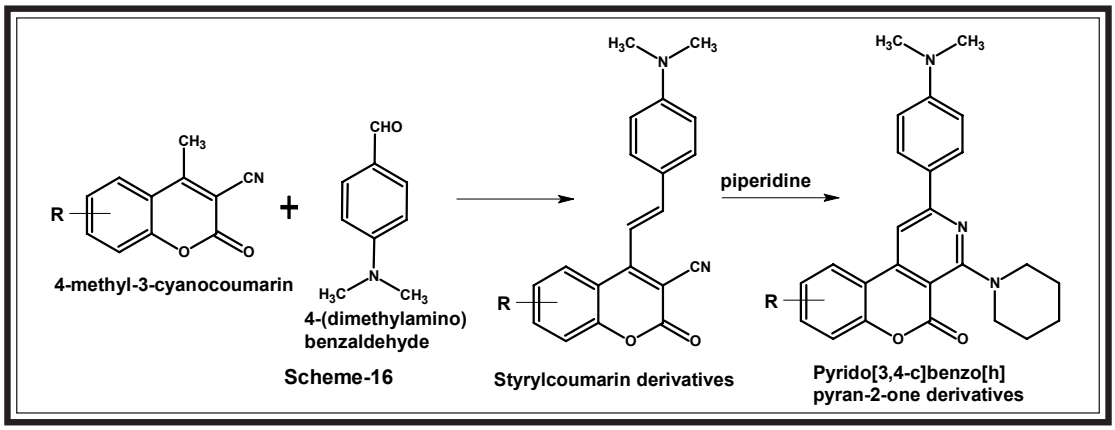
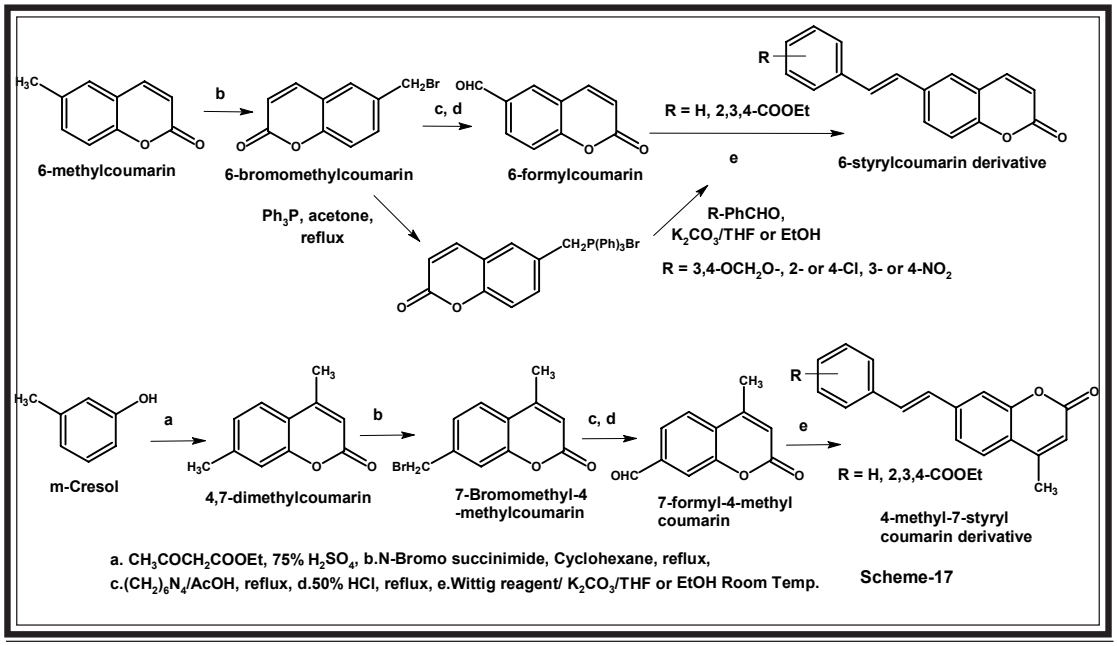
87.A.Gharde, B.Ghiya, *J.Ind.Chem.Soc.*, **69**,397(1992)



Scheme-16

Xu Song et al[25] have synthesized a series of styryl coumarin derivatives by phase transfer Wittig reaction.(Scheme-17)



Scheme-17

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93.T.Janecki, R.Bodalski,*Synthesis*, 719(1989)
94.M.Catellani, G.Chiusoli, M.Fagnola, G.Solari, *Tetrah. Lett.*, **35**, 5919(1994)
95. M.Catellani, G.Chiusoli, M.Fagnola, G.Solari, *Tetrah. Lett.*, **35**, 5923(1994)
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(b) G.Reynolds, J. van Allen, D.Daniel, *J Heterocycl Chem.*, **7**,1395(1970)
97(a) F.Umland, E.Hohaus, K.Brodte, *Chem Ber.,* **106**, 2427 (1973)
(b) J.Durden, D.Crosby, *J Org Chem.,* **30**, 1684 (1965)
98.G. Reynolds, J. van Allen, *J Heterocycl Chem.,* **6**, 375(1969)
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100.B.Varu, *Ph.D.Thesis*, Saurashtra University, 2001
101.P.Adlakha,*Ph.D.Thesis*, Saurashtra University, 2005
102.Aldrich advancing science (2005-2006)

Work Done At Our Laboratory

Earlier B.Varu[100] has synthesized 3-cyano-4-methylcoumarin derivatives by Kendall and Axford method[71] from 2-Hydroxyacetophenone or 5-methyl-2-hydroxyacetophenone and ethylcyanoacetate in the presence of triethyl amine, heated at 180°C for 7hrs. The 3-cyano-4-methylcoumarin derivatives thus obtained were reacted with different aromatic aldehydes to give the styrylcoumarins.

In continuation with this and in order to obtain substituted 2-hydroxyacetophenones, P.Adlakha[101] has studied the Fries rearrngement with polysubstituted phenols like 4-chloro-m-cresols. The substituted o-hydroxy acetophenone thus obtained were well characterized as 5-chloro-4-methyl-2-hydroxyacetophenone by it's physical properties and IR, NMR and Mass Spectral data as well as elemental analysis. The 5-chloro-4-methyl-2-hydroxyacetophenone thus obtained was again cyclized with ethylcyanoacetate to yield corresponding 4-methyl-3-cyanocoumarin followed by the styryl derivatization by the condensation with different aromatic aldehydes.

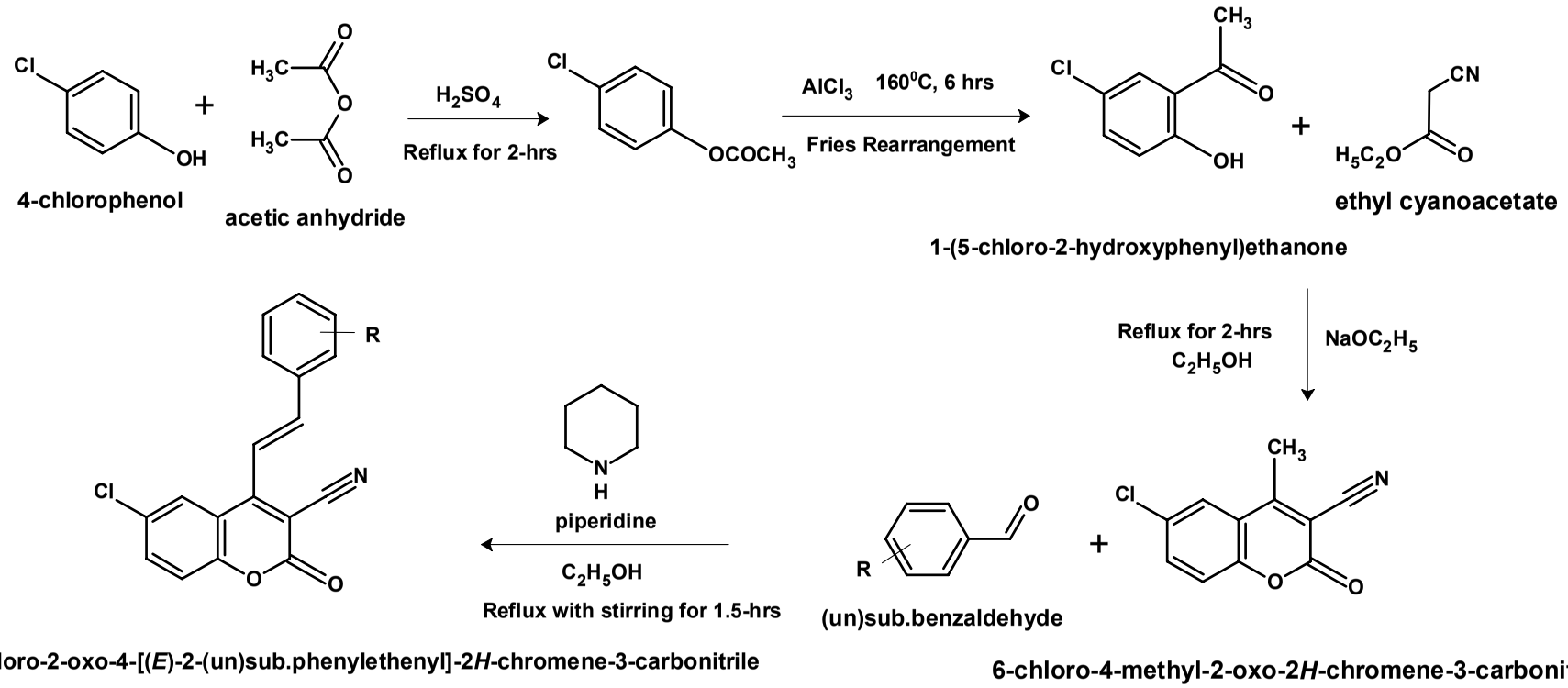
In the present chapter, it has been shown that acetyl ester derivative of 4-chlorophenol on Fries rearrangement exclusively gave only single isomer charachterized as 5-chloro-2-hydroxy acetophenone, which was in agreement with the theoritical basis, that in case of 4-substituted phenols, Fries rearrangement takes place with the migration of acetyl group only at ortho position as para position was already occupied with the substitution. Further, it was interesting to study the cyclization of the 2-hydroxyacetophenone along with the electron withdrawing substitution(5-Cl) with ethylcyanoacetate by using sodium ethoxide as catalyst. The method, we have earlier practiced suggested by Kendall and Axford method[71] was very drastic and time consuming. The current work presents a direct, efficient and operationally convenient approach to the synthesis of some novel styryl derivatives of the substituted 3-cyano-4-methyl coumarins. During the course of sodium methoxide catalysed cyclization it was observed that the rate of both the Aldol condensation and intramolecular Claisen condensation proceeds very smoothly and

speedily at mild reaction conditions and the reaction outcome as high purity of the product. Thus, the 6-chloro-3-cyano-4-methylcoumarin obtained was again examined for its characteristic property *inter alia*, the acidity of methyl group at C-4 position. When this compound was condensed with different substituted aromatic and heteroaromatic aldehydes in the presence of the basic catalyst, afforded the corresponding 6-chloro-3-cyano-4-styrylcoumarin derivatives.

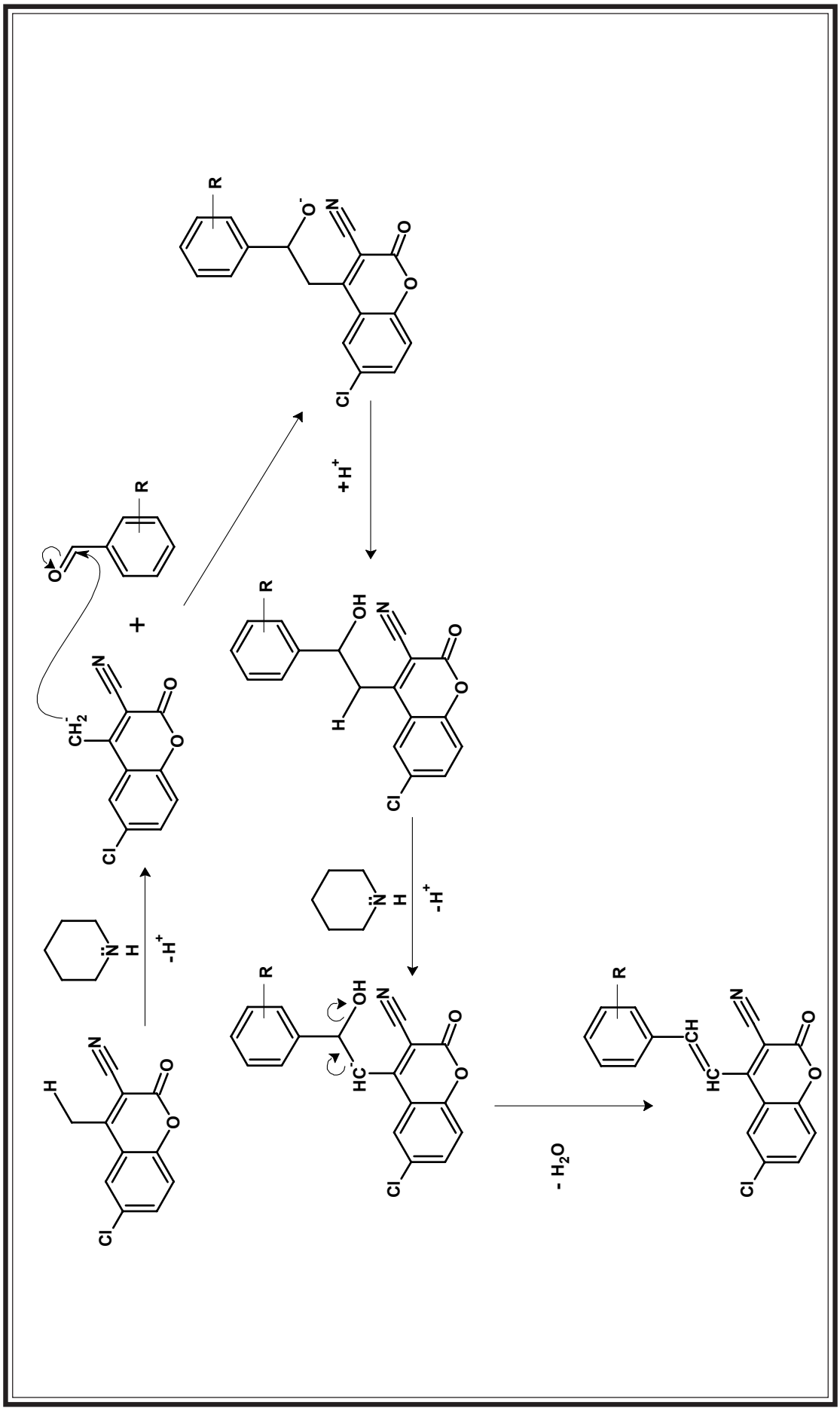
All the newly synthesized compounds were well characterized by IR, NMR and Mass spectral and elemental analysis.

In continuation with work on the styryl coumarins in this laboratory, which were studied as potent antibacterial agents, the current work encompasses with chloro substitutions in the benzenoid part so as to study the changes in the biological activity.The titled compounds were screened for antiviral, antimicrobial and antitubercular activity.

Reaction Scheme



Reaction Mechanism


R
O
+
CH2
N
O
O
Cl
H
-H+
+H+
OH
HC
- H2O
CH

Experimental Protocols

All the starting materials, p-chlorophenol, acetic anhydride, ethyl cyano acetate, sodium, methanol and substituted benzaldehydes were obtained from the commercial sources like Spectrochem Ltd., s.d.fine chem. Ltd., Sisco Research Labs., Allied Chem. Ltd., etc. Melting points of the synthesized compounds were recorded by open capillary method on controlled temperature using standard Zeal's thermometer and are uncorrected. The commencement of the reaction and purity of the synthesized compounds were ensured using thin layer chromatography (TLC) silica gel-G, used as stationary phase, the TLC plates were purchased from Merck India Ltd. by using acetone :benzene as the mobile phase. However other solvent systems like ethyl acetate:hexane and chloroform:methanol were also employed, but the best results observed with acetone :benzene (2 : 8) system.

Experimental

1. Synthesis of 4-chlorophenyl acetate

A mixture of 4-Chlorophenol (0.1 mole) and acetic anhydride (0.11 mole) along with catalytic amount of concentrated sulfuric acid was refluxed for 2 hours on boiling water bath. The progress and completion of the reaction was monitored by TLC. After completion of reaction, the reaction mass was poured into crushed ice. The reaction mass was extracted with dichloromethane after thorough wash with water. The organic layer was treated with anhydrous sodium sulphate in order to arrest traces of water. Solvent was removed from organic layer by vacuum distillation to yield pure 4-chlorophenyl acetate. Yield 90%, b.p. >300^{o}C (Reported b_{13} 105-8^{o}C[102]). Purity was checked by TLC using acetone:benzene (2 : 8) as mobile phase.

2. Synthesis of 5'-chloro-2'-hydroxy acetophenone

4-chlorophenyl acetate (0.1 mole) and anhydrous aluminium trichloride (0.11 mole) were mixed throughly in a round bottom flask. The reaction mixture was heated gradually at 120^{o}C till HCl gas was completely exhausted. The heating was extended for 2 hrs at 160^{o}C. The resulting aluminium complex was decompossed from crushed ice containing little amount of hydrochloric acid. The crude product was filtered dried and crystallized from methanol. Yield 60%, m. p. 53-55 ^{o}C(Reported m.p. was 53 ^{o}C[102]). Purity was checked by TLC using acetone:benzene(2 : 8).

3. Synthesis of 6-chloro-4-methyl-2-oxo-2*H*-chromene-3-carbonitrile

A mixture of 5'-Chloro-2-'hydroxy acetophenone (0.01 mole), ethyl cyano acetate (0.01 mole) and sodium methoxide in methanol (synthesized in situ by 0.2g sodium metal and 10ml ethanol) was heated to reflux for 2 hrs. The reaction mixture was cooled, the product separated from reaction mass was filtered, crystallized from ethanol to give pure 6-chloro-4-methyl-2-oxo-2*H*-chromene-3-carbonitrile.Yield 87%, m.p.221 ^{o} C. Purity was checked by TLC using acetone:benzene(2 : 8) as mobile phase.

4. Synthesis of 6-chloro-2-oxo-4-[substituted phenylvinyl]-2*H*-chromene-3-carbonitrile (General method)

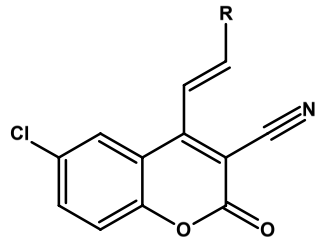
A mixture of 6-chloro-4-methyl-2-oxo-2*H*-chromene-3-carbonitrile (0.01 mole) and substituted aromatic or heteroaromatic aldehyde(0.01mole) were dissolved in minimum quantity of dichloro methane along with catalytic amount of piperidine. When no solid left, the reaction mixture was stirred till solid product precipitated. It was filtered and thoroughly washed with chilled methanol and crystallized from chloroform. Purity of each compound was checked by TLC using ethyl acetate: hexane(4:6) as mobile phase.

This method was employed to synthesize various 6-chloro-2-oxo-4-[sub. phenylvinyl]-2*H*-chromene-3-carbonitriles using different substituted aromatic aldehydes like benzaldehyde, o-/m-/p-nitro benzaldehyde, o-/m-/p-hydroxy benzaldehyde, o-/m-/p-chloro benzaldehyde, m-phenoxy benzaldehyde, m-bromo, 4-N,N-dimethyl benzaldehyde, cinnamaldehyde, furfural, 4-thiomethyl benzaldehyde and indole-3-carbaldehyde etc.

The elemental analysis of all the compounds performed by **PERKIN ELMER 470R** are in total agreement with the theoritical values.

The physical constants and elemental analysis of all compounds were shown in **Table 2.1**

Table 5.1 : Physical constants of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylethenyl]-2*H*-chromene-3-carbonitriles



Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analyses		
						C	H	N
1	KUS-1	Phenyl	$C_{18}H_{10}ClNO_2$	307.7	193-195	70.25 70.29	3.28 3.25	4.55 4.51
2	KUS-2	4-F Phenyl	$C_{18}H_9ClFNO_2$	325.7	246-249	66.37 66.33	2.79 2.76	4.30 4.34
3	KUS-3	2-Cl Phenyl	$C_{18}H_9Cl_2NO_2$	342.1	257-259	63.18 63.24	2.65 2.62	4.09 4.05
4	KUS-4	3-Cl Phenyl	$C_{18}H_9Cl_2NO_2$	342.1	239-242	63.18 63.24	2.65 2.62	4.09 4.05
5	KUS-5	4-Cl Phenyl	$C_{18}H_9Cl_2NO_2$	342.1	275-277	63.18 63.24	2.65 2.62	4.09 4.05

Note:- The underline values denote the calculated percentage of composition

Table 5.1 : Physical constants of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylethenyl]-2*H*-chromene-3-carbonitriles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C,H,N%)		
						C	H	N
6	KUS-6	3-Br Phenyl	$C_{18}H_9BrClNO_2$	386.6	235-237	55.92 55.98	2.35 2.32	3.62 3.65
7	KUS-7	2-NO2 Phenyl	$C_{18}H_9ClN_2O_4$	352.7	139-142	61.29 61.23	2.57 2.6	7.94 7.98
8	KUS-8	3-NO2 Phenyl	$C_{18}H_9ClN_2O_4$	352.7	236-237	61.29 61.23	2.57 2.6	7.94 7.98
9	KUS-9	3-OH Phenyl	$C_{18}H_{10}ClNO_3$	323.7	229-232	66.78 66.73	3.11 3.14	4.33 4.37
10	KUS-10	4-OH Phenyl	$C_{18}H_{10}ClNO_3$	323.7	160-163	66.78 66.73	3.11 3.14	4.33 4.37
11	KUS-11	2-OCH3 Phenyl	$C_{19}H_{12}ClNO_3$	337.7	157-159	67.56 67.62	3.58 3.54	4.15 4.11
12	KUS-12	4-OCH3 Phenyl	$C_{19}H_{12}ClNO_3$	337.7	>300	67.56 67.62	3.58 3.54	4.15 4.11
13	KUS-13	3,4-DiOCH3 Phenyl	$C_{20}H_{14}ClNO_4$	367.7	203-205	65.31 65.38	3.84 3.79	3.81 3.85

Note:- The underline values denote the calculated percentage of composition

Table 5.1 : Physical constants of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylethenyl]-2*H*-chromene-3-carbonitriles

Sr. No.	Code	Substituents	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Elemental Analysis (C,H,N%)		
						C	H	N
14	KUS-14	3,4,5-TriOCH3 Phenyl	$C_{21}H_{16}ClNO_5$	397.8	261-264	63.40 63.32	4.05 4	3.52 3.46
15	KUS-15	3-OC6H5 Phenyl	$C_{24}H_{14}ClNO_3$	399.8	221-223	72.10 72.17	3.53 3.58	3.50 3.44
16	KUS-16	4-SCH3 Phenyl	$C_{19}H_{12}ClNO_2S$	353.8	205-207	64.50 64.42	3.42 3.47	3.96 3.92
17	KUS-17	2-ethenyl Phenyl (Cinnamyl)	$C_{20}H_{12}ClNO_2$	333.7	257-260	71.97 71.91	3.62 3.67	4.20 4.14
18	KUS-18	9-Anthracyl	$C_{26}H_{14}ClNO_2$	407.8	115-117	76.57 76.5	3.46 3.42	3.43 3.47
19	KUS-19	2-furyl	$C_{16}H_8ClNO_3$	297.7	173-175	64.55 64.49	2.71 2.68	4.71 4.76
20	KUS-20	3-Indolyl	$C_{20}H_{11}ClN_2O_2$	346.7	158-161	69.27 69.2	3.20 3.15	8.08 8.12
21	KUS-21	4-N,N,Dimethyl Phenyl	$C_{20}H_{15}ClN_2O_2$	350.8	156-159	68.48 68.54	4.31 4.36	7.99 7.92
22	KUS-22	3-Mehoxy Phenyl	$C_{19}H_{12}ClNO_3$	337.7	202-205	67.56 67.62	3.58 3.54	4.15 4.11

Note:- The underline values denote the calculated percentage of composition

SPECTRAL CHARACTERIZATION

The constitution of newly synthesized compounds were supported by FT - IR, ^{1}H NMR and FAB-Mass spectral study.

IR Spectral Study

The wave like patern of all the frequences that make -up the infrared spectrum is known as interferogram. A mathematical operation which can seperate individual absorption frequency from the interferogram is known as **Fourier Transform (FT).** The instrument known as Fourier Transform Spectrophotometer acquires the interferogram in less than one second. When fourier transform is performed on the sum of accumulated interferograms, a spectrum with a better signal-to-noice ratio can be ploted. An FT-IR instrument is therefore capable of greater speed and sensitivity than a dispersion instrument.

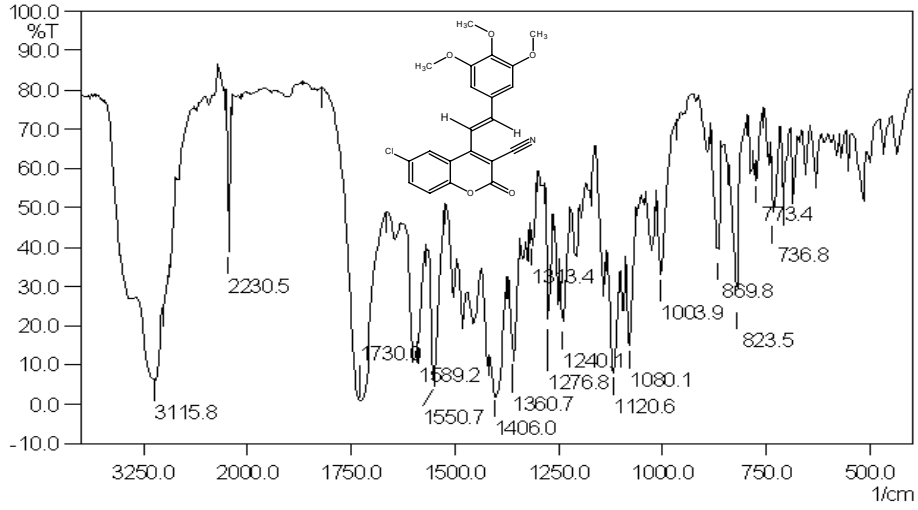
Instrument: SHIMADZU FT-IR 8400 Spectrophotometer

Sample technique: KBr pellet **Frequency range:** 400-4000 cm^{-1}

The 6-chloro-2-oxo-4-[(*E*)-2-sub.phenylvinyl]-2*H*-chromene-3-carbonitrile indicates the presence of very specific functional groups like cyano group, the vinyl group of styryl residue and carbonyl group of the lactone residue, aromatic ring skeleton etc., which absorb IR radiations of specific frequency and show sharp, medium or weak intense signals and hence supports in identifiction and confirmation of the molecule.

The IR spectrums of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylvinyl]-2*H*-chromene-3-carbonitriles shows common frequences as 3150-3050cm^{-1} for vinyl group, 2300-2200 cm^{-1} for cyano group, 1750-1710cm^{-1} for carbonyl group of lacton residue, aromatic ring skeleton observed between 1630-1480cm^{-1}, bands between 1280-1230cm^{-1} and 1010-890 for trans CH=CH group, C-O-C str. was observed between 1120-1030cm^{-1}, mono,di & tri phenyl substitutions observed between 870-750cm^{-1}, while C-Cl str. was observed between 750-790cm^{-1}. The IR spectral data of all compounds of KUS series are shown in **Table 2.2**

IR Spectrum of 6-Chloro-2-oxo-4-[(E)-2-(3,4,5-trimethoxyphenyl)vinyl]- 2H-chromene-3-carbonitrile (KUS-14)



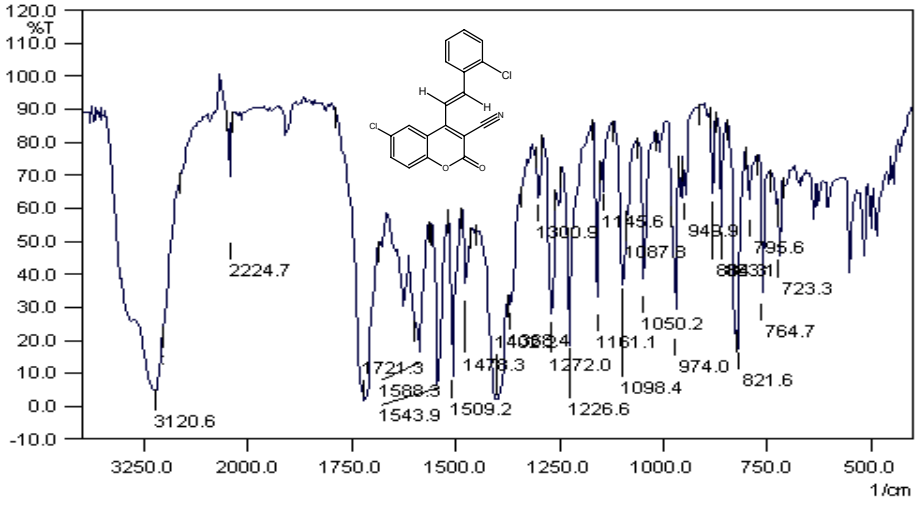
KUS - 14

Functional Group	Vibration Mode	Frequency (cm^{-1})
-CH = CH- (vinyl)	C-H str.	3115
	-CH=CH- i/p deformation	1276
	-CH=CH- o/p deformation	1003
-CN (cyano)	C=N str.	2230
-C=O (lactone)	-C=O str.	1730
Aromatic Skeleton	-C=C- str.	1589,1555,1486
	1:3:4:5 tetra substitution	869
C-O-C (cyclic)	-C-O-C- str.	1120
Halogen	C-Cl str.	773

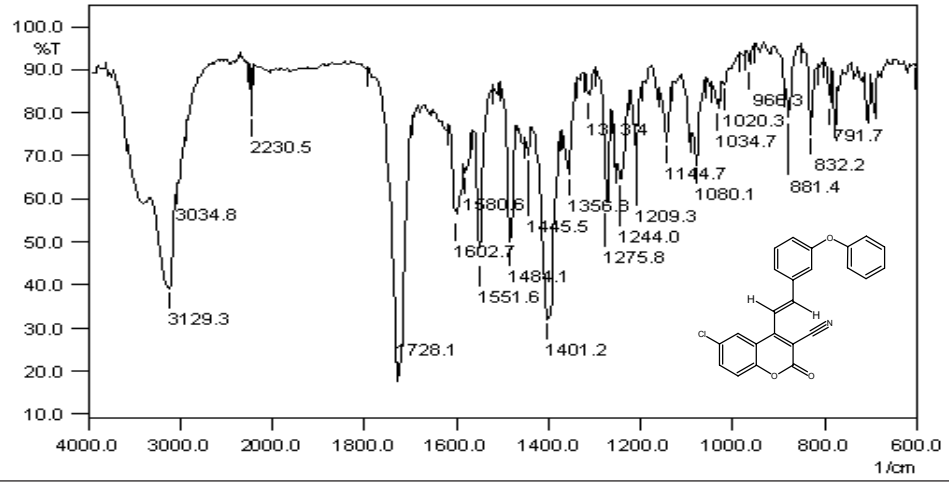
KUS - 3

Functional Group	Vibration Mode	Frequency (cm^{-1})
-CH = CH- (vinyl)	C-H str.	3120
	-CH=CH- i/p deformation	1272
	-CH=CH- o/p deformation	974
-CN (cyano)	C=N str.	2224
-C=O (lactone)	-C=O str.	1721
Aromatic Skeleton	-C=C- str.	1588,1543,1509
	1:2 di substitution	821
C-O-C (cyclic)	-C-O-C- str.	1098
Halogen	C-Cl str.	723

IR Spectrum of 6-Chloro-2-oxo-4-[(E)-2-(2-chlorophenyl)vinyl]- 2H-chromene-3-carbonitrile (KUS- 3)



IR Spectrum of 6-Chloro-2-oxo-4-[(E)-2-(3-phenoxyphenyl)vinyl]- 2H-chromene-3-carbonitrile (KUS-15)



KUS - 15

Functional Group	Vibration Mode	Frequency (cm^{-1})
-CH = CH- (vinyl)	C-H str.	3129
	-CH=CH- i/p deformation	1275
	-CH=CH- o/p deformation	966
-CN (cyano)	C=N str.	2230
-C=O (lactone)	-C=O str.	1728
Aromatic Skeleton	-C=C- str.	1602,1551,1484
	1:3 di substitution	832
C-O-C (cyclic)	-C-O-C- str.	1080
Halogen	C-Cl str.	791

KUS - 4

Functional Group	Vibration Mode	Frequency (cm^{-1})
-CH = CH- (vinyl)	C-H str.	3128
	-CH=CH- i/p deformation	1273
	-CH=CH- o/p deformation	975
-CN (cyano)	C=N str.	2231
-C=O (lactone)	-C=O str.	1731
Aromatic Skeleton	-C=C- str.	1603,1552,1481
	1:3 di substitution	824
C-O-C (cyclic)	-C-O-C- str.	1083
Halogen	C-Cl str.	788

IR Spectrum of 6-Chloro-2-oxo-4-[(E)-2-(2-chlorophenyl)vinyl]- 2H-chromene-3-carbonitrile (KUS- 4)

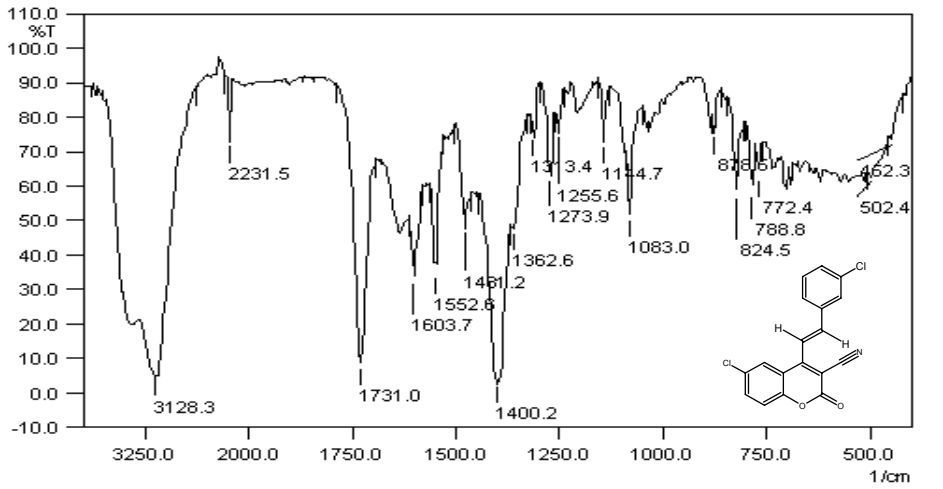
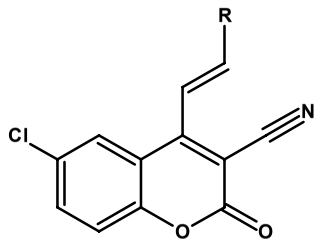
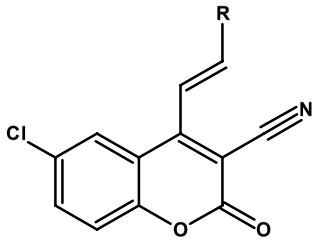


Table 5.2 : IR Spectral data of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylethenyl]-2*H*-chromene-3-carbonitriles



Code	Substitution (R)	Ar-H Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			Trans >CH=CH< i.p.deformation	>C-O-C< str.	Trans >CH=CH< o.o.p.deformation	Phenyl Substitution	Substitution	C-Cl str.
KUS-2	4-F	3107	2224	1721	1588	1509	1575	1272	1099	974	828 (1:4 di sub.)	1050 (C-F str)	760
KUS-5	4-Cl	3120	2227	1720	1624	1546	1577	1272	1090	974	820 (1:4 di sub.)	775 (C-Cl str)	770
KUS-6	3-Br	3124	2228	1723	1628	1581	1525	1272	1098	972	819 (1:3 di sub)	693 (C-Br str.)	753
KUS-7	2-NO_2	3143	2229	1708	1606	1471	1514	1272	1101	972	837 (1:2 di sub)	1344 (-N=O str.)	785
KUS-8	3-NO_2	3106	2230	1733	1602	1529	1502	1274	1080	892	877 (1:3 di sub.)	1345 (-N=O str.)	785
KUS-9	3-OH	3128	2226	1713	1628	1459	1504	1272	1097	969	842 (1:2 di sub)	3423 (O-H str) 1026(C-O str)	764
KUS-11	2-OCH_3	3125	2214	1738	1627	1488	1483	1245	1097	992	750 (1:2 di sub)	1026 (C-O-C str.)	754

Table 5.2 : IR Spectral data of 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylethenyl]-2*H*-chromene-3-carbonitriles



Code	Substitution (R)	Ar-H Str.	-CN Str.	>CO Str.	Aromatic Region (>C=C< str)			Trans >CH=CH< i.p.deformati-on	>C-O-C< str.	Trans >CH=CH< o.o.p.deformati-on	Phenyl Substitution Remarks	Substitution Remarks	C-Cl str.
KUS-12	4-OCH_3	3118	2232	1725	1631	1531	1579	1270	1032	976	830 (1:4 di sub)	1252 (C-O-C str.)	767
KUS-13	3,4-diOCH_3	3129	2237	1719	1620	1518	1492	1266	1097	974	840 (1:3:4 tri sub)	1239 (C-O-C str.)	760
KUS-16	4-SCH_3	3119	2224	1721	1624	1542	1494	1272	1091	974	808 (1:4 di sub)	3017,1405 (C-Hstr, i.p.def)	768
KUS-17	$CH=CHC_6H_5$	3126	2227	1735	1624	1520	1508	1232	1098	977	763 (mono sub.))	1257 (trans >CH=CH< i.p.def.) 992 (trans >CH=CH< o.o.p def)	762
KUS-19	2-Furyl	3124	2229	1720	1602	1469	1512	1269	1080	952	821 (mono sub.)	1043 (C-O-C str.furural)	765
KUS-20	3-Indolyl	3129	2222	1750	1608	1537	1502	1272	1095	975	824 (mono sub)	3320 (N-H str)	753
KUS-21	4-N,N-di CH_3	3126	2229	1732	1606	1481	1512	1274	1097	972	821 (1:4 di sub.)	3030(C-H str) 1402(C-H i.p.def) 1359(C-N str.)	769
KUQ-22	3-OCH_3	3078	2226	1718	1587	1479	1577	1271	1080	968	823 (1:3 sub)	1035 (C-O-C str)	783

^{1}H NMR Spectral Study

^{1}H NMR Spectrum of the 6-chloro-2-oxo-4-[(*E*)-2-substituted phenylvinyl]-2*H*-chromene-3-carbonitrile shows signals relevant to the number of protons and their electronic environment known as chemical shift. Chemical shift may move to either upfield (shielding) or down field(deshielding), according to the electronic environment of the corresponding proton. In addition to this, each ^{1}H NMR signal further splitted into number of subpeaks according to the number of neighbouring protons present in the skeleton of molecule. The splitting of the NMR signal further provides signification about the degree of interaction between neighbouring protons by means of spin-spin coupling constant J.

Instrument : BRUKER AC 300MHz FT-NMR

Internal Reference : TMS

Solvent : $CDCl_3$ or DMSO d_6

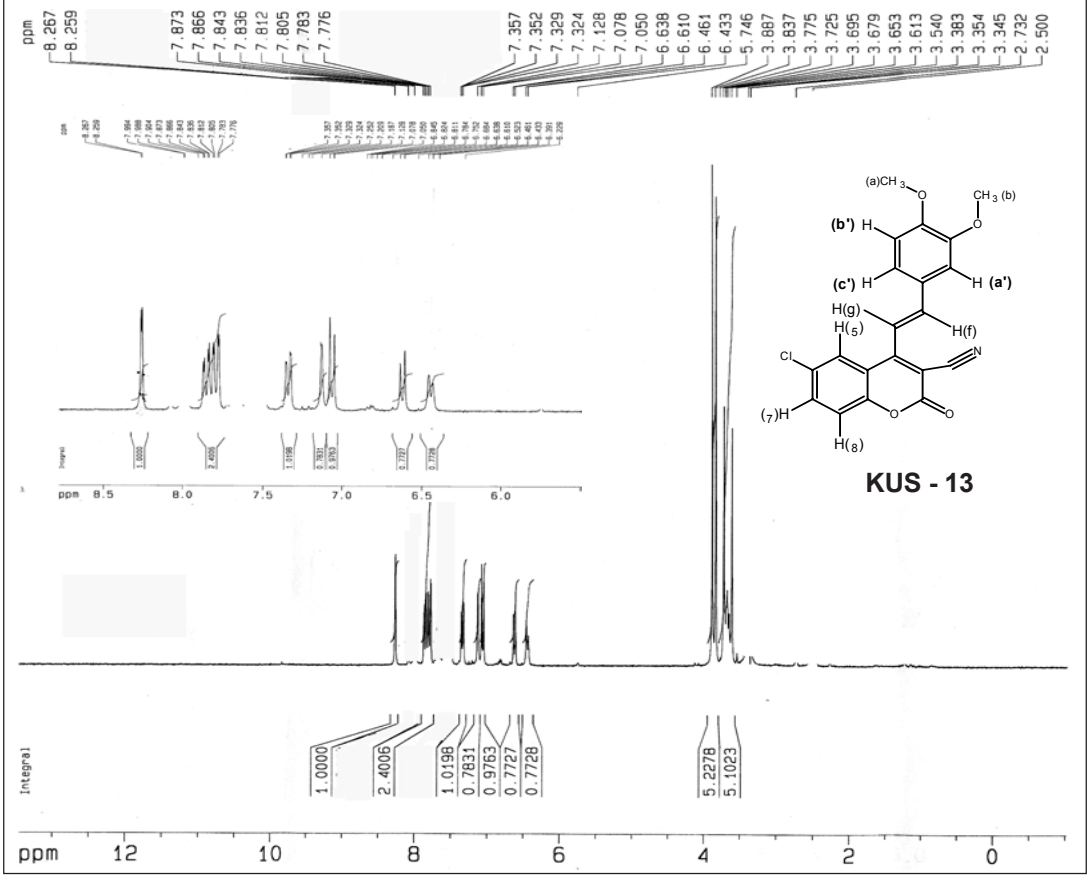
6-chloro-2-oxo-4-[(*E*)-2-(3,4,5-trimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile. (KUS-14)

In case of ^{1}H NMR Spectrum of KUS-14 shows two signals, relevant to three and six protons, of three methoxy groups appears at chemical shift value 3.359 & 3.364δ ppm. Two signals relevant to vinylic protons appears at chemical shift value 7.194 & 7.846δ ppm, both further splitted to give doublet, having coupling constant 7.77Hz & 6.3Hz respectively, which reveals that both vinylic protons are relatively in trans(*E*) position to each other. Phenyl residue having two chemically equivalent protons $H_{(a)}$ shows signal at 6.614δ ppm as singlet. Protons $H_{(5)}$, $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows signals at 8.07δ ppm for one proton as doublet with J value 2.1Hz, 7.514δ ppm for one proton as triplet with J value of 8.7 & 6.3Hz and 7.583δ ppm for one proton as doublet with J value of 9Hz respectively, which reveals that $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other where as $H_{(5)}$ & $H_{(7)}$ are relatively in meta position.

6-chloro-2-oxo-4-[(*E*)-2-(3,4-dimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile (KUS - 13)

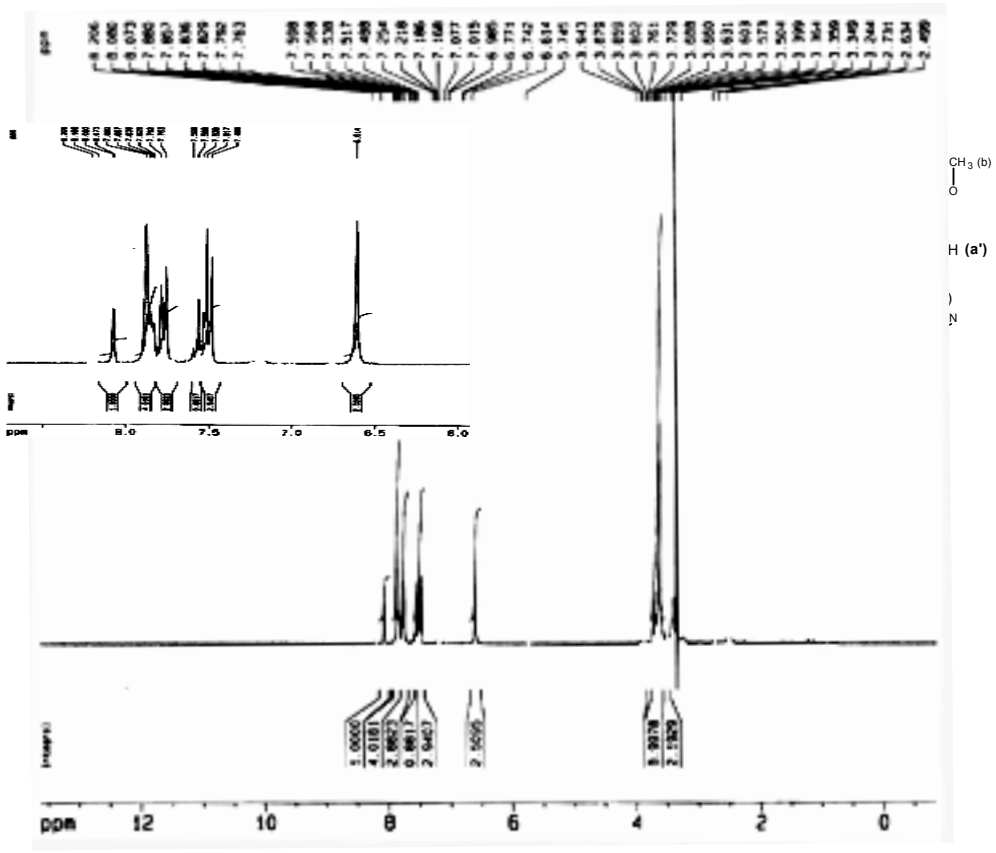
^{1}H NMR Spectrum of the KUS-13 shows two signals, each relevant to three protons, of two methoxy groups appears at chemical shift value 3.837 & 3.887δ ppm. Two signals relevant to vinylic protons appears at chemical shift value 6.412 & 6.661δ ppm, both further splitted to give doublet, with coupling constant 12.6Hz & 13.8Hz respectively, which reveals that both vinylic protons are relatively in trans(*E*) position to each other. Protons $H_{(a)}$, $H_{(b)}$ & $H_{(c)}$ of the phenyl residue shows signals at 7.128δ ppm for one proton as singlet, 7.064δ ppm for one proton as doublet with J value of 8.4Hz and 7.422δ ppm for one proton as double doublet with J value of 1.5, 6.9 & 1.5Hz respectively, which reveals that $H_{(b)}$ & $H_{(c)}$ are in ortho position to each other where as $H_{(a)}$ & $H_{(c)}$ are relatively in meta position. Protons $H_{(5)}$, $H_{(7)}$ & $H_{(8)}$ of the benzenoid part shows signals at 8.263δ ppm for one proton as doublet with J value 2.4Hz, 7.794δ ppm for one proton as double doublet with J value of 2.1, 6.6 & 2.1Hz and 7.854δ ppm for one proton as doubledoublet with J value of 1.2, 6.9 & 1.2Hz respectively, which reveals that $H_{(7)}$ & $H_{(8)}$ are in ortho position to each other where as $H_{(5)}$ & $H_{(7)}$ are relatively in meta position.

^{1}H NMR Spectrum of 6-chloro-2-oxo-4-[(*E*)-2-(3,4-dimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile (KUS - 13)



Type of Proton (Group)	Chemical Shift delta ppm	No. of Protons	Multiplicity	J Value (Hz)
O-$CH_{3\ (a)}$	3.837	3	s	-
O-$CH_{3(b)}$	3.887	3	s	-
Ar-$H_{(a)}$	7.128	1	s	-
Ar-$H_{(b)}$	7.064	1	d	8.4
Ar-$H_{(c)}$	7.422	1	dd	1.5, 6.9 & 1.5
CH=$CH_{(f)}$	6.412	1	d	12.6
CH=$CH_{(g)}$	6.661	1	d	13.8
Ar-$H_{(7)}$	7.794	1	dd	2.1, 6.6& 2.1
Ar-$H_{(8)}$	7.854	1	dd	1.2, 6.9 & 1.2
Ar-$H_{(5)}$	8.263	1	d	2.4

^{1}H NMR Spectrum of 6-chloro-2-oxo-4-[(*E*)-2-(3,4,5-trimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile (KUS - 14)



Type of Proton (Group)	Chemical Shift (delta ppm)	No. of Protons	Multiplicity	J Value (Hz)
O-$CH_{3\,(a)}$	3.359	3	s	-
O-$CH_{3(b)}$	3.364	6	s	-
2 Ar-$H_{(a')}$	6.614	2	s	-
CH=$CH_{(f)}$	7.194	1	d	7.777
CH=$CH_{(g)}$	7.846	1	d	6.3
Ar-$H_{(7)}$	7.514	1	t	8.7 & 6.3
Ar-$H_{(8)}$	7.583	1	d	9
Ar-$H_{(5)}$	8.07	1	d	2.1

Mass Spectral Study

The molecular ion peaks (M+) of the compounds in mass spectra were in total agreement with it's molecular weight.

Instrument: JEOL SX 102/DA-6000 Spectrograph for FAB

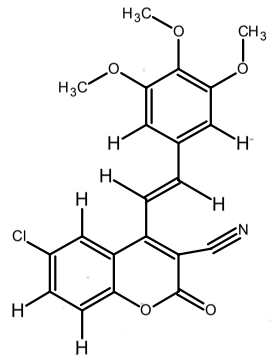
6-chloro2-oxo-4-[(*E*)-2-(3,4,5-trimethoxypheny)lvinyl]-2*H*-chromene-3-carbonitrile (KUS-14)

The FAB Mass spectrum of KUS-14 shows molecular ion peak at 398m/z (M^+ peak), while fragmantation pattern shows peaks at 384m/z (M^+-CH_3), 365m/z (M^+-OCH_3), 363m/z (M^+-Cl), 331m/z (M^+-Cl,-OCH_3), 307m/z (M^+-$3OCH_3$), 289m/z (M^+-Cl,-CN,-O i.e. rupture of coumarin ring), 273m/z (M^+-Cl,-$3OCH_3$), 257m/z (M^+-$3OCH_3$,-Cl,-O), 232m/z (M^+-$3OCH_3$,-Cl,-O,-CN), 154m/z (M^+-$3OCH_3$,-Cl,-O,-CN,-Phenyl)appears as base peak, 136m/z ($C_8H_8O_2$ or dimethoxy phenyl redical)

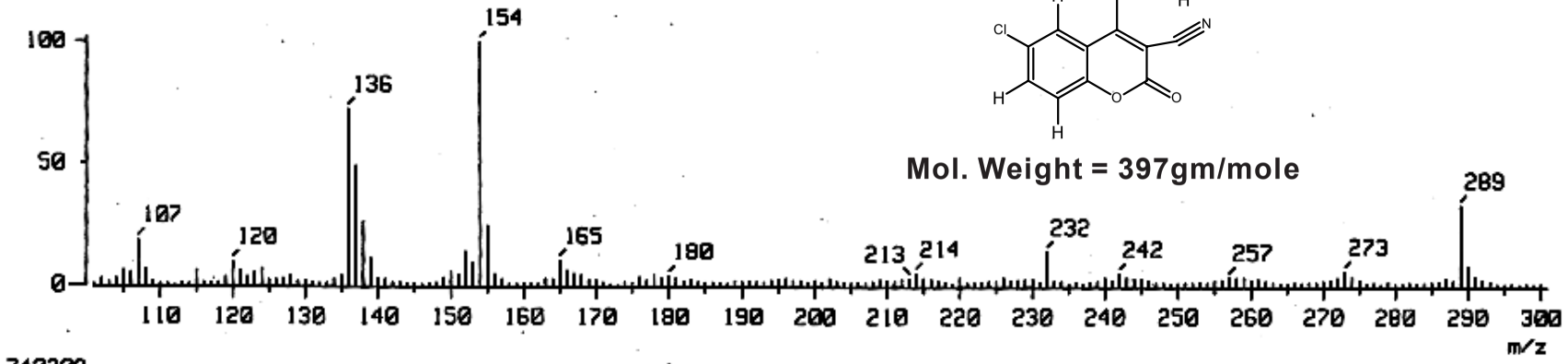
6-chloro2-oxo-4-[(*E*)-2-(3,4-dimethoxypheny)lvinyl]-2*H*-chromene-3-carbonitrile (KUS-13)

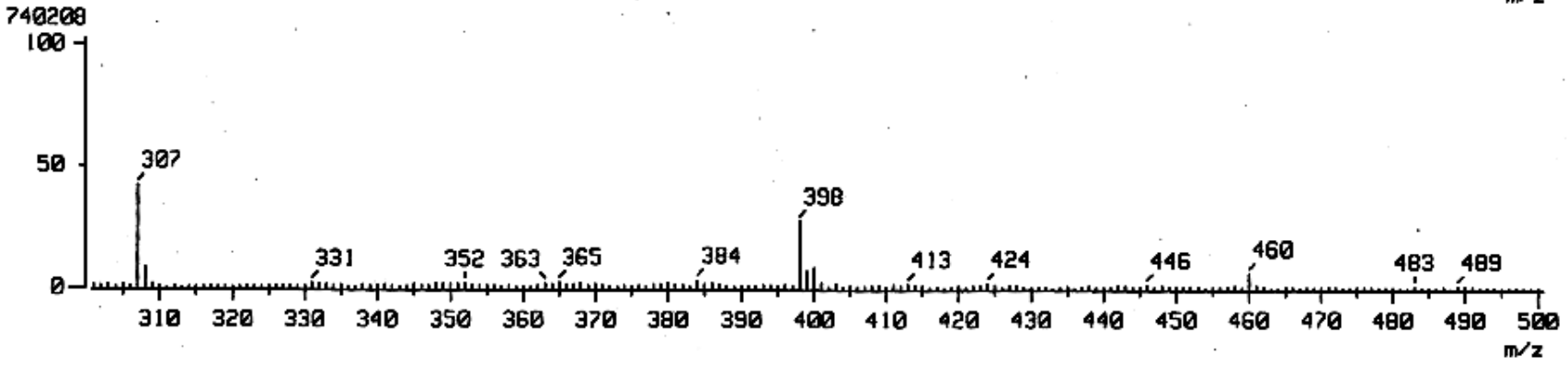
FAB Mass Spectrum of shows molecular ion peak at 368m/z (M^+ peak) and 368m/z (M^++1) peak, while fragmantation pattern shows peaks at 331m/z (M^+-Cl), 317 (M^+-Cl,-O ring rupture of coumarin ring), 307m/z (M^+-Cl,-CN or M^+-$2OCH_3$), 289m/z (M^+-Cl,-CN,-O or M^+-$2OCH_3$,-O), 279m/z (M^+-CN,-$2OCH_3$), 259m/z (M^+-Cl,-CN,-O,-OCH_3), 232m/z (M^+-Cl,-CN,-O,-CO,-OCH_3), 227m/z (M^+-Cl,-CN,-O,-$2OCH_3$), 202m/z (M^+-Cl,-CN,-O,-CO,-$2OCH_3$), 189m/z (M^+-CN,-O,-$2OCH_3$,-C_6H_3 redical), 165m/z (M^+-CN,-O,-$2OCH_3$,-C_6H_3 redical,-ethylene redical),154m/z (M^+-Cl,-CN,-O,-$2OCH_3$,-C_6H_3 redical or M^+-Cl,-O,-$2OCH_3$,-C_6H_3 redical,-ethylene redical) appears as base peak, 136m/z (M^+-CN,-CO,-O,-$2OCH_3$,-C_6H_3 redical,-ethylene redical)

FAB Mass Spectrum of 6-chloro-2-oxo-4-[(*E*)-2-(3,4,5-trimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile (KUS - 14)

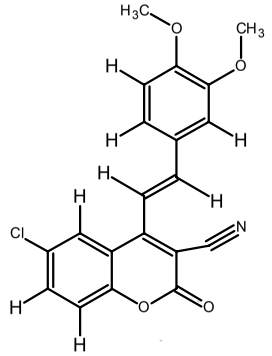


Mol. Weight = 397gm/mole

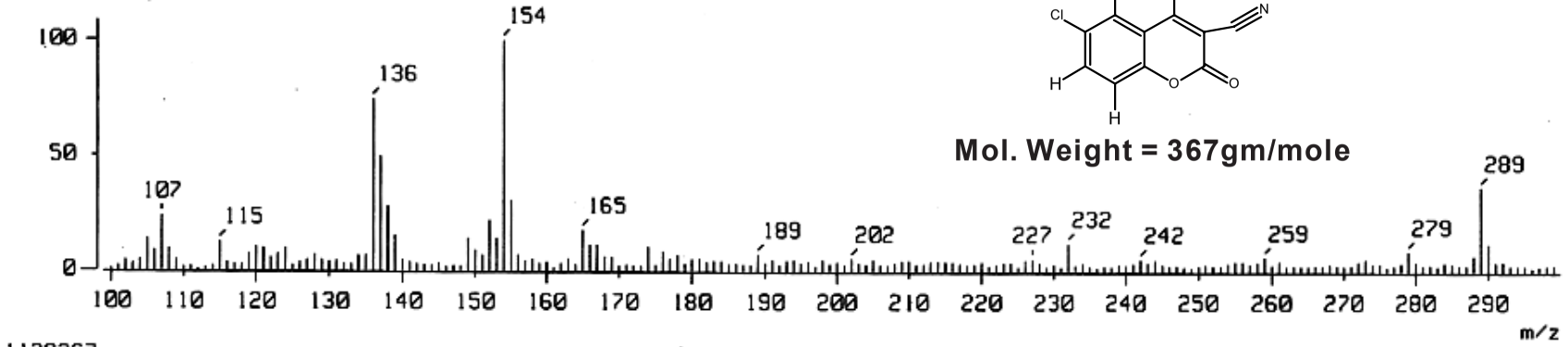


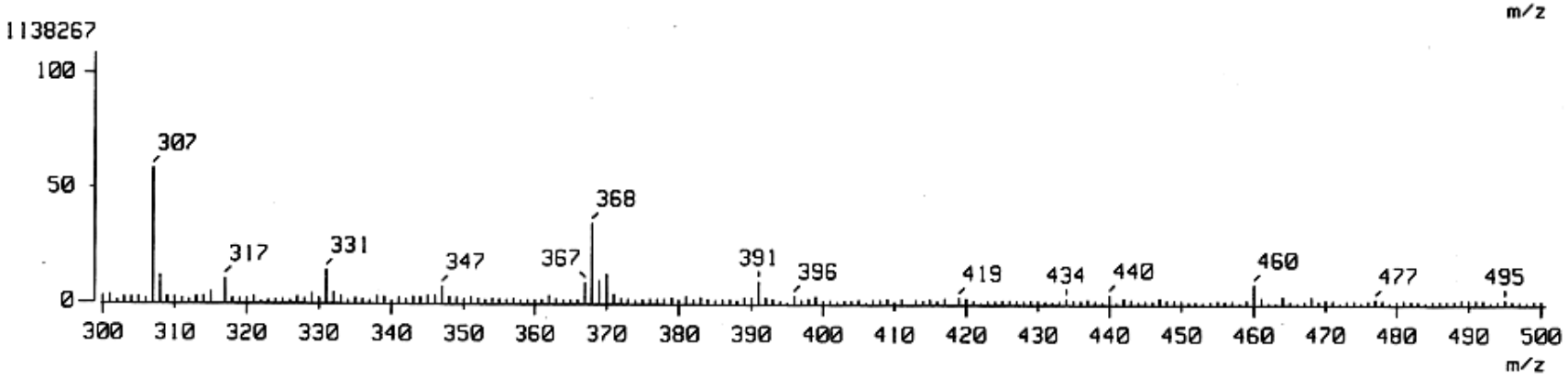


FAB Mass Spectrum of 6-chloro-2-oxo-4-[(*E*)-2-(3,4-dimethoxyphenyl)vinyl]-2*H*-chromene-3-carbonitrile (KUS - 13)



Mol. Weight = 367gm/mole





CHAPTER-6

Antitubercular and Antibacterial Study of Newly Synthesized Compounds

Introduction

The present chapter deals with the preliminary biological screening results of 120 compounds synthesized during the course of invention, which are heterocycles like triazolotriazinoindole, 1,5-benzothiazepine, pyrano[3,2-c]quinolone, 1,3,4-oxadiazol-2-yl-1H-pyrrole and 2-oxo-4-styryl-2H-chromene-3-carbonitrile.

In the current chapter, biological aspects of antitubercular and anti-bacterial screening were described along with the activity protocols and activity data obtained by preliminary screening *In vitro*.

Finally on the screening results a brief discussion is also narrated.

❖ Antitubercular Screening

The antitubercular screening of all the synthesized compounds of KUITT, KUT, KUQ & KUQP, KUPO and KUS series was carried out at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Alabama, USA.

Experimental protocols of *In vitro* Anti-*Mycobacterium tuberculosis* activity[1].

Level -1. Primary Screen.

The primary screen is conducted at 6.25 μg/ml(or molar euivalents of highest molecular weight compound in a series of congeners) against *Micobacterium tuberculosis* H_{37}Rv (ATCC 27294) in BACTET 12B medium using Microplate Almar Blue Assay (MABA)[1]. Compounds exhibiting fluorescence are tested in the BACTET 460-radiometric system[1]. Compounds effecting >99% inhibition in the primary screen (MIC < 6.25 μg/ml) are generally evaluated further.

Level-2.

Determination of Minimum Inhibitory Concentration (MIC)

Compounds demonstrating at least 90% inhibition in the primary screen are retested at lower concentration against *M. tuberculosis* H_{37}Rv to determinethe actual minimum inhibitory concentration(MIC) in the MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls like isoniazide(ATCC 35822), rifampin (ATCC 35838).

Determination of 50% Inhibitory Concentration (IC_{50})

Concurrent with the determination of MICs, compounds are tested for cytotoxicity(IC_{50}) in VERO cells at concentrations less than or equal to 62.5μg/ml or ten times the MIC for *M. tuberculosis* H_{37}Rv. After 72hours exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive cell proliferation assay.

Level-3

Determination of 90% and 99% Effective Concentration (EC_{90} & EC_{99})[2]

Compounds are tested for killing of *M. tuberculosis* Erdman(ATCC 35801) in monolayers of mouse bone marrow macrophages[2] (EC_{90} & EC_{99}; lowest concentration effecting a 90% and 99% reduction, respectively in colony forming units at seven days compared to drug free controls) at 4-fold concentrations equivalent to 0.25, 1, 4 and 16times the MIC. Compounds with EC_{90}>16×MIC are considered inactive in the model.

Determination of MIC against three strains of single drug resistant (SDR) *M. tuberculosis*

Concurrent with the testing of compounds in macrophages, MICs are determined in the MABA for three strains of drug-resistance *M. tuberculosis*(Each strain resistant to single TB drug). Typically all the compounds progressing to this stage of screening will be tested against *M. tuberculosis* strains resistant to isoniazide(ATCC 35822), rifampin (ATCC 35838), and one other drug resistant strain (the letter determined by the compound type) as well as the drug sensitive strains H_{37}Rv and Erdman. Generally MICs for SDR strains should not be greater than 10×MIC for non-resistant strains for compound evaluation to continue. However characteristics such as SAR, solubility and log P values are reviewed to determine to continue compound evaluation into *in vivo* testing.

Primary Screening Methodology

Antitubercular activity was determined using the *BACTEC 460* system as modified below. Stock solutions as test compounds were prepared in dimethylsulfoxide(DMSO) at 1 mg/ml and sterillized by passage through 0.22 um PFTE filters(Millex-FG, Millepore, Bedford MA). Fifty micro liters was added to 4ml radiometric 7H12 broth (BACTEC 12B; Bectron Dickinson Diagnostic Instrument system, Sparks, MD) to achieve a final concentration of 6.25μg/ml. Controls received 50 μl DMSO. Rifampin (Sigma Chemicals Co., St. Louis, MO) was included as a possitive drug control. Rifampin was solubilized and diluted in DMSO and adeed to *BACTEC-12* broth to achieve a range of concentration for concentration of minimum inhibitory concentration (MIC, lowest concentration inhibiting 99% inhibition of the inoculums).

M. Tuberculosis $H_{37}Rv$ (ACTT 27294; American type culture collection(Rockville, MD) was cultured at 37°C on a rotary shaker in middle brook 7H9 broth(Difco Laboratories, Detroiet, MI) supplemented with 0.2v/v glycerol and 0.05% v/v Tween 80 unit the culture turbidity achieved an optical density of 0.45-0.55 at 550nm. Bacteria were pellected centrifugation, washed twice and resuspended in one fifth of the origional volume in dulbecoo's phosphate buffered saline [PBS, Irvine Scientific, Santa Ana, (A)]. Large bacterial clumps were removed by passage through an 8μm filter (malgene, Rochester, NY) and aliquotes were frozen at -80°C. The cultures were showed and an appropriate dilution performed such that a *BACTEC-12B* broth containing the test compounds. An additional control vial was included which received a further 1:100 diluted inoculum (as well as 50ml DMSO) for use in calculating the MIC of the rifampicin, respectively by establishing procedures.

Cultures were incubated at 37°C and the Growth of Inhibition(GI) determined daily until control cultures acheived a GI of 999. Assays were usually completed in 5-8days. Percent inhibition was defined as 1-(GI of test sample/GI of control)×10. Minimum inhibitory concentration of compound effecting a reduction in daily change in GI, which was less than that observed with a 1:100 diluted control culture on day the later reached a GI of at least 30.

❖ Antibacterial Screening (Experimental protocols)

The antibacterial screening of all the synthesized compunds of present investigation was performed by two methods, described as under.

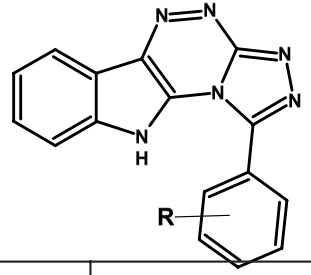
(Method - A) Measurment of Zone of Inhibition by Cup-plate method[3-5].

The screening assay included the nutrient agar broth prepared by usual method was inoculated especially 0.5ml for 24hrs. The bacterial cultures *E.coli / S.aureus* were taken in the seperate conical flask at 40-50^{o} and mixed well by gentle shaking. About 25ml of the content of the flask were poured and evenly spread in petridish(13cm in diameter) and 10mm bore in agar medium and filled with 0.05ml solution of sample in 10% dimethylsulfoxide(DMSO) in methanol. The plates were incubated at 37^{o}C for 24hrs and the control was also maintained with 10% DMSO in methanol in similar manner. The zone of inhibition of the bacterial growth were measured in mm diameter.

(Method - B) Measurment of Minimum Inhibitory Concentration by Agar dilution method[6].

The biological screening assay protocols discussed here are as per recommendation of NCCL, USA. Each compound was dissolved in dimethyl sulfoxide (DMSO, 20mg/ml).The required dilution are made to make the final concentration as 1000 μg/ml, 500μg/ml, 125μg/ml, 75μg/ml, 36μg/ml and 18μg/ml. The solvent control plate is inoculated with various bacterial cultures. The bacterial cultures are inoculated into Mullar Hilton broth and allowed to grow for 4hrs at 37^{o}C. For the standardization of terbidity of bacterial cultures Mac Forland solution is used to finalize the terbidities of cultures. The media used is Mullar Hilton agar medium. Negative control to check the successful growth of all cultures is used without adding solvent or compounds. The observations are made to check the visible growth of bacteria.

Table-1. Antitubercular and Antibacterial activity data of 1-substituted phenyl-10H-[1,2,4]tiazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole series (KUITT 1-7)



Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]		Antibacterial activity[6]					
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)		Minimum Inhibitory concentration (ug/ml)					
					E.Coli	*S.aureous*	*E.Coli*	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUITT-1	3,4-diOCH_3	Almar	>6.25	28	*	*	125	125	125	125	125	125
KUITT-2	4-OCH_3	Almar	>6.25	18	*	*	75	75	75	125	125	125
KUITT-3	4-SCH_3	Almar	>6.25	24	*	*	1000	125	125	1000	75	1000
KUITT-4	3-Br	Almar	>6.25	35	*	*	1000	500	1000	1000	500	1000
KUITT-5	4-F	Almar	>6.25	21	*	*	125	125	125	125	125	125
KUITT-6	2-Cl	Almar	>6.25	12	*	*	75	75	75	125	125	125
KUITT-7	4-Cl	Almar	>6.25	10	*	*	125	125	500	1000	1000	500

Note: * sign indicates full growth of microorganisms

Table-1. Antitubercular and Antibacterial activity data of 1-substituted phenyl-10H-[1,2,4]tiazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole series (KUITT 8-15)

Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]		Antibacterial activity[6]					
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)		Minimum Inhibitory concentration (ug/ml)					
					E.Coli	*S.aureous*	*E.Coli*	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUITT-8	3-NO_2	Almar	>6.25	15	*	*	250	250	250	250	250	1000
KUITT-9	4-NO_2	Almar	>6.25	18	*	*	250	250	250	250	250	250
KUITT-10	H	Almar	>6.25	32	*	*	500	500	75	75	500	1000
KUITT-11	2-NO_2	Almar	>6.25	23	*	*	1000	250	500	75	75	1000
KUITT-12	3-OC_6H_5	Almar	>6.25	19	*	*	75	500	500	125	500	250
KUITT-13	2, 5-diOCH_3	Almar	>6.25	16	*	*	-	75	1000	75	75	500
KUITT-14	3-OCH_3	Almar	>6.25	39	*	*	500	75	500	75	75	75
KUITT-15	2-OCH_3	Almar	>6.25	43	*	*	125	125	125	125	125	1000

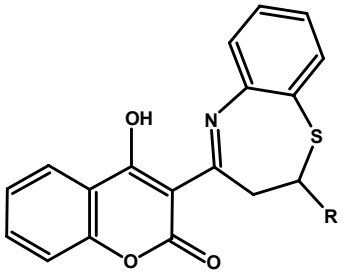
Note: - and * signs indicate full growth of microorganisms

Table-1. Antitubercular and Antibacterial activity data of 1-substituted phenyl-10H-[1,2,4]tiazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole series (KUITT 16-22)

Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]		Antibacterial activity[6]					
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)		Minimum Inhibitory concentration (ug/ml)					
					E.Coli	*S.aureous*	*E.Coli*	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUITT-16	3-Cl	Almar	>6.25	53	*	*	75	75	125	75	125	250
KUITT-17	2-OH	Almar	>6.25	26	*	*	75	75	75	75	125	500
KUITT-18	4-OH	Almar	>6.25	26	*	*	500	500	500	500	500	500
KUITT-19	3-OH	Almar	>6.25	27	*	*	75	500	75	75	75	500
KUITT-20	**3-OCH_3 -4-OH**	Almar	>6.25	42	*	*	75	75	**18**	75	**18**	75
KUITT-21	**4-CH_3**	Almar	>6.25	40	*	**12**	75	75	75	75	75	75
KUITT-22	9-Anthracyl	Almar	>6.25	62	*	*	75	75	75	75	75	75

Note: * sign indicates full growth of microorganisms.

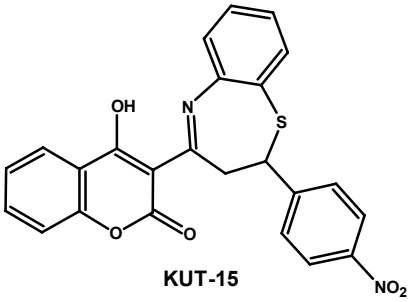
Table-2. Antitubercular and Antibacterial activity data of 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one series(KUT 1-24)



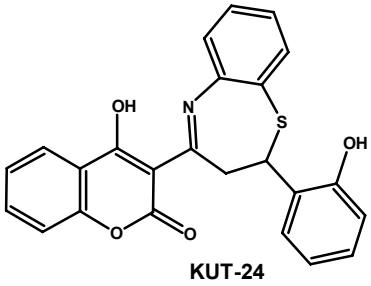
Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]	
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)	
					E.Coli	*S.aureous*
KUT-1	Phenyl	Almar	>6.25	-	*	*
KUT-2	2-NO_2 Phenyl	Almar	>6.25	4	*	*
KUT-3	3-NO_2 Phenyl	Almar	>6.25	2	*	*
KUT-4	2-OCH_3 Phenyl	Almar	>6.25	8	*	*
KUT-5	3-OCH_3 Phenyl	Almar	>6.25	3	*	*
KUT-6	4-OCH_3 Phenyl	Almar	>6.25	2	*	*
KUT-7	3-Br Phenyl	Almar	>6.25	-	*	*
KUT-8	2-Cl Phenyl	Almar	>6.25	60	*	*
KUT-9	3-Cl Phenyl	Almar	>6.25	8	*	*
KUT-10	4-Cl Phenyl	Almar	>6.25	13	*	*
KUT-11	2-ethenyl Phenyl	Almar	>6.25	-	*	*
KUT-12	**4-F Phenyl**	Almar	>6.25	**72**	*	*
KUT-13	4-N,N DiCH_3 Phenyl	Almar	>6.25	-	*	*
KUT-14	4-OH Phenyl	Almar	>6.25	32	*	*
KUT-15	**4-NO_2 Phenyl**	Almar	>6.25	**93**	*	*
KUT-16	4-SCH_3 Phenyl	Almar	>6.25	-	*	*
KUT-17	3,4-DiOCH_3 Phenyl	Almar	>6.25	-	*	*
KUT-18	**3,4,5-TriOCH_3 Phenyl**	Almar	>6.25	9	**12**	*
KUT-20	3-OH Phenyl	Almar	>6.25	3	*	*
KUT-21	**3-Indolyl**	Almar	>6.25	**80**	*	*
KUT-22	**9-Anthracyl**	Almar	>6.25	-	*	**24**
KUT-24	**2-OH**	Almar	<6.24	**94**	*	*

Note: - and * signs indicate full growth of microorganisms.

Table-3. Antitubercular screening Level-2 data of 4-hydroxy-3-(2-(4-nitrophenyl)-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one (KUT-15) and 4-hydroxy-3-(2-(2-hydroxy phenyl)-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one (KUT-24)



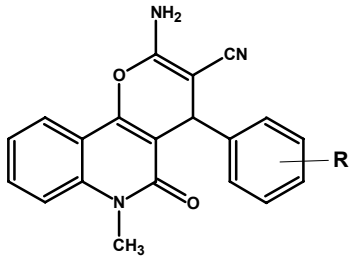
KUT-15



KUT-24

Compd. Code	Antitubercular activity				
	Assay	MIC (ug/ml)	% Inh.	IC_{50} (ug/ml)	Comment
KUT-15	Almar	>6.25	93	>10	High MIC
KUT-24	Almar	>6.25	94	3.63	High MIC

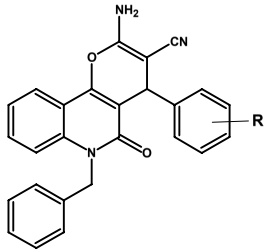
Table-4. Antitubercular and Antibacterial activity data of 2-amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitrile series (KUQ 1-26)



Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]	
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)	
					E.Coli	*S.aureous*
KUQ-1	H	Almar	>6.25	*	*	*
KUQ-2	2-Cl	Almar	>6.25	2	*	*
KUQ-3	3-Cl	Almar	>6.25	9	*	*
KUQ-4	4-Cl	Almar	>6.25	13	*	*
KUQ-5	2-NO_2	Almar	>6.25	7	*	*
KUQ-6	3-NO_2	Almar	>6.25	10	*	*
KUQ-7	4-NO_2	Almar	>6.25	-	*	*
KUQ-8	4-F	Almar	>6.25	-	*	*
KUQ-9	3-Br	Almar	>6.25	-	*	*
KUQ-10	3-OC_6H_5	Almar	>6.25	21	*	*
KUQ-11	4-SCH_3	Almar	>6.25	8	*	*
KUQ-13	3-OH	Almar	>6.25	-	*	*
KUQ-14	4-OH	Almar	>6.25	-	*	*
KUQ-15	4-N,N-diCH_3	Almar	>6.25	18	*	*
KUQ-16	2-OCH_3	Almar	>6.25	42	*	*
KUQ-17	4-OCH_3	Almar	>6.25	9	*	*
KUQ-18	3,4,5-triOCH_3	Almar	>6.25	13	*	*
KUQ-19	3,4-diOCH_3	Almar	>6.25	-	*	*
KUQ-21	9-Anthracyl	Almar	>6.25	-	*	*
KUQ-24	3-OC_2H_5 -4-OH	Almar	>6.25	-	*	*
KUQ-25	3-Indolyl	Almar	>6.25	21	*	*
KUQ-26	3-OCH_3	Almar	>6.25	14	*	*

Note: - and * signs indicate full growth of microorganisms.

Table-5. Antibacterial activity data of 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbo nitrile series (KUQP 1-7)

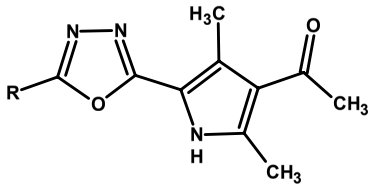


Compd. Code	Substitution (R)	Antibacterial activity[6]					
		Minimum Inhibitory concentration (ug/ml)					
		E.Coli	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUQP-1	4-OCH_3	500	500	500	500	500	500
KUQP-2	4-F	250	250	500	500	500	500
KUQP-3	4-Cl	500	500	500	500	500	500
KUQP-4	4-NO_2	500	500	500	125	500	500
KUQP-5	3,4-diOCH_3	125	125	125	125	125	125
KUQP-6	3,4,5-triOCH_3	500	250	500	250	250	250
KUQP-7	2,5-diOCH3	500	500	500	500	500	500

Table-5. Antibacterial activity data of 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbo nitrile series (KUQP 8-16)

Compd. Code	Substitution (R)	Antibacterial activity[6]					
		Minimum Inhibitory concentration (ug/ml)					
		E.Coli	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUQP-8	3-NO_2	250	500	500	500	250	500
KUQP-9	2-NO_2	250	250	250	250	250	250
KUQP-10	2-OH	250	250	250	250	250	250
KUQP-11	3,4-diOH	500	500	250	500	500	500
KUQP-12	2-OCH_3	75	75	500	500	500	500
KUQP-13	3-OCH_3	125	125	125	125	125	125
KUQP-14	3-Br	125	250	250	250	250	250
KUQP-15	4-CH_3	125	125	125	125	125	125
KUQP-16	4-SCH_3	250	250	250	250	250	250

Table-6. Antitubercular and Antibacterial activity data of 1-{2,4-dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1H-pyrrol-3-yl}ethanone series (KUPO 1-7)



Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]		Antibacterial activity[6]					
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)		Minimum Inhibitory concentration (ug/ml)					
					E.Coli	*S.aureous*	*E.Coli*	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUPO-1	phenyl	Almar	>6.25	56	*	*	250	125	125	125	250	250
KUPO-2	2-Cl phenyl	Almar	>6.25	36	*	*	500	500	500	500	500	500
KUPO-3	**4-Cl phenyl**	Almar	>6.25	**88**	*	*	**18**	**18**	250	250	250	250
KUPO-4	4-NO_2 phenyl	Almar	>6.25	69	*	*	500	500	500	500	500	500
KUPO-5	2-OH phenyl	Almar	>6.25	29	*	*	500	500	125	125	500	500
KUPO-7	**4-OCH_3 phenyl**	Almar	>6.25	**70**	*	*	**37.5**	**37.5**	**37.5**	**37.5**	**37.5**	**37.5**

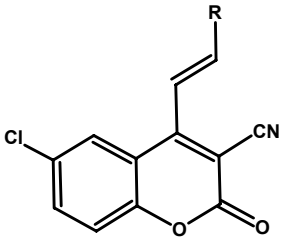
Note: * sign indicates full growth of microorganisms.

Table-6. Antitubercular and Antibacterial activity data of 1-{2,4-dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1H-pyrrol-3-yl}ethanone series (KUPO 9-16)

Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]		Antibacterial activity[6]					
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)		Minimum Inhibitory concentration (um/ml)					
					E.Coli	*S.aureous*	*E.Coli*	*Enterobacter sp.*	*P. aeruginosa*	*S.aureous*	*K. pneumoniae*	*B.subtilis*
KUPO-9	3-NO_2 phenyl	Almar	>6.25	66	*	*	250	250	125	125	125	250
KUPO-10	**2-CH_3 phenyl**	Almar	>6.25	54	*	*	**18**	**37**	500	500	500	500
KUPO-11	**4-CH_3 phenyl**	Almar	>6.25	**84**	*	*	125	125	75	75	75	125
KUPO-12	**2-$OHC_{10}H_6$**	Almar	>6.25	8	*	**18**	**18**	**18**	125	125	125	125
KUPO-13	**2-Indolyl**	Almar	>6.25	**81**	*	**12**	**18**	250	250	250	250	250
KUPO-14	**2-$OCOCH_3$ phenyl**	Almar	>6.25	38	*	**18**	75	75	75	75	75	75
KUPO-15	**4-Br phenyl**	Almar	>6.25	**83**	*	*	125	125	75	75	75	125
KUPO-16	**2-NHC_6H_5 phenyl**	Almar	>6.25	**70**	*	**18**	75	75	75	75	75	125

Note: * sign indicates full growth of microorganisms.

Table-7. Antitubercular and Antibacterial activity data of 6-chloro-2-oxo-4-[(E)-2-substituted phenyl vinyl]-2H-chromene-3-carbonitrile series (KUS 1-22)



Compd. Code	Substitution (R)	Antitubercular activity			Antibacterial activity[3-5]	
		Assay	MIC (ug/ml)	% Inh.	Zone of Inhibition (10 ug/ml)	
					E.Coli	*S.aureous*
KUS-1	Phenyl	Almar	>6.25	55	*	*
KUS-2	4-F Phenyl	Almar	>6.25	-	*	*
KUS-3	2-Cl Phenyl	Almar	>6.25	-	*	*
KUS-4	3-Cl Phenyl	Almar	>6.25	69	*	*
KUS-5	4-Cl Phenyl	Almar	>6.25	-	*	*
KUS-6	3-Br Phenyl	Almar	>6.25	23	*	*
KUS-7	**2-NO2 Phenyl**	Almar	>6.25	11	*	**12**
KUS-8	3-NO2 Phenyl	Almar	>6.25	69	*	*
KUS-9	**3-OH Phenyl**	Almar	>6.25	-	*	**12**
KUS-10	**4-OH Phenyl**	Almar	>6.25	-	*	**12**
KUS-11	2-OCH3 Phenyl	Almar	>6.25	24	*	*
KUS-12	4-OCH3 Phenyl	Almar	>6.25	33	*	*
KUS-13	3,4-DiOCH3 Phenyl	Almar	>6.25	63	*	*
KUS-14	**3,4,5-TriOCH3 Phenyl**	Almar	>6.25	**87**	*	*
KUS-15	**3-OC6H5 Phenyl**	Almar	>6.25	**72**	*	*
KUS-16	4-SCH3 Phenyl	Almar	>6.25	-	*	*
KUS-17	2-ethenyl Phenyl	Almar	>6.25	-	*	*
KUS-18	**9-Anthracyl**	Almar	>6.25	**81**	*	*
KUS-19	2-furyl	Almar	>6.25	63	*	*
KUS-20	**3-Indolyl**	Almar	>6.25	-	**12**	**12**
KUS-21	**4-N,N,Dimethyl Phenyl**	Almar	>6.25	**79**	*	*
KUS-22	**3-Mehoxy Phenyl**	Almar	>6.25	31	*	**12**

Note: - and * signs indicate full growth of microorganisms

Results and Discussion

Looking to the biological screening results (as per shown in Table 1-7) of antitubercular and antibacterial activity of all the synthesized compounds of the current investigation leads to the following conclusion.

Compounds of 1-substituted phenyl-10H-[1,2,4]tiazolo[3',4':3,4][1,2,4] triazino[5,6-b]indole (KUITT 1-22) series possess moderate to weak anti-tubercular activity(Table-1).

The screening results revealed that KUITT-22 (R = 9-anthracyl) was shown maximum inhibition(62%), while that of KUITT (R = 3-chloro) was 53% at MIC value >6.25μg/ml.

The antibacterial activity profile of the series(Table-1) revealed that KUITT-2(R = 4-methoxy), KUITT-6(R = 2-chloro), KUITT-12(R = 3-phenoxy), KUITT-16 (R = 3-chloro), KUITT-17(R = 2-hydroxy), KUITT-19(R = 3-hydroxy), KUITT-21(R = 4-methyl) and KUITT-22(R = 9-anthracyl) have shown to inhibit the growth of *E.coli*. at MIC value of 75μg/ml, while KUITT-2(R = 4-methoxy), KUITT-6(R = 2-chloro), KUITT-13(R = 2,5-dimethoxy), KUITT-14(R = 3-methoxy), KUITT-16 (R = 3-chloro), KUITT-17(R = 2-hydroxy), , KUITT-21(R = 4-methyl) and KUITT-22(R = 9-anthracyl) were active against *Enterobacter sp.* upto 75μg/ml. Candidates of the series KUITT-2(R = 4-methoxy), KUITT-6(R = 2-chloro), KUITT-10(R = H), KUITT-17(R = 2-hydroxy), KUITT-19(R = 3-hydroxy), KUITT-21(R = 4-methyl) and KUITT-22(R = 9-anthracyl) were inhibited growth of *P.aeruginosa* at MIC value of 75μg/ml, on the other hand KUITT-10(R = H), KUITT-11(R = 2-nitro), KUITT-13(R = 2,5-dimethoxy), KUITT-14(R = 3-methoxy), KUITT-16 (R = 3-chloro), KUITT-17(R = 2-hydroxy), KUITT-19(R = 3-hydroxy), KUITT-21(R = 4-methyl) and KUITT-22(R = 9-anthracyl) were active against *S.auereous* at the minimum concentration level of 75μg/ml.

In addition to this **KUITT-21((R = 4-methyl)** was found to control growth of ***S.aureus*** upto **12mm** diameter at **10μg/ml** concentration.

Compounds KUITT-3(4-thiomethyl), KUITT-11(R = 2-nitro), KUITT-13(R = 2,5-dimethoxy), KUITT-14(R = 3-methoxy), KUITT-21(R = 4-methyl) and

KUITT-22(R = 9-anthracyl) were inhibit the growth of *K.pneumoniae* at 75μg/ml and KUITT-14(R = 3-methoxy), KUITT-21(R = methyl) and KUITT-22(R = 9-anthracyl) were active against *B.subtilis* upto 75μg/ml.

Over and above to this **KUITT-20(R = 3-methoxy,4-hydroxy)** was found to be **most potent** antibacterial candidate of the series as it is effective to inhibit the growth inhibition of all the gram possitive and gram negative bacterial species used in screening viz., *E.coli, Enterobacter sp., P. aeruginosa, S. aureus, K. pneumoniae* and *B.subtilis* at minimum inhibitory concentration level of 75μg/ml, more over it inhibits growth of ***P. aeruginosa*** and ***K.pneumoniae*** at lowest minimum inhibitory concentration level of **18μg/ml**.

In case of 4-hydroxy-3-(2-substituted phenyl-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one series(KUT 1-24), two candidates viz.**4-hydroxy-3-(2-(4-nitrophenyl)-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one (KUT-15)** and **4-hydroxy-3-(2-(2-hydroxy phenyl)-2,3-dihydro-1,5-benzothiazipine-4-yl)-2H-chromen-2-one (KUT-24)** were found to be most potent antitubercular candidates of the present invention **(Table-2 and 3)**. In primary screening both **KUT-15(R = 4-nitro)** and **KUT-24(R = 2-hydroxy)** have shown **93%** and **94%** inhibition of ***Mycobacterium tuberculosis H_{37}Rv(TTCC 27294)***at **MIC** value of **>6.25μg/ml and <6.24μg/ml** respectively in BACTET 12B medium using the Microplate Almar Blue Assay (MABA)[1].

Hence, KUT-15(R = 4-nitro) and KUT-24(R = hydroxy) were further evaluated for determination of its actual minimum inhibitory concentration (MIC) and determination of 50% inhibitory concentration(IC_{50})(Table-3). Unfortunately IC_{50} value of both the compounds in Level-2 screening were determined to be >10μg/ml and 3.63μg/ml respectively, which are to low values to evaluate them in further screening for the determination of their effective concentrations on *M. tuberculosis*.

The anti bacterial profile of the series **(Table-2)** reveals that **KUT-18(R = 3,4,5-trimethoxy phenyl)** was found to control growth of ***E.coli*** upto **12mm**

diameter while that of ***S.aureous*** is found to be **24mm** by **KUT-22 (R = 9-anthracyl)** at **10μg/ml** concentration.

The entire series of 2-amino-6-methyl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbonitriles was neither active against *M.tuberculosis* nor showed antibaterial activity(Table-4). On the other hand one candidate of 2-amino-6-benzyl-5-oxo-4-substituted phenyl-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbo nitrile series (KUQP 1-16)(Table-5) viz. 2-amino-6-benzyl-5-oxo-4-(2-methoxy phenyl)-5,6-dihydro-4H-pyrano[3,2-c]quinoline-3-carbo nitrile(KUQP-12) was found to inhibit the growth of *E.coli* and *Enterobacter sp.* at minimum inhibitory concentration level of 75μg/ml.

There are number of compounds of 1-{2,4-dimethyl-5-[(5-substituted phenyl-1,3,4-oxadiazol-2-yl)carbonyl]-1H-pyrrol-3-yl}ethanone series (KUPO 1-16) were exhibited moderate antitubercular activity(Table-6).

The primary screening results obtained from Microplate Almar Blue Assay (MABA)[1], suggested that **KUPO-3(R = 4-chloro phenyl)** to be most potent candidate of the series as it shows **88%** inhibition of the growth of ***M.tuberculosis*** $H_{37}Rv$, while **KUPO-11(R = 4-methyl phenyl), KUPO-15(R = 4-bromo phenyl), KUO-13(R = 2-indolyl)** shows **84%,83%,81%** inhibition respectively while **KUO-7(R = 4-OCH_3 phenyl)** and **KUO-16 (R = 2-N-phenylamino phenyl)** shows **70%** inhibition at MIC value > 6.25μg/ml.

The antibacterial activity profile of the series(Table-6) reveals that **KUPO-3(R = 4-chloro phenyl), KUPO-10(R = 2-methylphenyl), KUPO-12 (R = 2-hydroxy naphthyl), KUPO-13 (R = 2-Indolyl)** are the **most active** antimicrobial candidates of the series as **KUPO-3(R = 4-chloro phenyl)** and **KUPO-12 (R = 2-hydroxy naphthyl)** inhibits growth of ***E.coli*** and ***Enterobacter sp.*** while **KUPO-10(R = 2-methyl phenyl)** and **KUPO-13 (R = 2-Indolyl)** inhibits growth of ***E.coli***, at minimum inhibitory concentration value of **18μg/ml**. Where as **KUPO-7(R = 4-methoxy phenyl)** inhibits growth of all bacterial species of the screening and **KUO-10(R = 2-methyl phenyl)** inhibits growth of ***Enterobacter sp.*** at MIC value of **37.5μg/ml**.

However KUPO-3(R = 4-chloro phenyl), KUPO-11(R = 4-methyl phenyl), KUPO-14(R = 2-acetoxy phenyl), KUPO-15(R = 4-bromo phenyl) and KUO-16 (R = 2-N-phenylamino phenyl) inhibits growth of P. aeruginosa, KUPO-14(R = 2-acetoxy phenyl) and KUO-16 (R = 2-N-phenylamino phenyl) inhibit growth of both *E.coli* and *Enterobacter sp*., KUPO-11(R = 4-methyl phenyl), KUPO-14(R = 2-acetoxy phenyl), KUPO-15(R = 4-bromo phenyl) and KUO-16 (R = 2-N-phenylamino phenyl) inhibits growth of both *S.aureous* and *K. pneumoniae* and KUPO-14(R = 2-acetoxy phenyl) inhibits growth of *B.subtilis* at minimum inhibitory concentration level of 75µg/ml.

On the other hand **KUPO-12 (R = 2-hydroxy naphthyl)**, **KUPO-13 (R = 2-Indolyl)**, **KUPO-14(R = 2-acetoxy phenyl)** and **KUO-16 (R = 2-N-phenylamino phenyl)** were found to control growth of ***S.aureous*** upto **18, 12, 18** and **18** mm diameter respectively at **10µg/ml** concentration.

The series of 6-chloro-2-oxo-4-[(E)-2-substituted phenyl vinyl]-2H-chromene-3-carbonitrile series (KUS 1-22) shows moderate activity profile(Table-7) of antitubercular activity against ***M.tuberculosis $H_{37}Rv$***. **KUS-14(R = 3,4,5-trimethoxy phenyl)** is considered to be most potent candidate as it shows highest inhibition of **87%**, subsequently **KUS-18(R = 9-anthracyl)**, **KUS-21(R = 4-N,N-dimethyl phenyl)** and **KUS-15(R = 3-phenoxy)** shows **81%**, **79%** and **72%** inhibition respectively.

The antibacterial activity screening (Table-7) reveals that **KUS-7(R = 2-nitro phenyl)**, **KUS-9(3-hydroxy phenyl)**, **KUS-10(4-hydroxy phenyl)**, **KUS(3-methoxy phenyl)** control the growth of ***S.aureous***, **KUS-20(3-Indolyl)** control growth of both ***E.coli*** and ***S.aureous*** upto **12 mm** diameter all at **10µg/ml** concentration.

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List of Paper Presentation at National & International Conferences:

1. 9th National Conference on "Bioactive Heterocycles & Drug Discovery Paradigm" including One Day International Symposium on 'Recent Trends in Drugs Discovery'
   (a) Novel Synthesis & Biological Evaluation of Substituted 4-Hydroxy-3-(2-Substituted Phenyl-2,3-Dihydro-1H-1,5-Benzodiazepine-4-yl)-2H-Chromen-2-ones
   Kuldip Upadhyay, Kena Raval, Gautam Patel & Denish Karia
   (b) Synthesis & x-Ray Crystallographic Studies of Biologically Active asymmetric 1,4-Dihydro Pyridines
   Priti Adlekha, Kuldip Upadhyay, Harsukh Gaveriya & Anamik Shah

2. XVIII Gujarat Science Congress
   Microwave assisted Synthesis & X-Ray Crystallographic Study of ethyl 2-oxo-2*H*-chromene-3-carboxylate

3. 10th International Conference of ISCB on 'Drug Discovery: Perspectives and Challenges(Including Symposium on infectious diseases) and International Satellite Symposium on Medicinal Plants and Functional Foods in the Management of the Diabetes, Obesity and Cardiovascular Diseases'
   Synthesis of Substituted 4H-1,4-Benzothiazines
   Kuldip Upadhyay, Ravi Chaniyara, Bharat Savaliya, Hardevsinh Vala, Bhavin Marvaniya and Anamik Shah

Conferences / Workshops Attended:

1. National Workshop on Spectroscopy & Theoretical Chemistry organized by Department of Chemistry, The Maharaja Sayajirao University of Baraoda, Vadodara during 8-12th February 2005
2. 7th National Conference of Indian Society of Chemists & Biologists organized by Central Drug Research Institute, Lucknow during 26-28th February, 2003.
3. 9th National Conference on "Bioactive Heterocycles & Drug Discovery Paradigm" including One Day International Symposium on 'Recent Trends in Drugs Discovery' jointly Organized by Department of Chemistry, Saurashtra University, Rajkot & Central Drug Research Institute, Lucknow during 8-10th January, 2005.
4. XVIII Gujarat Science Congress Organized by Gujarat Science Academy at 13th March, 2004.
5. National Workshop on "Nanotechnology: Opportunities & Challenges" jointly organized by Saurashtra University, Rajkot and Gujarat Council of Science & Technology (GUJCOST), Gandhinagar on 17th Oct' 2005.
6. International Conference on Building Bridges, Forging Bonds for 21st Century Organic Chemistry and Chemical Biology (ACS-CSIR OCCB 2006) jointly Organized by American Chemical Society and Council of Scientific & Industrial Research, New Delhi at National Chemical Laboratory, Pune on 6 – 9 Jan' 2006.
7. UGC Sponsored One Day National Level Seminar on "Bioinformatics – Emerging Trends" organized by Shri M. & N. Virani Science College, Rajkot on 1st Jan' 2006.
8. DST Sponsored National Workshop on "Green Chemistry" Organized by Institute of Pharmacy and Faculty of Science, Nirma University of Science & Technology, Ahmedabad on 25-26 Nov' 2005.
9. 10th International Conference of ISCB on Drug Discovery: Perspectives and Challenges(Including Symposium on infectious diseases) and International Satellite Symposium on Medicinal Plants and Functional Foods in the Management of the Diabetes, Obesity and Cardiovascular Diseases, jointly organized by Indian Society of Chemists(India) & Biologists and University of Toronto(Canada) at Central Drug Research Institute, Lucknow on 24-26th Feb'-2006.