STUDIES ON BIOACTIVE HETEROCYCLES

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IN

CHEMISTRY

BY

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UNDER THE GUIDANCE OF

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

The work included in the thesis is done by me under the supervision of Dr. Yogesh T. Naliapara and the contribution made thereof is my own work.

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Place:Rajkot **Mahesh M. Savant**

Certificate

This is to certify that the present work "**Studies on Bioactive Heterocycles**" submitted for the Ph. D. Degree Chemistry of Saurashtra University, Rajkot, Gujarat, India by Mr. Mahesh M. Savant has been the result of work carried out under my supervision and is a significant contribution in the field of synthetic organic chemistry and medicinal chemistry.

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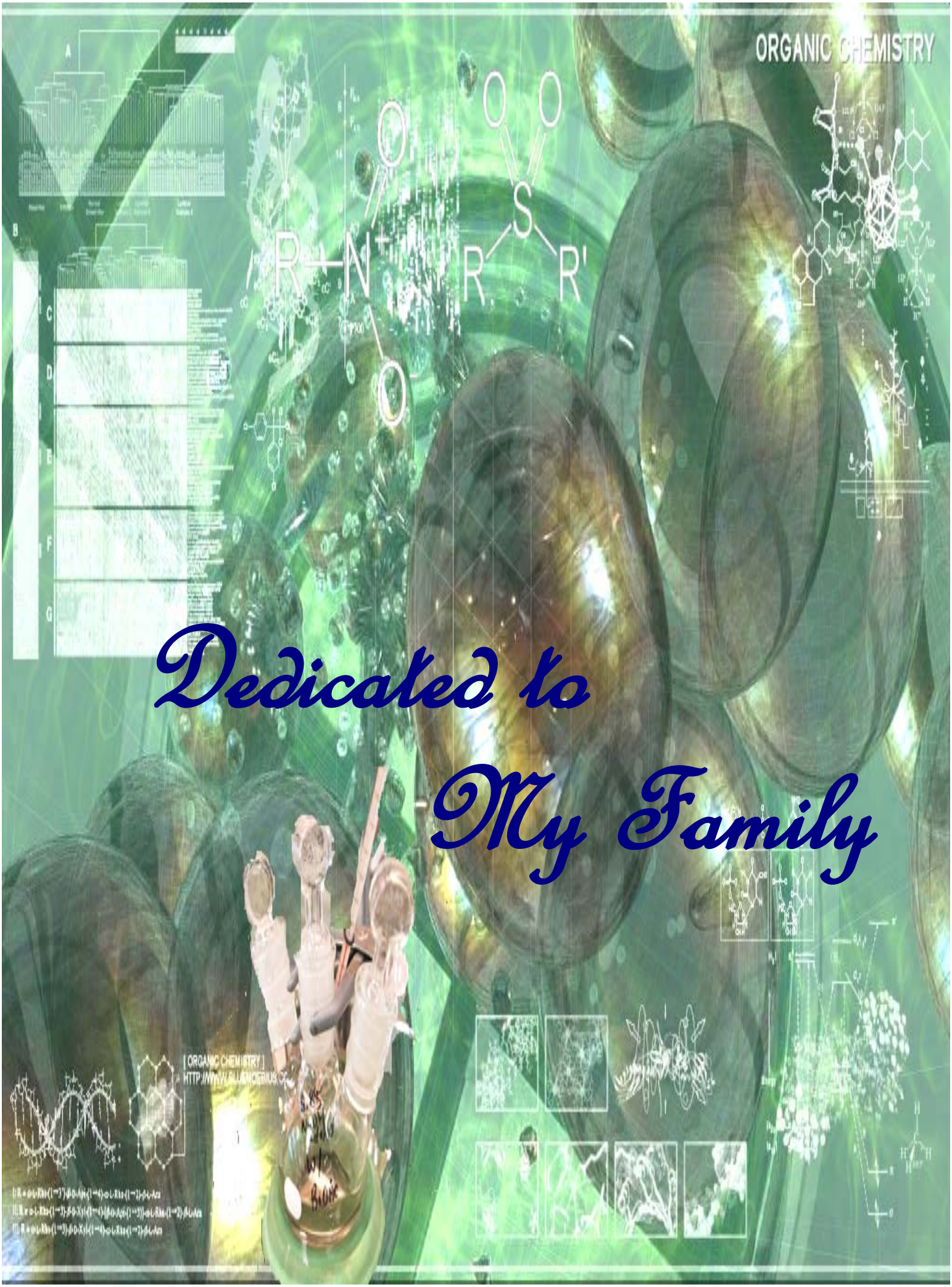
ORGANIC CHEMISTRY
Dedicated to
My Family

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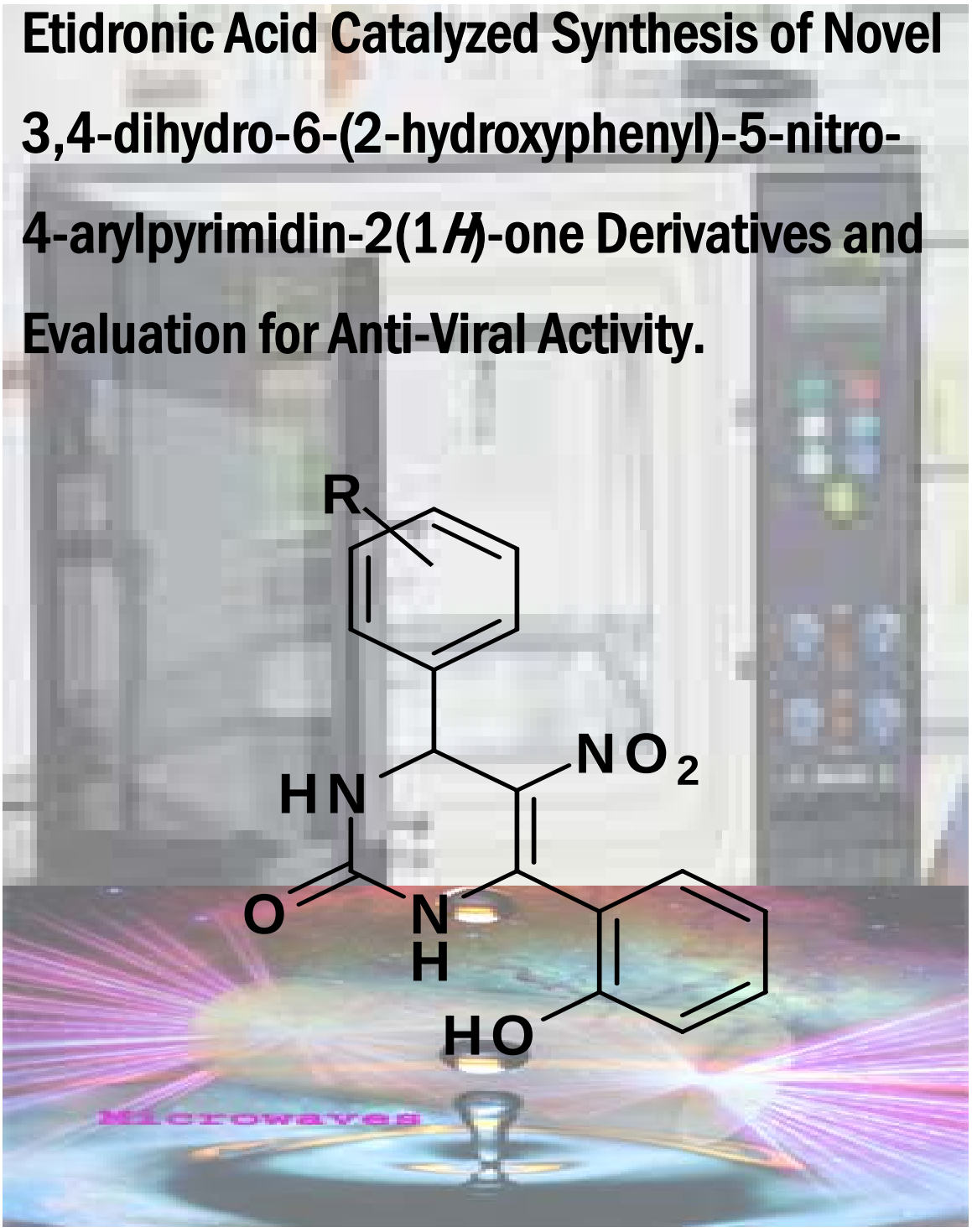
Abbreviations

2HNA	2-hydrxoy-ω-nitroacetophenone
AIDS	Aquired immune deficiency syndrome
αAKDTA	α-acyl ketene dithioacetal
CS_2	Carbon disulfide
DMSO	Dimethyl sulfoxide
DMS	Dimethyl sulfate
DHP	Dihydropyridine
DHPM	Dihydropyrimidine
EDA	Etidronic acid
HIV	Human immunodeficiency virus
MDC	Methylene dichloride
*m*CPBA	*meta*-chloro perbenzoic acid
SPC	Sodium percarbonate
SPB	Sodium perborate
THF	Tetrahydrofuran
TMS	Trimethyl silane
UPLC	Ultra performance liquid chromatography
*i*PA	*iso*-propyl alcohol
h	hour (time)
min	minute (time)
rt	room temperature
mp	melting point

CHAPTER 1

Etidronic Acid Catalyzed Synthesis of Novel 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-arylpyrimidin-2(1*H*)-one Derivatives and Evaluation for Anti-Viral Activity.



1.1 INTRODUCTION

The pyrimidine fragment is present in the molecules of a series of biologically active compounds, many of which have found use in medical practice (soporific, anti-inflammatory, antitumor, and other products).[1,2] In this connection, great attention has recently been paid to derivatives of pyrimidine, including their hydrogenation products. The first investigations into the synthesis of such compounds appeared more than a hundred years ago (e.g., the Biginelli reaction),[3] and for a long time they remained unused. Only in the last decade have methods been developed specifically for the production of hydrogenated pyrimidine systems and their physicochemical properties been studied. This is explained by the high reactivity and wide range of biological activity with these scaffolds. Thus, for example, 2-substituted 5-alkoxycarbonyl-4-aryl-l,4-dihydropyrimidines, which are structural analogs of hantzsch esters, are modulators of the transport of calcium through membranes.[4-7] Many hydrogenated pyrimidines exhibit antimicrobial,[8] hypoglemic,[9] herbicidal,[10] and pesticidal[11] activity. Publications devoted to these problems have been summarized in a number of reviews.[9-14]

Of great interest among the investigated compounds are the nitro-substituted dihydropyrimidines. They readily undergo various chemical transformations, among which the unique ability to undergo recyclization to heterocyclic and carbocyclic compounds should be noted in particular. The interest in the nitrodihydropyrimidines is also due to the fact that these compounds represent the active principle or act as metabolites responsible for the physiological action of nitropyrimidines. Recently, products having antimicrobial,[15] antiviral[16-18] activity and also products suitable for the treatment of cardiovascular diseases[19-22] have been found among them.

5-Nitrodihydropyrimidines can be described by five structures, having one (1,4-, 1,6-, and 1,2-) or two (2,5- and 4,5-dihydropyrimidine systems) geminal centers, the carbon atoms of which are characterized by sp^3 hybridization (**Figure 1**).

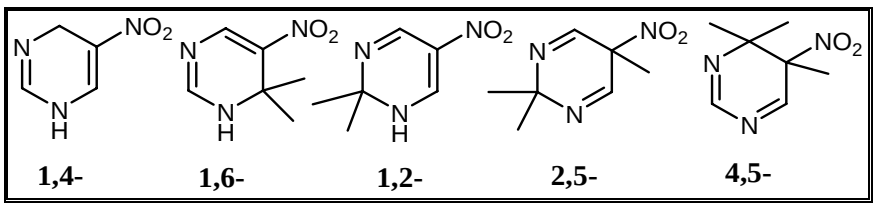


Figure 1

The 5-nitro-l,2-, 5-nitro-l,4-, and 5-nitro-l,6-dihydropyrimidines are cyclic enamines, in which the electron pair of the sp^3-hybridized nitrogen atom is in conjugation with the four π

electrons of the C=C and C=N double bonds. On account of the mobility of the hydrogen atom of the *NH* group, the 5-nitro-1,4- and 5-nitro-1,6-dihydropyrimidines can be in tautomeric equilibrium. At the same time the 5-nitro-2,5- and 5-nitro-4,5-dihydropyrimidines are cyclic imines, in which there is no conjugation.[23]

1.2. Methods for the preparation of nitrodihydropyrimidines

The methods for the production of dihydropyrimidines described in the literature can be divided into two main groups such as: synthesis from acyclic compounds and transformations based on pyrimidine derivatives (**Figure 2**). Analysis of the published data makes it possible to conclude that the first methods have advantages over the other. However, the second methods are used more widely if an electron withdrawing group, and particularly a nitro group, is introduced into the pyrimidine molecule. (One of the acyclic compounds contains a nitro group.)

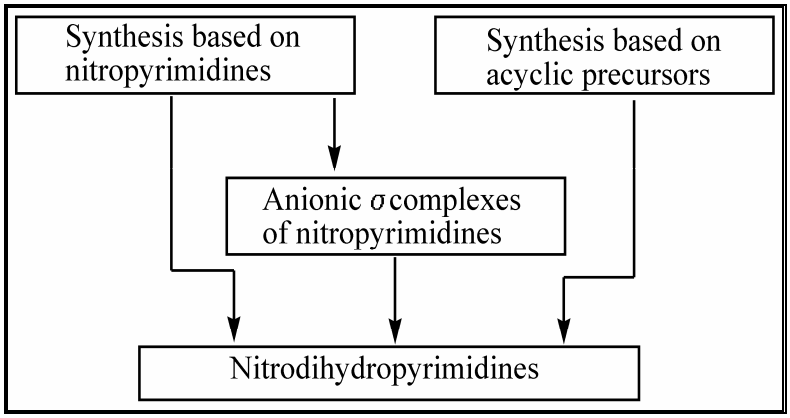


Figure 2

❖ **Synthesis from acyclic compounds**

Only derivatives of 5-nitro-l,4-dihydropyrimidine have been obtained from acyclic compounds (**Figure 3**). Three-component (**A**) and two-component (**B**, **C**) versions of cyclocondensation, based on the Biginelli reaction,[17,24-26] and also intramolecular cyclization of the already prepared six-membered chain (version **D**) have been used for this. Nitro ketones (versions **A**, **C**) and 1-arylidene-l-nitropropan-2-ones (version **B**) are used as nitro components in these reactions and derivatives of urea, arninopyrazole and amidines are used as N-C-N fragments.

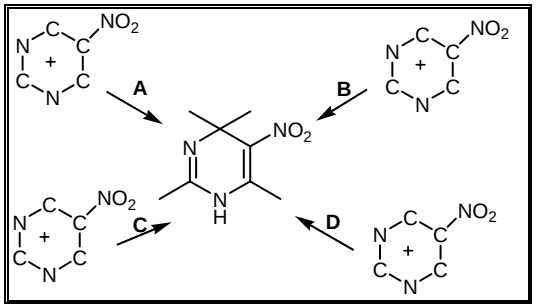


Figure 3

The proposed methods of cyclocondensation are interrelated. As a rule, realization of the reactions by one of the methods leads not to the final product but to an intermediate compound, which is in turn the starting compound for another method of cyclization.

The formation of 5-nitro-4,6-diphenyl-l,4-dihydropyrimidin-2(1*H*)-one (**Figure 4**) from benzylidenebisurea and *α*-nitroacetophenone was first described in 1972.[27] According to the mechanism of the Biginelli reaction,[26] at the first stage the urea fragment is clearly substituted by the nitroketone residue, and this is followed by cyclization of the six-membered intermediate in the acidic medium.

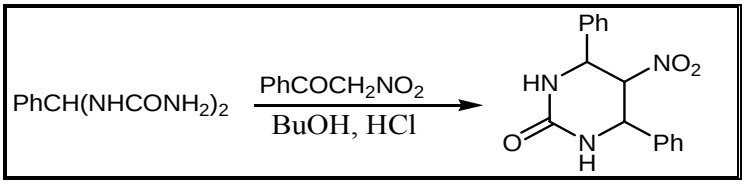


Figure 4

In the reaction of aromatic aldehydes with nitro acetone and a two-fold excess of urea or *N*-methyl urea in boiling ethanol in the presence of HCI 4-aryl-6-methyl- or 4-aryl-l,6-dimethyl-5-nitro-l,4-dihydropyrimidin-2(1*H*)-ones were obtained (**Figure 5**).[23,28] The latter are also formed as a result of the two-component cyclization of the respective 1-arylidene-l-nitropropan-2-ones with urea or *N*-methyl urea.

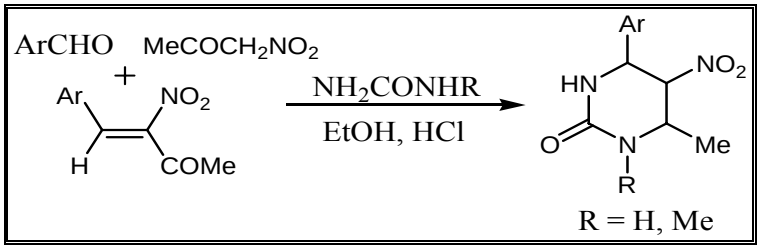


Figure 5

By analysis of the spectral characteristics it is possible to assign compounds (**Figure 5**) the 1,2,3,4-tetrahydropyrimidine structure. It should be noted that almost any aromatic aldehydes enter into the described transformations. This is important during comparison of the

pharmacological activity of compounds of this series with the corresponding derivatives of 4-aryl-l,4-dihydropyridines.

❖ **Synthesis based on nitropyrimidines**

Owing to their reactivity, nitropyrimidines, which contain accepting nitro groups and "pyrimidines" nitrogen atoms, have found use as synthons for the production of various derivatives of pyrimidine and also various other types of organic compounds whose synthesis by other methods is difficult or practically impossible.[29-31]

5-Nitropyrimidines do not form covalent σ adducts with uncharged O-nucleophiles. The amination of highly π-deficient six-membered nitroaza aromatic compounds was conducted successfully in the liquid ammonia-potassium permanganate system.[32] When dissolved in liquid NH_3, depending on the temp., 5-nitropyrimidine forms σ adducts at positions 2 and 4 of the heterocycle, and they are detected spectrally (**Figure 6**).[33]

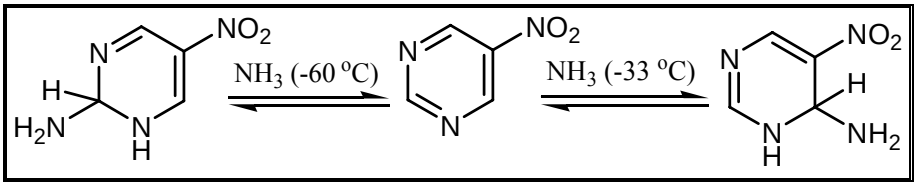


Figure 6

1.3. Biological activity of some 4-aryl-1,4-dihydropyridines and 5-nitro DHPMs

4-Aryl-1,4-dihydropyridines (DHPs, e.g. nifedipine) are the most studied class of organic calcium channel modulators. More than 30 years after the introduction of nifedipine many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market.[34,35]

Nowadays, interest has also focused on aza-analogs such as dihydropyrimidines (DHPMs) which shows a very similar pharmacological profile to classical dihydropyridine calcium channel modulators.[5-7, 36-43] Over the past few years several lead-compounds were developed (*i.e.* SQ 32,926) that are superior in potency and duration of antihypertensive activity to classical DHP drugs, and compare favorable with second-generation analogs such as amlodipine and nicardipine (**Figure 7**).

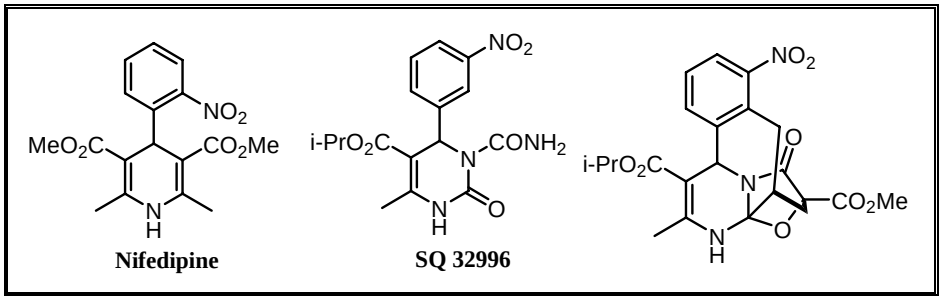


Figure 7

Barrow et al reported *in vitro* and *in vivo* evaluation of dihydropyrimidinone C-5 amides as potent and selective r1A receptor antagonists for the treatment of benign prostatic hyperplasia (**Figure 8**). R1 Adrenergic receptors mediate both vascular and lower urinary tract tone, and R1 receptor antagonists such as terazosin are used to treat both hypertension and benign prostatic hyperplasia (BPH). Recently, three different subtypes of this receptor have been identified, with the R1A receptor being most prevalent in lower urinary tract tissue. Barrow et al reported 4-aryldihydropyrimidinones attached to an aminopropyl-4-arylpiperidine *via* a C5 amide as selective R1A receptor subtype antagonists. In receptor binding assays, these types of compounds generally display *K*i values for the R1a receptor subtype <1 nM while being greater than 100-fold selective versus the R1b and R1d receptor subtypes. Many of these compounds were also evaluated *in vivo* and found to be more potent than terazosin in both a rat model of prostate tone and a dog model of intra-urethral pressure without significantly affecting blood pressure. While many of the compounds tested displayed poor pharmacokinetics, one compound was found to have adequate bioavailability (>20%) and half-life (>6 h) in both rats and dogs. Due to its selectivity for the R1a over the R1b and R1d receptors as well as its favorable pharmacokinetic profile, it has the potential to relieve the symptoms of BPH without eliciting effects on the cardiovascular system.[44,45]

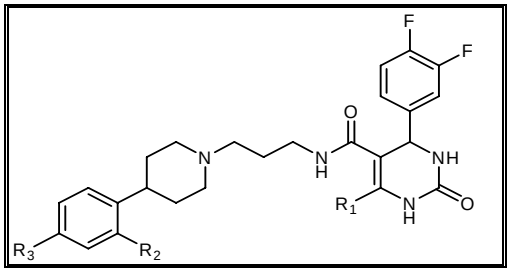


Figure 8

The 4-aryldihydropyrimidinone heterocycles attached to an aminopropyl-4-arylpiperidine *via* a C5 amide has proved to be an excellent template for selective R1A receptor subtype antagonists. These types of compounds are exceptionally potent in both cloned receptor binding studies as well as *in vivo* pharmacodynamic models of prostatic tone.

Atwal et al have examined a series of novel dihydropyrimidine calcium channel blockers that contain a basic group attached to either C5 or N3 of the heterocyclic ring (**Figure 9**). Structure-activity studies show that l-(phenylmethyl)-4-piperidinyl carbamate moiety at N3 and sulfur at C2 are optimal for vasorelaxant activity *in vitro* and impart potent and long-acting antihypertensive activity *in vivo*. One of these compounds was identified as a lead, and the individual enantiomers were synthesized. Two key steps of the synthesis were (1) the efficient separation of the diastereomeric ureido derivatives and (2) the high-yield transformation of 2-methoxy intermediate to the (*p*-methoxybenzyl)thio intermediates. Chirality's was demonstrated to be a significant determinant of biological activity, with the DHP receptor recognizing the enamines ester moiety but not the carbamate moiety. DHPM is equipotent to nifidepine and amlodipine *in vitro*. In the spontaneously hypertensive rat, DHPM is more potent and longer acting than both nifidepine and the long-acting amlodipine (DHP derivative). DHPM has the potential advantage of being a single enantiomer.[46,47]

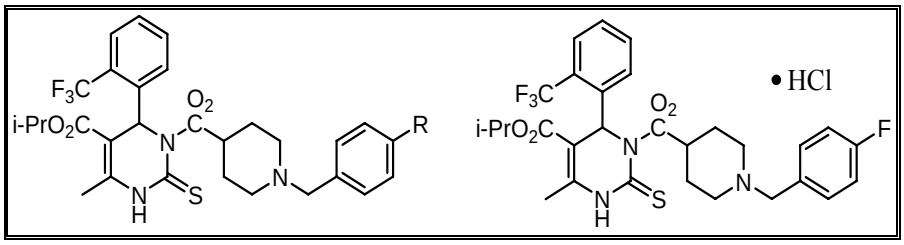


Figure 9

In order to explain the potent antihypertensive activity of the modestly active (ICw = 3.2 pM) DHPM calcium channel blocker**,** Atwal et al carried out drug metabolism studies in the rat and found it is metabolized. Two of the metabolites (ICw = 16 nM) and (ICw = 12 nM), were found to be responsible for the antihypertensive activity of compound**.** Potential metabolism *in vivo* precluded interest in pursuing compounds related to it. Structure-activity studies aimed at identifying additional aryl-substituted analogues led to comparable potential *in vivo*, though these compounds were less potent *in vitro*. To investigate the effects of absolute stereochemistry on potency, authors resolved *via* diastereomeric ureas, prepared by treatment with (R)-*α*-methylbenzylamine. The results demonstrate that the active R-(-)-enantiomer is more potent and longer acting than nifedipine as an antihypertensive agent in the SHR. The *in vivo* potency and duration is comparable to the long-acting DHP amlodipine. The superior oral antihypertensive activity compared to that of previously described carbamates (R_2=COOEt) could be explained by its improved oral bioavailability, possibly resulting from increased stability of the urea functionality (**Figure 10**).[6]

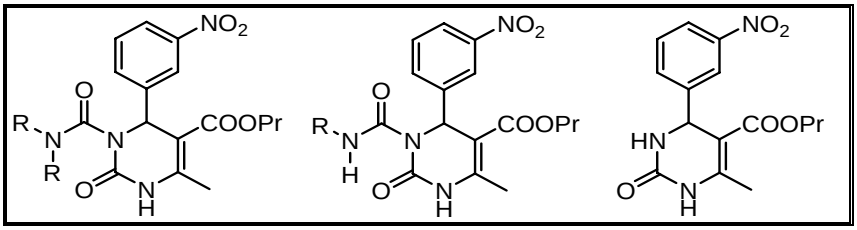


Figure 10

Authors modified the structure of previously described DHPM i.e. 3-substituted 1,4-dihydropyrimidines**.** Structure-activity studies using potassium-depolarized rabbit aorta show that ortho, meta-disubstituted aryl derivatives are more potent than either ortho or meta-monosubstituted compounds. While vasorelaxant activity was critically dependent on the size of the **C5** ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, and alkyl) were tolerated at N3. The results show DHPMs are significantly more potent than corresponding 2- heteroalkyl-l,4-dihydropyrimidines and only slightly less potent than similarly substituted 2-heteroalkyl-1-4-dihydropyridines (**Figure 11**). Where as DHP enantiomer usually show 10-15-fold difference in activity, the enantiomer of DHPM show more than a 1000-fold difference in activity. These results strengthen the requirement of an enaminoester for binding to the dihydropyridine receptor and indicate a nonspecific role for the N3-substituent.

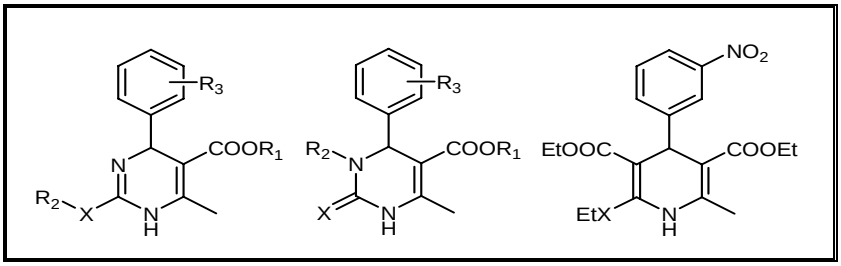


Figure 11

2-Heterosubstituted-4-aryl-l,4-dihydro-6-methyl-5-pyrimidinecarboxylicesters (**Figure 12**), which lack the potential symmetry of DHP calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit aorta and radio ligand binding techniques showed that some of these compounds are potent mimics of DHP calcium channel blockers.[35]

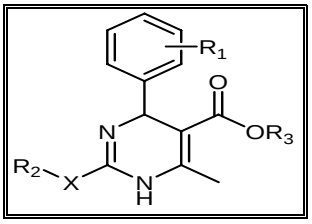


Figure 12

Bryzgalov A. O. et. al. have been studied the antiarrhythmic activity of 4,6-di(het)aryl-5-nitro-3,4-dihydropyrimidin-(1*H*)-2-ones(**Figure 13**) toward two types of experimental rat arrhythmia. With $CaCl_2$ induced arrhythmia model, several agents have demonstrated high Antiarrhythmic activity and the lack of influence on arterial pressure of rats.[48]

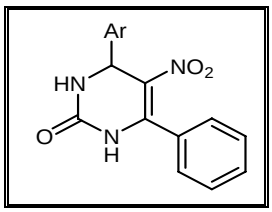


Figure 13

Remennikov G. Y. et al,[20] have been synthesized some novel 4-aryl-5-nitro substituted DHPMs (**Figure 14**) using nitroacetone and screened as calcium modulators. They have studied the pharmacological properties of 6-methyl- and 1,6-dimethyl-4-aryl-5-nitro-2-oxo-l,2,3,4-tetrahydropyrimidines with different substituents in the aryl fragment, i.e. unsubstituted, *ortho, meta, para, di*, and *tri*-substituted compounds and observed that 5-nitro DHPMs bearing unsubstituted, ortho and tri-substitution on aryl moieties at C4 position reduced blood pressure and inhibited myocardial contractile activity. The second group consisted *meta, para* and *di*-substituted aryl moieties with DHPMs increased blood pressure and had positive inotropic effects. The compounds with the highest hypotensive activity were containing substituents in the *ortho* position of the phenyl fragment. Thus, compounds having substitution on aryl moieties which had pronounced vasodilator and weak cardio depressive actions, increased cardiac pump function (SV). When inhibition of myocardial contractile function predominated, there was a reduction in SV. The effect of compounds of the first group on heart rate was variable, though most reduced heart rate. In addition, a reflex increase in heart rate might be expected because of the reduction in blood pressure. The reference preparation for compounds of this group was the calcium antagonist nifedipine. The pharmacological profile of compounds of the first group were analogous to that of nifedipine. This suggests that they share a common mechanism of action - blockade of calcium ion influx

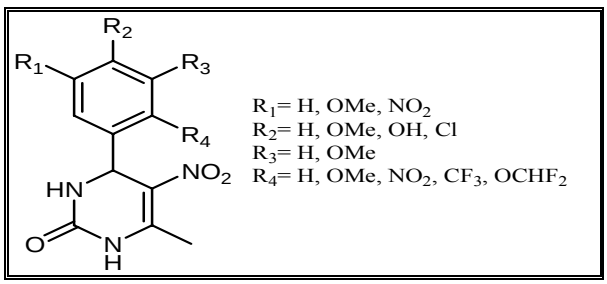


Figure 14

1.4 Alternative synthetic routes for better yield, shorter reaction time to synthesize new analogs

Various modifications have been applied to Biginelli reaction to get better yield and to synthesize biologically active analogs. Different catalysts have been reported to increase the yield of the reaction. Microwave synthesis strategies have also been applied to shorten the reaction time. Solid phase synthesis and combinatorial chemistry has made possible to generate library of DHPM analogs. The various modifications are discussed in the following section.

❖ Catalysts

Min Yang and coworkers[49] have synthesized the different DHPMs by using different inorganic salts as a catalyst (**Figure 15**). They found that the yields of the one-pot Biginelli reaction can be increased from 20-50% to 81-99%, while the reaction time shorted for 18-24 h to 20-30 min. This report a new and simple modification of the Biginelli type reaction by using $Yb(OTf)_3$ and $YbCl_3$ as a catalyst under solvent free conditions. One additional important feature of this protocol is the catalyst can be easily recovered and reused.

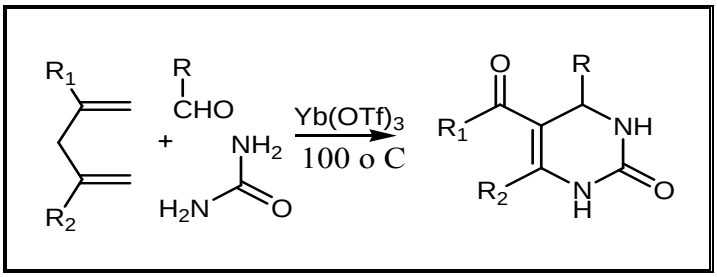


Figure 15

Indium (III) chloride was emerged as a powerful Lewis catalyst imparting high region and chemo selectivity in various chemical transformations. B. C. Ranu and co-workers[50] reported indium (III) chloride ($InCl_3$) as an efficient catalyst for the synthesis of 3,4-dihydropyrimidn-2(1*H*)-ones (**Figure 16**). A variety of substituted aromatic, aliphatic and heterocyclic aldehydes have been subjected to this condensation very efficiently. Thiourea has been used with similar success to provide the corresponding dihydropyrimidin-2(1*H*)-thiones.

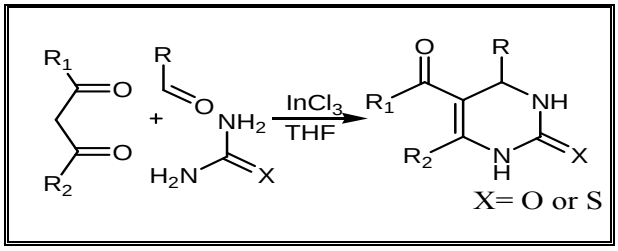


Figure 16

Majid M. Heravi et al. have reported a simple, efficient and cost-effective method for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones/thiones by one pot three-component cyclocondensation reaction of a 1,3-dicarbonyl compound, an aldehyde and urea or thiourea using 12-tungstophosphoric acid[51] and 12-molybdophosphoric acid[52] as recyclable catalyst (**Figure 17**).

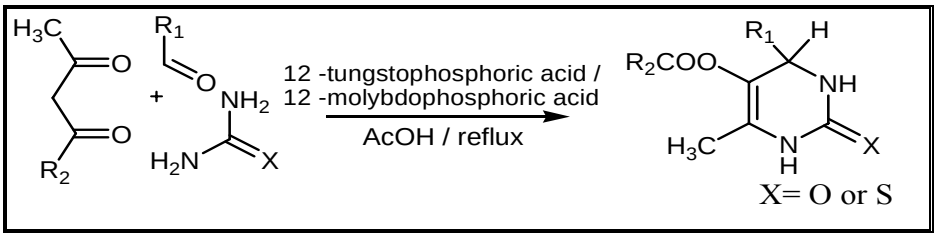


Figure 17

An improved approach has been found to carry out the Biginelli reaction for the synthesis of 3,4- dihydropyrimidine- 2(1*H*)-one derivatives. This synthesis was performed in the presence of hydrochloric acid and *β*-cyclodextrin in ethanol solution. Compared with the classical Biginelli reaction conditions, this new approach has the advantage of excellent yields and short reaction time.[53]

An efficient synthesis of 3,4-DHPMs from the aldehyde, *β*-keto ester and urea in ethanol, using ferric chloride hexahydrate or nickel chloride hexahydrate as the catalyst, was described. Compared with the classical Biginelli reaction conditions, this new method has the advantage of excellent yields (53-97%) and short reaction time (4-5 hours).[54]

5-Alkoxycarbonyl-4-aryl-3,4-dihydropyrimidin-2-ones were synthesized by the one-pot reactions of aldehydes, *β*-ketoesters and urea using a catalytic amount of phosphotungstic acid (PTA) in ethanol. The modified Biginelli cyclocondensation not only shortens the reaction period and simplifies the operation, but also improves the yields.[55]

Ruthenium (III) chloride efficiently catalyzes the three-component Biginelli reaction of an aldehyde, a *β*-keto ester, and urea or thiourea under solvent-free conditions to afford the corresponding 3,4-dihydropyrimidine-2-(1*H*)-ones in excellent yields.[56]

The Biginelli reaction, a one-pot condensation of aldehydes, urea or thiourea and *β*-dicarbonyl compounds, is efficiently catalyzed by samarium diiodide. The biologically active dihydropyrimidinones are easily synthesized in moderate to excellent yields under solvent-free conditions.[57]

Hydroxyapatite doped with $ZnCl_2$, $CuCl_2$, $NiCl_2$ and $CoCl_2$ efficiently catalyses the three components Biginelli reaction between an aldehyde, ethyl acetoacetate and urea in refluxing toluene to afford the corresponding dihydropyrimidinones in high yields.[58]

Sc(III)triflate efficiently catalyzes the three-component condensation reaction of an aldehyde, a *β*-ketoester and urea in refluxing acetonitrile to afford the corresponding 3,4-dihydropyrimidin-2(1H)-ones in excellent yields (**Figure 18**). The catalyst can be recovered and reused, making this method friendly and environmentally acceptable.[59]

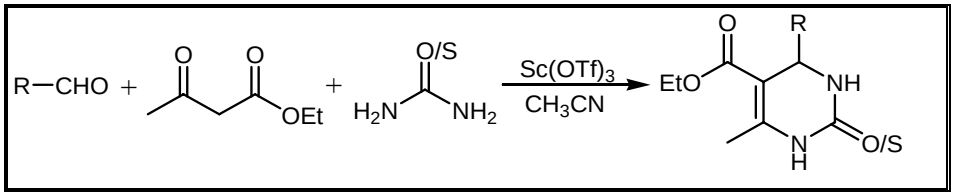


Figure 18

Very recently, chiral phosphoric acid is reported as highly enantioselective catalyst for Biginelli reaction. Reaction is reported in presence of 10 mol % of chiral phosphoric acid to produce desired enantioselective product. This is the first organocatalytic asymmetric Biginelli reaction. The optimal chiral phosphoric acid afforded the reaction in high yields with excellent enantioselectivities of up to 97% ee. A wide variety of substrates, including aldehydes and α-keto esters, could be tolerated. This reaction has an advantage of avoiding the contamination of transition metals in the manufacture of the medicinally relevant chiral 3,4-dihydropyrimidin-2-(1*H*)-ones (**Figure 19**).[60]

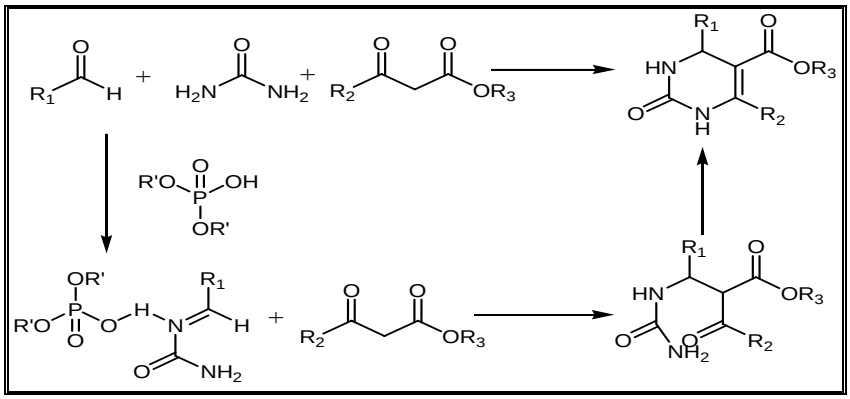


Figure 19

Shkurko, O. P. et al have been synthesized 4,6-diaryl-5- nitro-3,4-dihydropyrimidin-2(1*H*)-ones and *N*-benzoyl-*N*′-(1-aryl-2-nitroethyl)ureas (**Figure 20**) using *ω*-nitro acetophenone, aromatic aldehydes and urea in the presence of iron(III), cobalt(II), nickel(II), and copper(II) salts as catalyst with moderate to poor yields.[61]

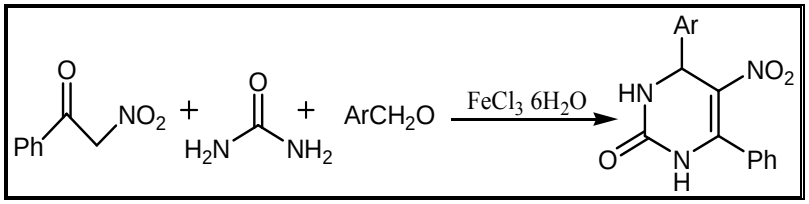


Figure 20

An efficient three-component synthesis of 3,4-dihydropyrimidinones using trichloroisocyanuric acid (TCCA) as mild, homogeneous and neutral catalyst for Biginelli reaction in ethanol or DMF under reflux condition.[62] Many researchers[63-68] have investigated Biginelli reaction under solvent-free conditions for one-pot synthesis of 3,4-dihydropyrimidine-2-(1*H*)ones/thiones using various catalyst as described under.

❖ **Solid phase synthesis**

The generation of combinatorial libraries of heterocyclic compounds by solid phase synthesis is of great interest for accelerating lead discovery and lead optimization in pharmaceutical research. Multi-component reactions (MCRs)[29,69-75] leading to heterocycles are particularly useful for the creation of diverse chemical libraries, since the combination of any 3 small molecular weight building blocks in a single operation leads to high combinatorial efficiency. Therefore, solid phase modifications of MCRs are rapidly become the cornerstone of combinatorial synthesis of small-molecule libraries.

The first solid-phase modification of the Biginelli condensation was reported by Wipf and Cunningham[76] in 1995 (**Figure 21**). In this sequence, γ-aminobutyric acid derived urea was attached to Wang resin using standard procedures. The resulting polymer-bound urea was condensed with excess *β*-ketoester and aromatic aldehydes in THF at 55 °C in the presence of a catalytic amount of HCl to afford the corresponding immobilized DHPMs. Subsequent cleavage of product from the resin by 50 % trifluoroacetic acid (TFA) provided DHPMs in high yields and excellent purity.

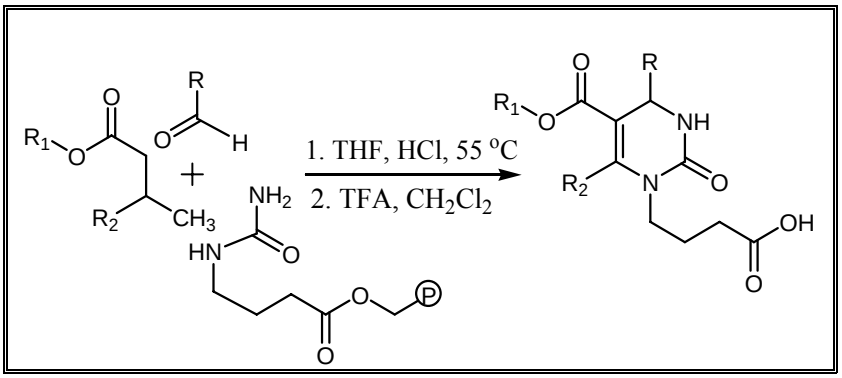


Figure 21

Li W. and Lam Y.[77] have described the synthesis of 3,4-dihydropyrimidin-2-(1*H*)ones/thiones using sodium benzenesulfinate as a traceless linker (**Figure 22**). The key steps involved in the solid-phase synthetic procedure were sulfinate acidification, condensation of urea or thiourea with aldehydes and sulfinic acid and traceless product release by a one-pot cyclization-dehydration process. Since a variety of reagents can be used, the overall strategy appears to be applicable to library generation.

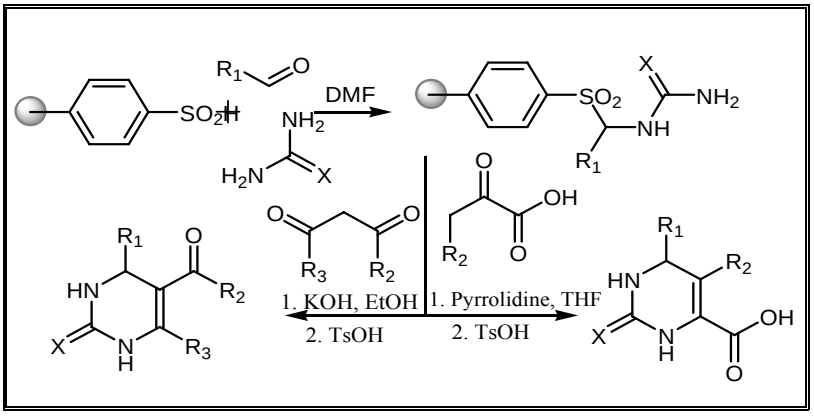


Figure 22

Gross et al.[78] developed a protocol to increase the diversity of DHPM which based on immobilized *α*-ketoamides (**Figure 23**). The resulting synthetic protocol proved to be suitable for the preparation of a small library using different building blocks. They found that the aromatic aldehyde and *α*-ketoamide building blocks were formed the expected DHPM derivatives in high purity and yield. The usage of an aliphatic aldehyde leads to an isomeric DHPM mixture. Purities and yields were not affected, when thiourea was used instead of urea.

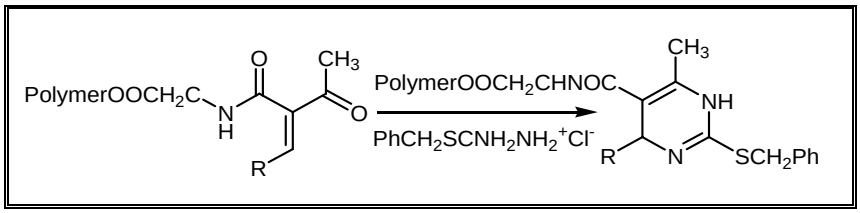


Figure 23

❖ **Liquid phase synthesis**

In the solid phase synthesis there are some disadvantages of this methodology compared to standard solution-phase synthesis, such as difficulties to monitor reaction progress, the large excess of reagents typically used in solid-phase supported synthesis, low loading capacity and limited solubility during the reaction progress and the heterogeneous reaction condition with solid phase.[79] Recently, organic synthesis of small molecular

compounds on soluble polymers, i.e. liquid phase chemistry has increasingly become attractive field.[80] It couples the advantages of homogeneous solution chemistry with those of solid phase chemistry.

Moreover owing to the homogeneity of liquid-phase reactions, the reaction conditions can be readily shifted from solution-phase systems without large changes and the amount of excessive reagents is less than that in solid-phase reactions. In the recent years, Task Specific room temperature Ionic Liquids (TSILs) has emerged as a powerful alternative to conventional molecular organic solvents or catalysts. Liu Z. et al[81] have reported cheap and reusable TSILs for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones *via* one-pot three component Biginelli reaction.

Ionic liquid-phase bound acetoacetate reacts with thiourea and various aldehydes with a cheap catalyst to afford ionic liquid-phase supported 3,4-dihydropyrimidin-2(1*H*)-thiones, which reported by Bazureau J. P. and co-workers[82] (**Figure 24**). 3,4-Dihydropyrimidinones were synthesized in one-pot, by the reaction of aldehydes, *β*-dicarbonyl compounds and urea, catalyzed by non-toxic room temperature ionic liquid 1-*n*-butyl-3-methylimidazolium saccharinate (BMImSac).[83]

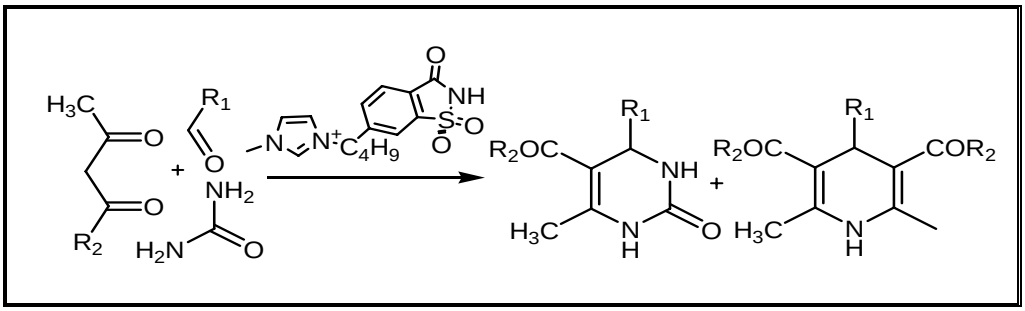


Figure 24

❖ **Microwave assisted synthesis**

In general, the standard procedure for the Biginelli condensation involves one pot condensation of the three building blocks in a solvent such as ethanol using a strongly acidic catalyst that is hydrochloric acid. One major drawback of this procedure, apart from the long reaction time involving reflux temperature, is the moderate yields frequently observed when using more complex building blocks. Microwave irradiation (MW) has become accepted tool in organic synthesis, because the rate enhancement, higher yields and often, improved selectivity with respect to conventional reaction conditions.[84] The publication by Dandia A. et al[85] described microwave-enhanced solution-phase Biginelli reactions employing ethyl acetoacetate, thiourea and a wide variety of aromatic aldehydes as building blocks (**Figure**

25). Upon irradiation of the individual reaction mixtures (ethanol, catalytic HCl) in an open glass beaker inside the cavity of a domestic microwave oven the reaction times were reduced from 2–24 hours of conventional heating 80 °C, reflux to 3–11 minutes under microwave activation (ca. 200 –300 W). At the same time the yields of DHPMs obtained were distinctly improved compared to those reported earlier using conventional conditions.

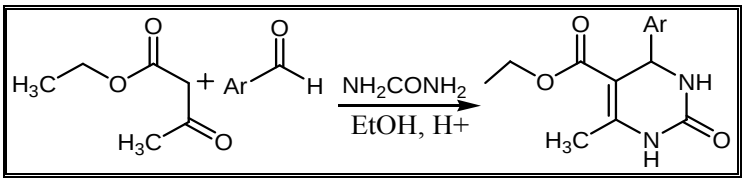


Figure 25

In recent years, solvent free reactions using either organic or inorganic solid supports have received more attention.[86] There are several advantages to perform synthesis in dry media: (i) short reaction times, (ii) increased safety, (iii) economic advantages due to the absence of solvent. In addition, solvent free MW processes are also clean and efficient. Gopalakrishnan M. and co-workers have reported Biginelli reaction under microwave irradiation in solvent-free conditions using activated fly ash as catalyst, an industrial waste (pollutant) is an efficient and novel catalyst for some selected organic reactions in solvent free conditions under microwave irradiation.[87]

❖ **Ultrasound assisted synthesis**

Ultrasound as a green synthetic approach has gradually been used in organic synthesis over the last three decades. Compared with the traditional methods, it is more convenient, easier to be controlled and consumes less power. With the use of ultrasound irradiation, a large number of organic reactions can be carried out in milder conditions with shorter reaction time and higher product yields.[88] Ultrasound irradiated and amidosulfonicacid (NH_2SO_3H) catalyzed synthesis of 3,4-dihydropyrimidi-2-(1*H*)ones have reported by Li J. T. and co-workers[89] using aldehydes, *β*-ketoester and urea.

Liu C. et al.[90] have synthesized a novel series of 4-substituted pyrazolyl- 3,4-dihydropyrimidin-2(1*H*)-thiones under ultrasound irradiation using magnesium perchlorate [$Mg(ClO_4)_2$] as catalyst (**Figure 26**), by the condensation of 5-chloro/phenoxyl-3-methyl-1-phenyl-4-formylpyrazole, 1,3-dicarbonyl compound and urea or thiourea in moderate yields. The catalyst exhibited remarkable reactivity and can be recycled.

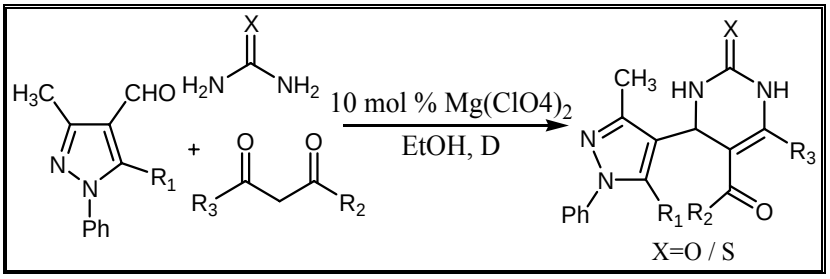


Figure 26

Sonication of aromatic aldehydes, urea and ethyl acetoacetate in presence of solvent (ethanol) or solvent-less dry media (bentonite clay) by supporting-zirconium chloride ($ZrCl_4$) as catalyst at 35 kHz gives 6-methyl-4-substitutedphenyl-2-oxo-1,2,3,4- tetrahydropyrimidine-5-carboxylic acid ethyl esters proficiently in high yields, which reported by Harish Kumar et al. (**Figure 27**).[91]

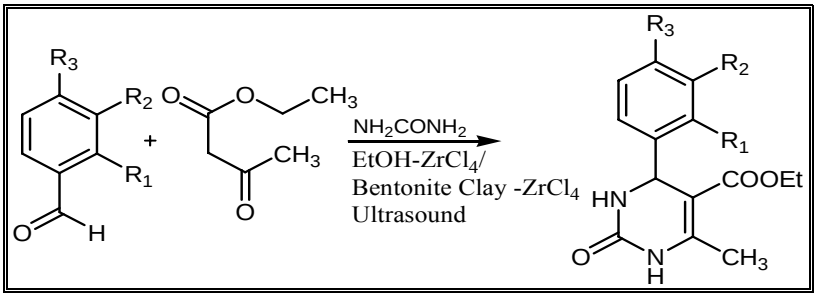


Figure 27

1.5 CURRENT RESEARCH WORK

Our group is involved in the development of various synthetic methodologies for the synthesis of functionalized 1,4-DHPs and DHPMs for last few years. Substitution of Nitro functionality for COOAlk in the DHPM moiety may alters their biological action. Reports reveal that nitro group functionalized dihydropyrimidines which might have potential biological activities were less studied. Very promising results may obtain with these modifications to DHPM skeleton. This concept prompted us to introduce NO_2 group at C5 position in DHPM skeleton. For this modification *ω*-nitroacetophenone was required as a precursor which was synthesized by reported the procedure in literature.[92]

During the course of our ongoing interest on the development of useful synthetic methodologies by utilizing acid catalysts[93], we were observed that bisphosphonic acid is an efficient catalyst for the synthesis of pyrimidines *via* biginelli condensation. In extension of this work and to explore further, the utility of etidronic acid as a catalyst in multicomponent cyclocondensation reaction, we have synthesized some novel nitro group at C5 bearing dihydropyrimidine derivatives using 2-hydroxy-*ω*-nitro acetophenone instead of 1,3 diketone. The reaction of various aryl aldehydes, urea and substituted *ω*-nitro acetophenone under microwave irradiation using etidronic acid as a catalyst and THF as solvent afforded nitro functionalized novel DHPMs with excellent yield. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. Among them, compound 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one was confirmed by X-Ray Diffraction Technique. The detail study of X-Ray Crystallography study is described in chapter 6. All the synthesized compounds were screened for *in vitro* anti-viral activity against III_B and ROD strains.

1.6 RESULTS AND DISCUSSION

Etidronic acid [(1-hydroxyethylidene) bisphosphonic acid] is one of the bisphosphonic acid derivative and also known as bisphosphonate having molecular formula $C_2H_8O_7P_2$. The two PO_3 (phosphonate) groups covalently linked to carbon atom (**Figure 28**).

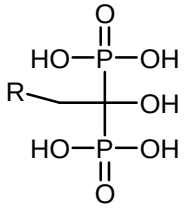


Figure 28: Bisphosphonic acid

It is differ from Polyphosphate ester and polyphosphoric acid. Various bisphosphonic acids are known.[94,95] Etidronic acid is mild enough as compare to another strong acid such as polyphosphoric acid etc. moreover, the catalyst did not affect acid sensitive aldehydes.

Scheme-1: Etidronic acid catalyzed one-pot synthesis of nitro functionalized dihydropyrimidines under microwave irradiation.

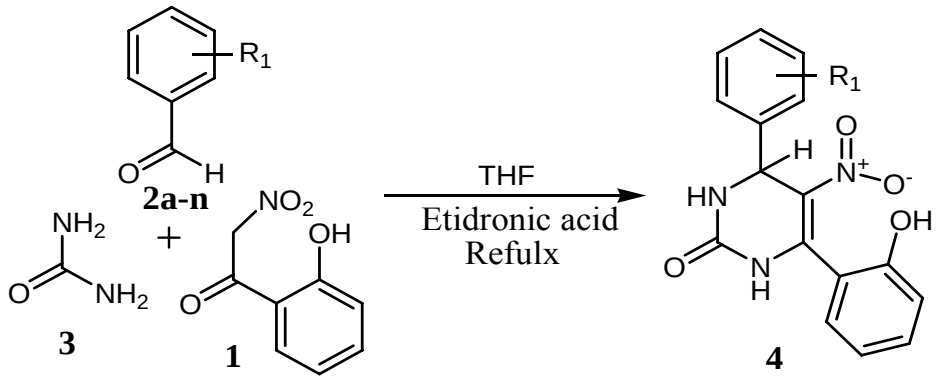


Table 1: Optimization of the reaction conditions for the synthesis of 4a

Entry	Catalyst(equiv)	Solvent	Yield %	Time
1	EDA(1.0)	THF	89	4.0 h
2	EDA(1.0)	THF	95	3.5 min
3	EDA(0.1)	THF	86	6.0 h
4	EDA(0.1)	THF	93	3.0 min
5	EDA(0.1)	MeOH	73	11.5 h
6	EDA(0.1)	MeOH	82	6.5 min
7	EDA(0.1)	EtOH	78	9.0 h
8	EDA(0.1)	EtOH	86	5.5 min

Indeed, condensation of the 1-(2-hydroxyphenyl)-2-nitroethanone **1** with benzaldehyde **2a** and urea **3** took place smoothly in the presence of EDA in THF resulted in the formation of dihydropyrimidine **4a** in 89% yield (entry 1, Table 1). We found that the final product obtained was dihydro biginelli product **4a** (Scheme-1). The condensation of **1** with **2a** and **3** to generate **4a** was investigated under a variety of conditions (Table 1), as a test case, to optimize the yield, and the results are gathered in Table 1. The condensation took place even with a catalytic amount of EDA (10%, entry 3). Though the condensation reaction with a catalytic amount of EDA was cleaned, it took a longer time (6 h). On the other hand, the reaction was relatively fast (4 hr) when one equiv. of EDA was employed (entry 1). However, the reaction carried out under microwave irradiation gave excellent yields (entry 4, 2). The yield of desired product **4a** was moderate when methanol and ethanol was used as solvent (entry 5-8).

Table 2: Synthesis of nitro dihydropyrimidines 4a using various catalyst and THF under microwave irradiation.

Entry	R	Catalyst (equiv)	Yield %	Time min
1	-H	Etidronic acid (0.1)	93	3.0
2	$-CH_2NH_2$	Pamidronic acid(0.1)	65	9.5
3	$-CH_2NH_2$	Pamidronic acid(1.0)	68	8.0
4	$-(CH_2)_2NH_2$	Alendronic acid (0.1)	66	8.0
5	$-(CH_2)_2NH_2$	Alendronic acid (1.0)	67	9.5
6	-3-Pyridyl	Risedronic acid (0.1)	55	11.0
7	-3-Pyridyl	Risedronic acid (1.0)	59	9.5
8	-1-imidazolyl	Zoledronic acid (0.1)	45	11.5
9	-1-imidazolyl	Zoledronic acid (1.0)	51	11.0

With the optimized conditions in hand, the reactions of **1** with benzaldehyde **2a** and urea **3** with various bisphosphonic acids were examined to explore the utility of these catalysts in multicomponent cyclocondensation reaction under microwave irradiation in THF. We found that bisphosphonic acid linked with alkyl amines (Table 2, entry 2-4) or with heterocyclic moieties (Table 2, entry 5-8) showed very poor catalytic activity compared to EDA (Table 2, entry 1). This can be explained by the fact that electron donating moieties attached with bisphosphonic acid at C2 position may decreases the reactivity of these catalysts leading to moderate or poor yields of **4a**. Thus, it is clear from the aforementioned experiments that the best yield of compound **4a** could be obtained by employing catalytic amount of etidronic acid in THF under microwave irradiation.

Table 3: Synthesis of nitro functionalized dihydropyrimidines using etidronic acid (catalyst) and THF under microwave irradiation.

Entry	R_1	Products	Yields (%)	Time min
MMS-1	H	**4a**	93	3.0
MMS-2	3-Cl	**4b**	86	4.5
MMS-3	4-OCH_3	**4c**	91	4.0
MMS-4	4-Cl	**4d**	89	4.0
MMS-5	4-F	**4e**	90	4.5
MMS-6	3-OCH_3	**4f**	92	3.0
MMS-7	2-Cl	**4g**	86	4.5
MMS-8	4-NO_2	**4h**	93	2.5
MMS-9	3-NO_2	**4i**	91	3.0
MMS-10	3,4-di-OCH_3	**4j**	93	3.5
MMS-11	4-OH	**4k**	88	4.0
MMS-12	3-OH	**4l**	86	4.5
MMS-13	3-Br	**4m**	86	4.0
MMS-14	2,4-di-Cl	**4n**	89	3.5
MMS-15	2,5-di-OCH_3	**4o**	91	4.5
MMS-16	2-NO_2	**4p**	78	3.5
MMS-17	4-CH_3	**4q**	88	3.0
MMS-18	2-OH	**4r**	75	4.0
MMS-19	2-OCH_3	**4s**	82	3.5
MMS-20	2-OCH_3, 4-NO_2	**4t**	72	4.0

When the reaction of the 1-(2-hydroxyphenyl)-2-nitroethanone **1** with various arylaldehydes **2a** and urea **3** was conducted it was observed that the electron deficiency and nature of the substituents on the aromatic ring aldehydes effect the conversion rate; aromatic aldehydes having electron-withdrawing groups on the aromatic ring (Table 3, entries 6, 8, 9) reacted faster than electron-donating groups (Table 3, entries 2, 11, 12). The synthesized compounds were characterized by spectroscopy analysis. In mass spectrum of **4a** molecular ion peak

appears at 311 m/z which reveal the formation of dihydropyrimidine. The ^{1}H NMR spectrum of **4a** displayed one characteristic doublet for the methane proton at 5.74 δ ppm and one –OH proton at 9.64 δ ppm. The overall study indicates the catalyst is efficient to synthesize nitro dihydropyrimidines. The anti-viral activity results are depicted in table 5. The mechanism for the formation of DHPM[96] involves acid-catalyzed formation of an *N*-acyliminium ion intermediate of type (**Figure 29**) from the aldehyde (**1**) and urea (**2**) precursors. Interception of the iminium ion (**4**) by ω-nitroacetophenone (**5**), presumably through its active methylene produces an open chain ureide (**6**) which subsequently cyclizes to hexahydropyrimidine (**7**). Acid-catalyzed elimination of water from (**7**) ultimately leads to the final DHPM product (**8**).

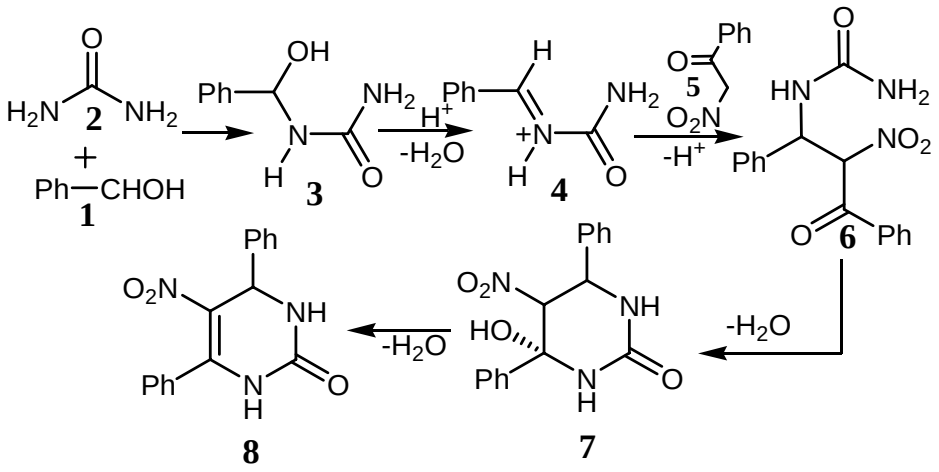


Figure 29: Mechanism for the formation of DHPM

1.7 ANTIVIRAL ACTIVITY

Table 5: **The *in-vitro* anti-viral activity against HIV-1 III_B and ROD strains using MTT method.**

Code Name	Strain	Exp.no.	IC_{50}(µg/ml)	CC_{50}(µg/ml)	SI	Max Prot (%)	Appr.	Average IC_{50}(µg/ml)	Average CC_{50}(µg/ml)	SD	SI	Remarks
MMS-01	III_B	P3.4836	> 57.3	57.3	1	11	1					
		P3.4842	>60.4	60.4	1	0	1	>60.95	60.95	5.36	<1	
	ROD	P3.4837	>68.7	68.7	1	6	1					
		P3.4843	>57.4	57.4	1	1	1	>60.95	60.95	5.36	<1	
MMS-02	III_B	P3.4836	>22.5	22.5	1	15	1					
		P3.4842	>22.5	22.5	1	4	1	>24.28	24.28	5.20	<1	
	ROD	P3.4837	>31.9	31.9	1	21	1					
		P3.4843	>20.2	20.2	1	11	1	>24.28	24.28	5.20	<1	
MMS-03	III_B	P3.4836	>20.6	20.6	1	13	1					
		P3.4842	>45.9	45.9	1	1	1	>40.18	40.18	14.23	<1	
	ROD	P3.4837	>54	54	1	13	1					
		P3.4843	>40.2	40.2	1	7	1	>40.18	40.18	14.23	<1	
MMS-04	III_B	P3.4836	>10.5	10.5	1	5	1					
		P3.4842	>27	27	1	6	1	>21.88	21.88	10.16	<1	
	ROD	P3.4837	>33.2	33.2	1	9	1					
		P3.4843	>16.8	16.8	1	5	1	>21.88	21.88	10.16	<1	
MMS-05	III_B	P3.4836	>15.8	15.8	1	9	1					
		P3.4842	>24.9	24.9	1	0	1	>21.03	21.03	4.01	<1	
	ROD	P3.4837	>20.1	20.1	1	8	1					
		P3.4843	>23.3	23.3	1	10	1	>21.03	21.03	4.01	<1	

MMS-06	III_B	P3.4836	>68.4	68.4	1	14	1				
		P3.4842	>65.3	65.3	1	8	1	>64.83	64.83	9.99	<1
	ROD	P3.4837	>74.6	74.6	1	19	1				
		P3.4843	>51	51	1	8	1	>64.83	64.83	9.99	<1
MMS-07	III_B	P3.4836	>72.1	72.1	1	7	1				
		P3.4842	>61.1	61.1	1	0	1	>65.60	65.60	12.56	<1
	ROD	P3.4837	>78.9	78.9	1	11	1				
		P3.4843	>50.3	50.3	1	0	1	>65.60	65.60	12.56	<1
MMS-08	III_B	P3.4836	>75.3	75.3	1	16	1				
		P3.4842	>59.7	59.7	1	6	1	>67.30	67.30	8.09	<1
	ROD	P3.4837	>73.2	73.2	1	10	1				
		P3.4843	>61	61	1	1	1	>67.30	67.30	8.09	<1
MMS-09	III_B	P3.4842	>44.3	44.3	1	2	1				
		P3.4848	>50.9	50.9	1	0	1	>52.55	52.55	7.52	<1
	ROD	P3.4843	>62.5	62.5	1	10	1				
		P3.4849	>52.5	52.5	1	3	1	>52.55	52.55	7.52	<1
MMS-10	III_B	P3.4842	>52.9	52.9	1	11	1				
		P3.4848	>53.3	53.3	1	0	1	>50.53	50.53	4.39	<1
	ROD	P3.4843	>51.9	51.9	1	9	1				
		P3.4849	>44	44	1	4	1	>50.53	50.53	4.39	<1
MMS-11	III_B	P3.4842	>41.8	41.8	1	8	1				
		P3.4848	>41.3	41.3	1	2	1	>41.68	41.68	4.25	<1
	ROD	P3.4843	>36.6	36.6	1	17	1				
		P3.4849	>47	47	1	13	1	>41.68	41.68	4.25	<1
MMS-12	III_B	P3.4842	>8.75	8.75	1	4	1				

		P3.4848	>9.04	9.04	1	7	1	>10.75	10.75	2.20	<1
	ROD	P3.4843	>12	12	1	8	1				
		P3.4849	>13.2	13.2	1	6	1	>10.75	10.75	2.20	<1
MMS-13	III_B	P3.4842	>45	45	1	7	1				
		P3.4848	>62.8	62.8	1	1	1	>61.20	61.20	11.58	<1
	ROD	P3.4843	>64.6	64.6	1	10	1				
		P3.4849	>72.4	72.4	1	9	1	>61.20	61.20	11.58	<1
MMS-14	III_B	P3.4842	>47.2	47.2	1	9	1				
		P3.4848	>49.8	49.8	1	3	1	>49.60	49.60	2.11	<1
	ROD	P3.4843	>52.3	52.3	1	11	1				
		P3.4849	>49.1	49.1	1	**30**	1	>49.60	49.60	2.11	<1
MMS-15	III_B	P3.4842	>60	60	1	6	1				
		P3.4848	>69.1	69.1	1	4	1	>66.35	66.35	6.96	<1
	ROD	P3.4843	>61.4	61.4	1	9	1				
		P3.4849	>74.9	74.9	1	4	1	>66.35	66.35	6.96	<1
MMS-16	III_B	P3.4842	>49	49	1	5	1				
		P3.4848	>68.3	68.3	1	8	1	>57.15	57.15	8.07	<1
	ROD	P3.4843	>55.8	55.8	1	7	1				
		P3.4849	>55.5	55.5	1	0	1	>57.15	57.15	8.07	<1
MMS-17	III_B	P3.4842	>70.7	70.7	1	12	1				
		P3.4848	>88.5	88.5	1	0	1	>83.13	83.13	10.66	<1
	ROD	P3.4843	>78.5	78.5	1	12	1				
		P3.4849	>94.8	94.8	1	16	1	>83.13	83.13	10.66	<1
MMS-18	III_B	P3.4842	>53.7	53.7	1	10	1				
		P3.4848	>61.9	61.9	1	0	1	>57.80	57.80	6.48	<1

	ROD	P3.4843	>51	51	1	5	1				
		P3.4849	>64.6	64.6	1	7	1	>57.80	57.80	6.48	<1	
MMS-19	III_B	P3.4842	>15.1	15.1	1	16	1					
		P3.4848	>14.8	14.8	1	2	1	>15.63	15.63	1.60	<1	
	ROD	P3.4843	>14.6	14.6	1	7	1					
		P3.4849	>18	18	1	0	1	>15.63	15.63	1.60	<1	
MMS-20	III_B	P3.4842	>62.3	62.3	1	9	1					Cryst.
		P3.4848	>96.3	96.3	1	11	1	>86.38	86.38	16.13	<1	observ. at
	ROD	P3.4843	>94.6	94.6	1	22	1					1 µg/ml
		P3.4849	>92.3	92.3	1	**30**	1	>86.38	86.38	16.13	<1	

From the above results, it has been concluded that the synthesized compounds are inactive against III_B and ROD strains.

1.8 CONCLUSION

In summary, we have demonstrated a simple route for the synthesis of nitro group containing dihydropyrimidines *via* cyclocondensation reaction using etidronic acid as an efficient homogeneous catalyst. The use of etidronic acid was well tolerated with a range of aldehydes. This protocol is general and provides dihydropyrimidines in good to excellent yields depending on the reactivity of arylaldehydes. Thus, the present synthesis of pyrimidines will serve as an exclusive method of preparative importance for this class of compounds. However, the newly synthesized compounds were inactive against HIV-1 III_B and ROD strains.

1.9 EXPERIMENTAL SECTION

^{1}HNMR (400 MHz) and ^{13}CNMR (100 MHz) spectra were recorded in DMSO, and TMS was used as an internal reference on a Bruker AVANCE II spectrometer. Mass spectra were determined using direct inlet probe on a GCMSQP2010 mass spectrometer. IR spectra were recorded on KBr discs, using FTIR-8400 spectrophotometer. The syntheses were carried out in a Questron Technologies Corporation QPro-M microwave synthesizer. Melting points were measured in open capillaries and are uncorrected.

❖ **General procedure for the synthesis of nitro dihydropyrimidines MMS 1-20.**

To a mixture of various aromatic aldehydes (10 mmol, **2a-n**) and urea (10 mmol) in dry THF (5 mL) was added etidronic acid (0.1 mmol) and stirred it for 5 min at rt to this add 1-(2-hydroxyphenyl)-2-nitroethanone (10 mmol) and subjected to microwave irradiation at 360 W for appropriate time (Table 3). The reaction was being monitored by TLC. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The separated solid was washed with water and followed by methanol, filtered, dried and crystallized from glacial acetic acid to furnish analytically pure products.

❖ **Spectral data of the synthesized compounds MMS 1-20**

3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one (MMS-1):
Lemon yellow solid; mp 241-243 °C; IR (KBr): 3624, 3076, 2976, 1674, 1554, 1201 cm^{-1} ; ^{1}H NMR: δ 5.74 (d, 1H, *J*=3.16 Hz), 6.90-7.59 (m, 9H, Ar-H), 7.68 (s, 1H, NH), 8.97 (s, 1H, NH), 9.64 (s, 1H, OH); ^{13}C NMR: 55.30, 114.54, 116.08, 119.31, 120.38, 126.99, 128.73, 131.61, 142.12, 146.22, 151.53, 166.05; MS *m/z*: 311(M^{+}); Anal. Calcd. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50%. Found: C, 61.58; H, 4.08; N, 13.33%.

4-(3-chlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-2): Yellow solid; mp 255-257 °C; IR (KBr): 3556, 3290, 2901, 1672, 1494, 1218 cm^{-1}; ^{1}H NMR: δ 5.71 (d, 1H, *J*=3.52 Hz), 6.91-7.93 (m, 8H, Ar-H), 7.94 (s, 1H, NH), 9.17 (s, 1H, NH), 9.78 (s, 1H, OH); ^{13}C NMR: 54.40, 115.52, 117.18, 120.25, 122.31, 127.63, 129.63, 134.63, 145.22, 148.34, 153.53, 163.12; MS *m/z*: 345(M^{+}); Anal. Calcd. for $C_{16}H_{12}ClN_3O_4$: C,55.58; H, 3.50; N, 12.15;. Found: C, 55.42; H, 3.38; N, 12.03%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(4-methoxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-3): Pale yellow solid; mp 246-248 °C; IR (KBr): 3649, 3292, 2899, 1678, 1504, 1193 cm^{-1}; ^1H NMR: δ 3.79 (s, 1H, OCH_3), 5.65 (d, 1H, *J*=3.36 Hz), 6.86-7.49 (m, 8H, Ar-H), 7.95 (s, 1H, NH), 9.42 (s, 1H, NH), 9.71 (s, 1H, OH); ^{13}C NMR: 54.65, 55.28, 113.91, 115.97, 119.24, 128.22, 128.61, 131.22, 134.45, 135.44, 136.30, 151.52, 159.28, 172.96, 181.34; MS *m/z*: 341(M^+); Anal. Calcd. for $C_{17}H_{15}N_3O_5$: C, 59.83; H, 4.43; N, 12.31%. Found: C, 59.66; H, 4.32; N, 12.18%.

4-(4-chlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-4): Lemon yellow solid; mp 250-252 °C; IR (KBr): 3610, 3088, 2928, 1714, 1531, 1176 cm^{-1}; MS *m/z*: 345(M^+); Anal. Calcd. for $C_{16}H_{12}ClN_3O_4$: C, 55.58; H, 3.50; N, 12.15%. Found: C, 55.47; H, 3.38; N, 12.13%.

4-(4-fluorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1H)-one (MMS-5): Yellow solid; mp 262-264 °C; IR (KBr): 3621, 3288, 2968, 1689, 1531, 1186 cm^{-1}; MS *m/z*: 329(M^+); Anal. Calcd. for $C_{16}H_{12}ClN_3O_4$: C, 58.36; H, 3.67; N, 12.76%. Found: C, 58.25; H, 3.60; N, 12.65%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(3-methoxyphenyl)-5-nitropyrimidin-2(1H)-one (MMS-6): Pale yellow solid; mp 255-257 °C; IR (KBr): 3639, 3312, 2814, 1698, 1494, 1093 cm^{-1}; MS *m/z*: 341(M^+); Anal. Calcd. for $C_{17}H_{15}N_3O_5$: C, 59.83; H, 4.43; N, 12.31%. Found: C, 59.69; H, 4.35; N, 12.20%.

4-(2-chlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-7): Yellow solid; mp 248-250 °C; IR (KBr): 3567, 3308, 2941, 1721, 1587, 1162 cm^{-1}; MS *m/z*: 345(M^+); Anal. Calcd. for $C_{16}H_{12}ClN_3O_4$: C, 55.58; H, 3.50; N, 12.15%. Found: C, 55.50; H, 3.37; N, 12.03%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(4-nitrophenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-8): Lemon yellow solid; mp 268-270 °C; IR (KBr): 3487, 3247, 2864, 1688,1499, 1057 cm^{-1}; ^1H NMR: δ 5.82 (d, 1H, *J*=3.64 Hz), 6.92-7.84 (m, 6H, Ar-H), 8.14 (s, 1H, NH), 8.20-8.22 (q, 2H, Ar-H), 9.31(s, 1H, NH), 9.76 (s, 1H, OH); MS *m/z*: 356 (M^+); Anal. Calcd. for $C_{16}H_{12}N_4O_6$: C, 53.94; H, 3.39; N, 15.73%. Found: C, 53.75; H, 3.24; N, 15.56%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(3-nitrophenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-9): Lemon yellow solid; mp 247-249 °C; IR (KBr): 3605, 3221, 2928, 1724, 1511, 1084 cm^{-1} ; MS *m/z*: 345(M^+); Anal. Calcd. for $C_{16}H_{12}ClN_3O_4$: C, 55.58; H, 3.50; N, 12.15%. Found: C, 55.46; H, 3.46; N, 12.08%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(3,4-dimethoxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-10): Yellow solid; mp 264-266 °C; IR (KBr): 3610, 3314, 2957, 1698, 1521, 1096 cm^{-1}; MS *m/z*: 371(M^+); Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 58.22; H, 4.61; N, 11.32%. Found: C, 58.17; H, 4.48; N, 11.18%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(4-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-11): Yellow solid; mp 237-239 °C; IR (KBr): 3622, 3274, 2904, 1724, 1531, 1210 cm^{-1}; MS *m/z*: 327(M^+); Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84%. Found: C, 58.59; H, 3.88; N, 12.71%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(3-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-12): Yellow solid; mp 248-250 °C; IR (KBr): 3654, 3225, 2965, 1741, 1564, 1157 cm^{-1}; MS *m/z*: 327(M^+); Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84%. Found: C, 58.61; H, 3.86; N, 12.74%

4-(3-bromophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-13): Lemon yellow solid; mp 262-264 °C; IR (KBr): 3610, 3242, 2928, 1714, 1531, 1176 cm^{-1}; MS *m/z*: 390(M^+); Anal. Calcd. for $C_{16}H_{12}BrN_3O_4$: C, 49.25; H, 3.10; N, 10.77%. Found: C, 49.17; H, 2.98; N, 10.70%.

4-(2,4-dichlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-14): Lemon yellow solid; mp 244-246 °C; IR (KBr): 3610, 3258, 2932, 1726, 1491, 1154 cm^{-1}; MS *m/z*: 380(M^+); Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O_4$: C, 50.55; H, 2.92; N, 11.05%. Found: C, 50.47; H, 3.04; N, 11.03%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(2,5-dimethoxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-15): Yellow solid; mp 255-257 °C; IR (KBr): 3574, 3294, 2857, 1718, 1521, 1161 cm^{-1}; MS *m/z*: 371(M^+); Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 58.22; H, 4.61; N, 11.32%. Found: C, 58.15; H, 4.52; N, 11.18%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(2-nitrophenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-16): Lemon yellow solid; mp 262-264 °C; IR (KBr): 3514, 3317, 2924, 1698,1598, 1107 cm^{-1}; MS *m/z*: 356 (M^+); Anal. Calcd. for $C_{16}H_{12}N_4O_6$: C, 53.94; H, 3.39; N, 15.73%. Found: C, 53.77; H, 3.29; N, 15.58%.

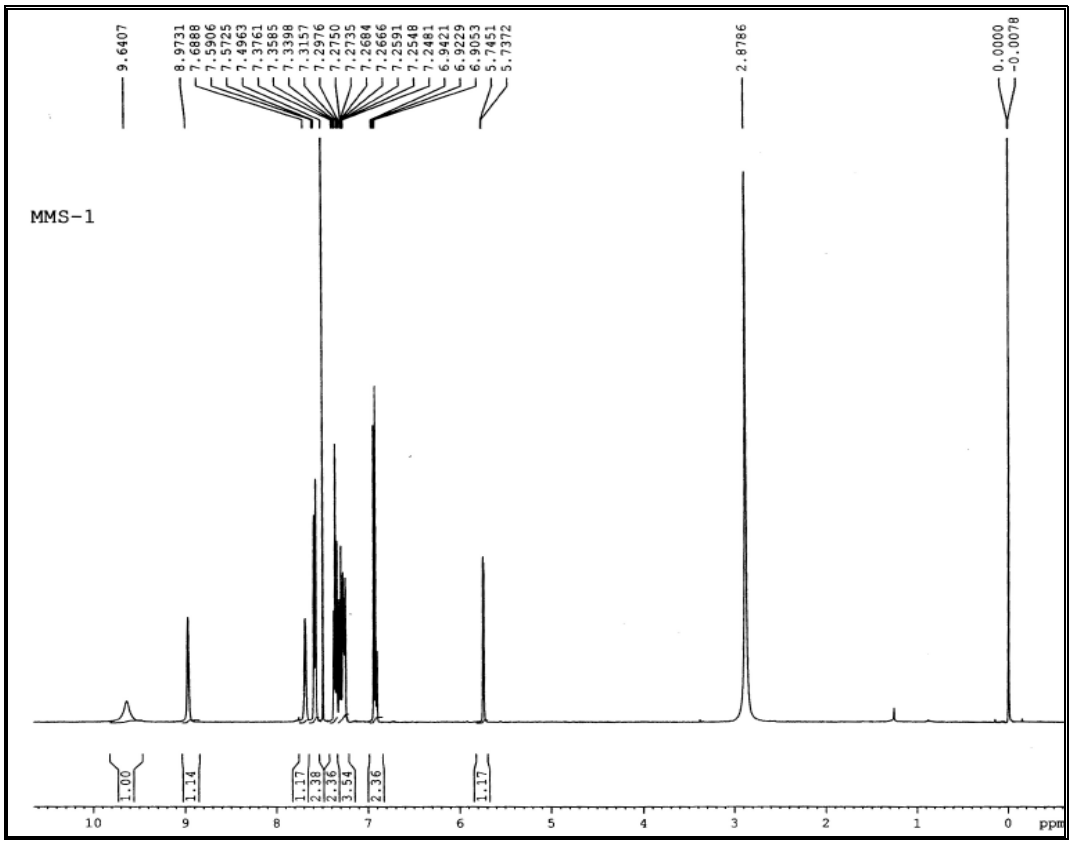
3,4-dihydro-6-(2-hydroxyphenyl)-4-(4-methylphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-17): Pale yellow solid; mp 238-240 °C; IR (KBr): 3608, 3223, 2928, 1734, 1521, 1167 cm^{-1}; MS *m/z*: 325(M^+); Anal. Calcd. for $C_{17}H_{15}N_3O_4$: C, 62.76; H, 4.65; N, 12.92%. Found: C, 62.67; H, 4.52; N, 12.83%.

4-(4-(dimethylamino)phenyl)-3,4-dihydro-6-(2-hydroxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-18): Lemon yellow solid; mp 250-252 °C; IR (KBr): 3628, 3247, 2936, 1678, 1511, 1086 cm^{-1}; MS *m/z*: 354(M^+); Anal. Calcd. for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81%. Found: C, 61.05; H, 5.04; N, 15.73%.

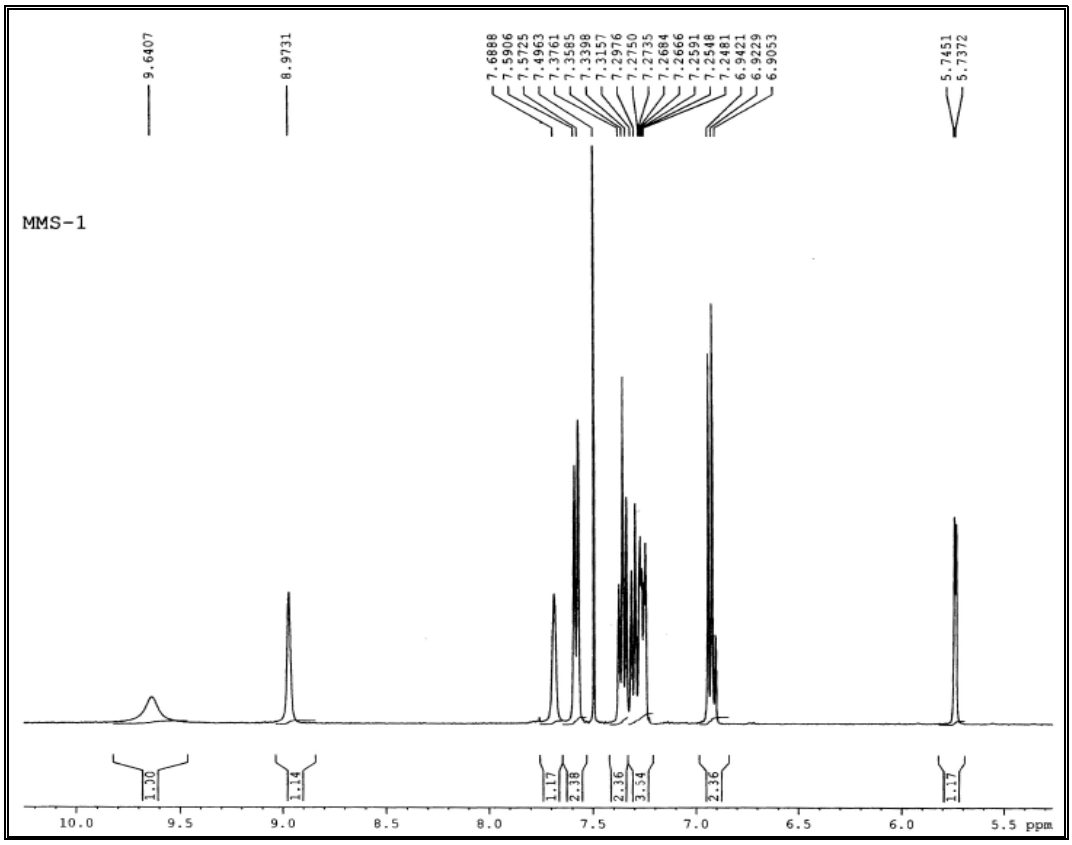
3,4-dihydro-6-(2-hydroxyphenyl)-4-(2-methoxyphenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-19): Yellow solid; mp 248-250 °C; IR (KBr): 3658, 3312, 2914, 1727, 1533, 1171 cm^{-1}; MS *m/z*: 341(M^+); Anal. Calcd. for $C_{17}H_{15}N_3O_5$: C, 59.83; H, 4.43; N, 12.31%. Found: C, 59.70; H, 4.38; N, 12.25%.

3,4-dihydro-6-(2-hydroxyphenyl)-4-(2-methoxy-4-nitrophenyl)-5-nitropyrimidin-2(1*H*)-one (MMS-20): Lemon yellow solid; mp 258-260 °C; IR (KBr): 3605, 3373, 2934, 1722, 1565, 1106 cm^{-1}; MS *m/z*: 386(M^+); Anal. Calcd. for $C_{17}H_{14}N_4O_7$: C, 52.85; H, 3.65; N, 14.50%. Found: C, 52.67; H, 3.58; N, 14.43%.

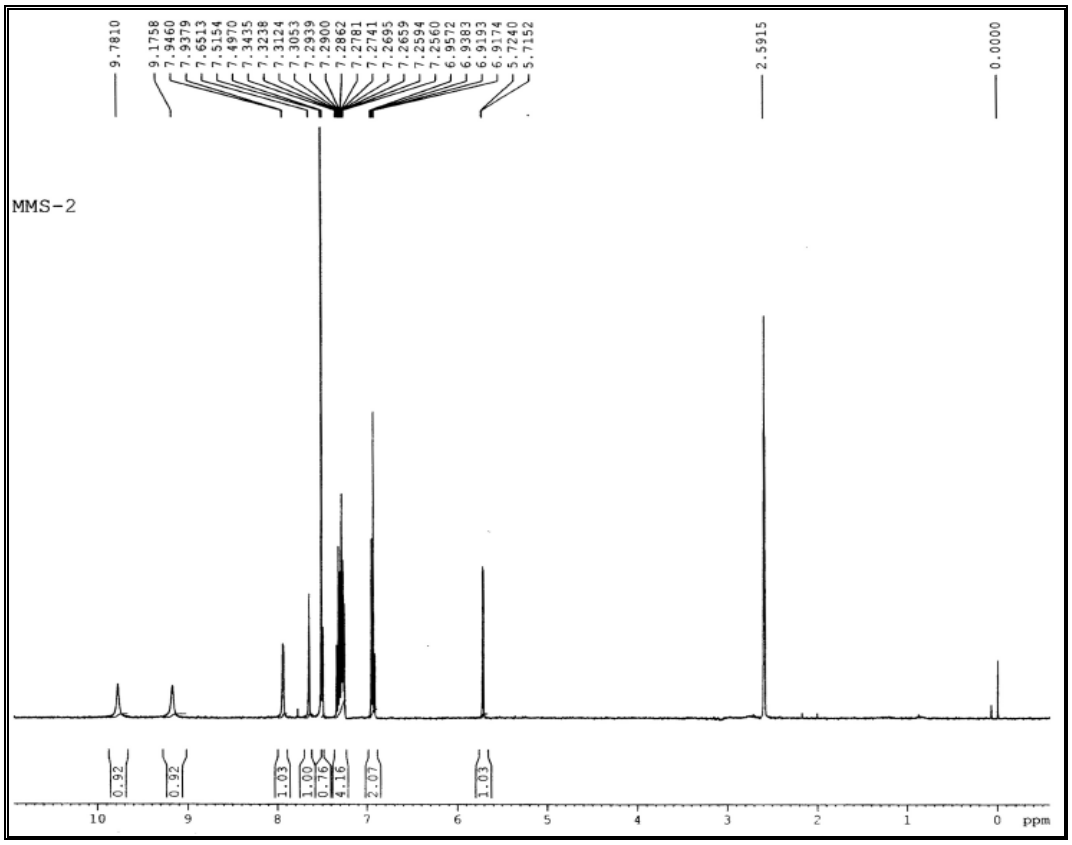
^{1}H NMR spectrum of compound MMS-1



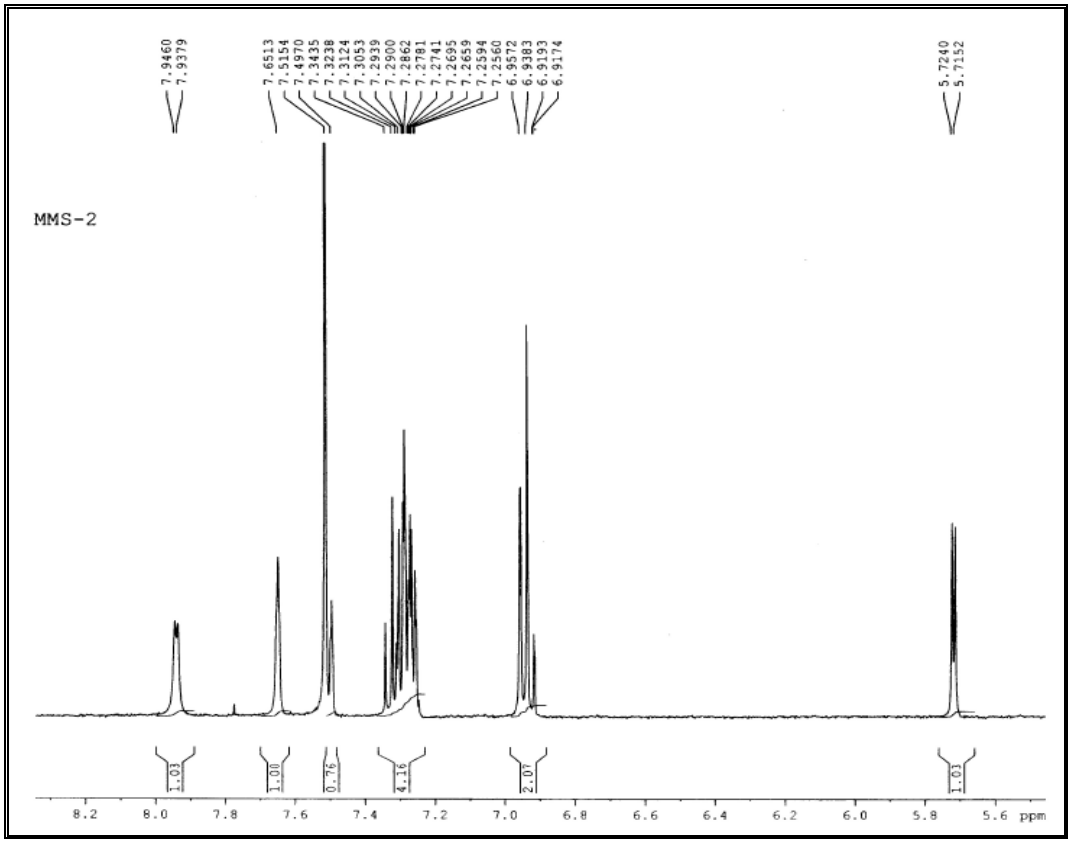
Expanded 1H NMR spectrum of compound MMS-1



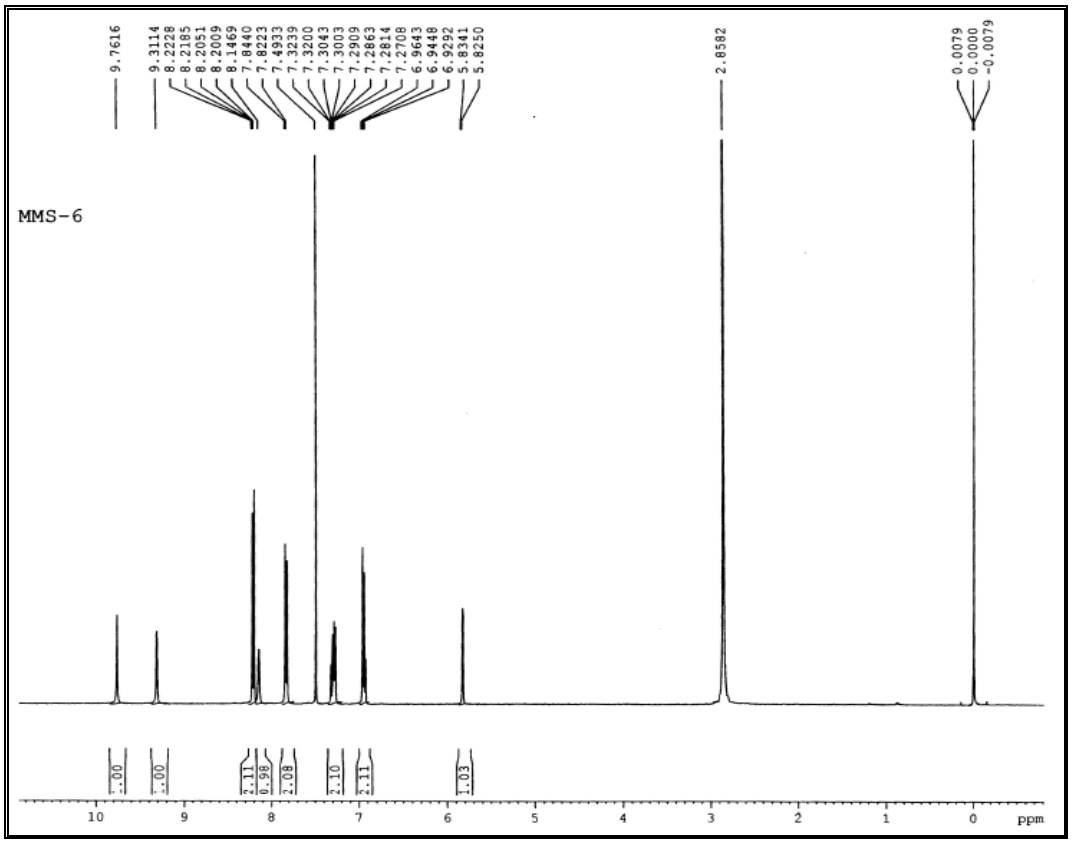
^{1}H NMR spectrum of compound MMS-2



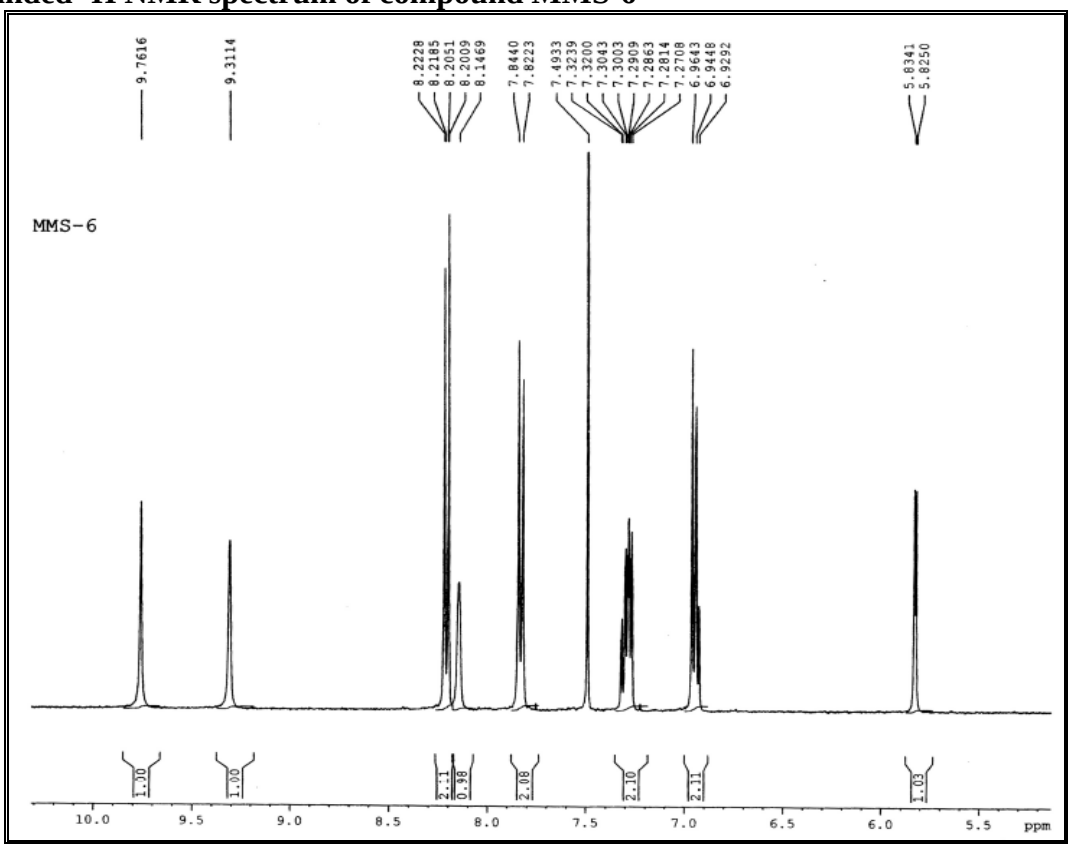
Expanded ^{1}H NMR spectrum of compound MMS-2



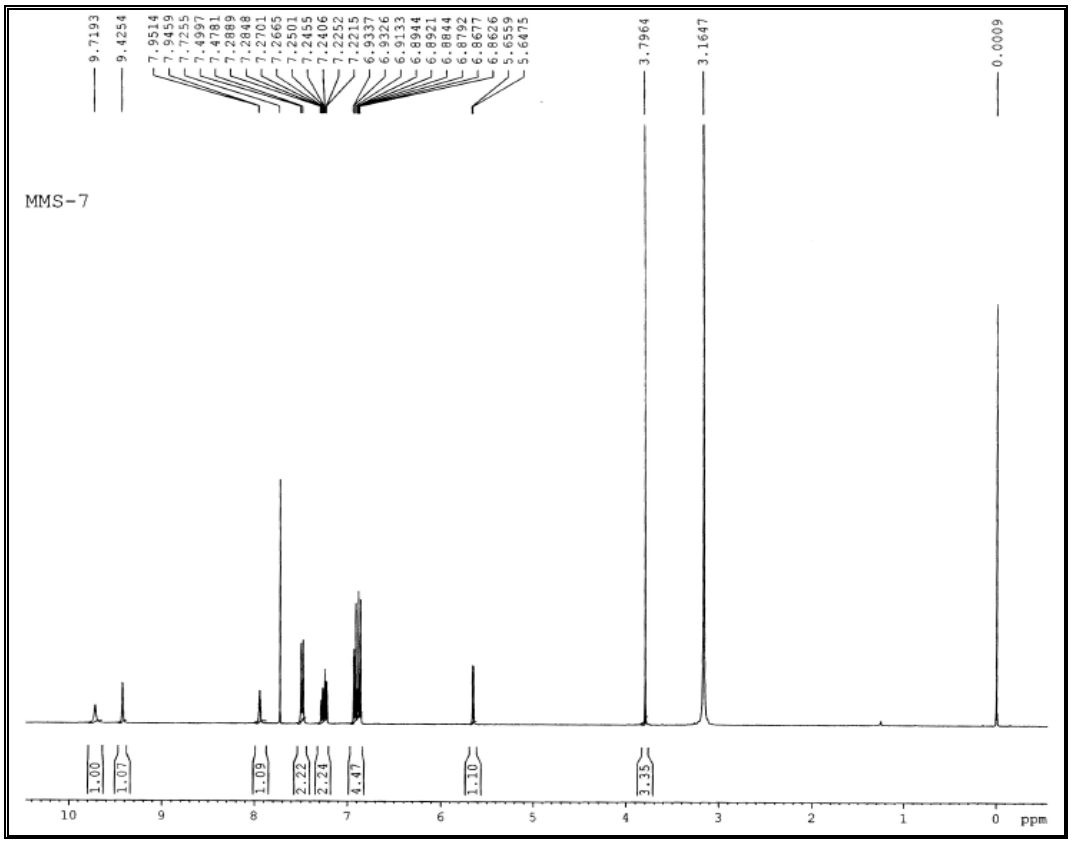
^{1}H NMR spectrum of compound MMS-6



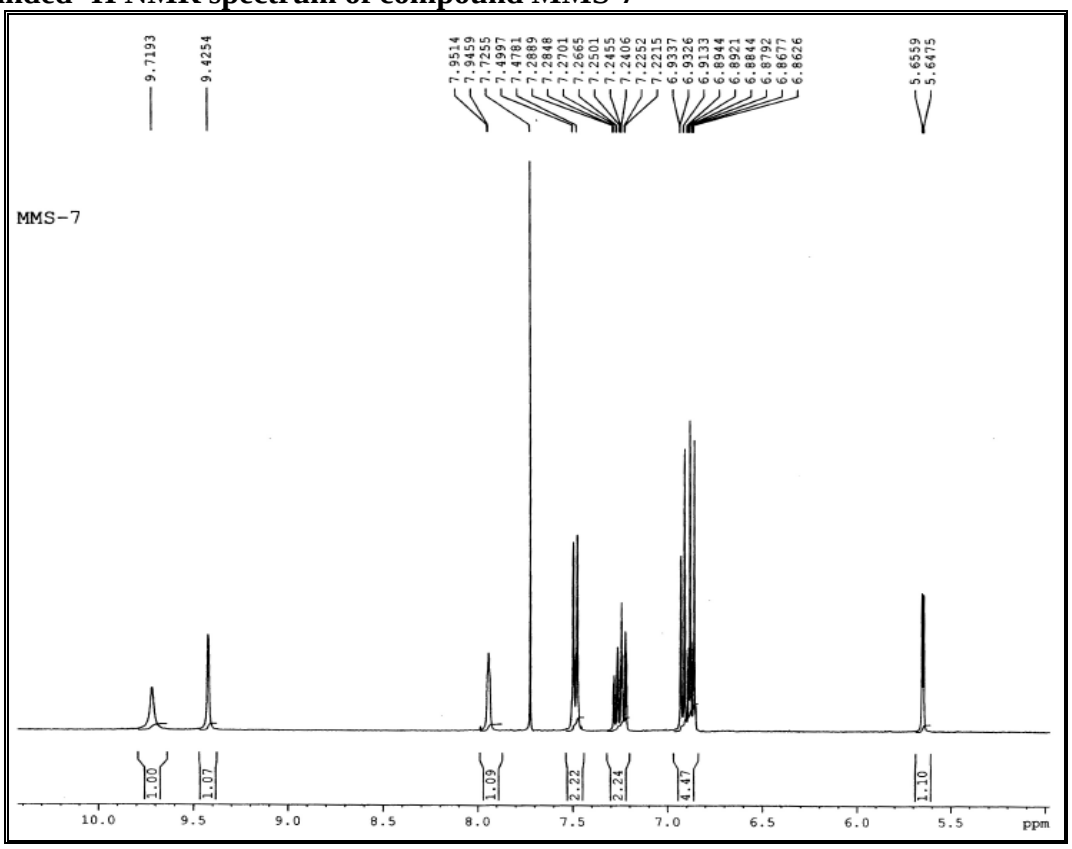
Expanded ^{1}H NMR spectrum of compound MMS-6



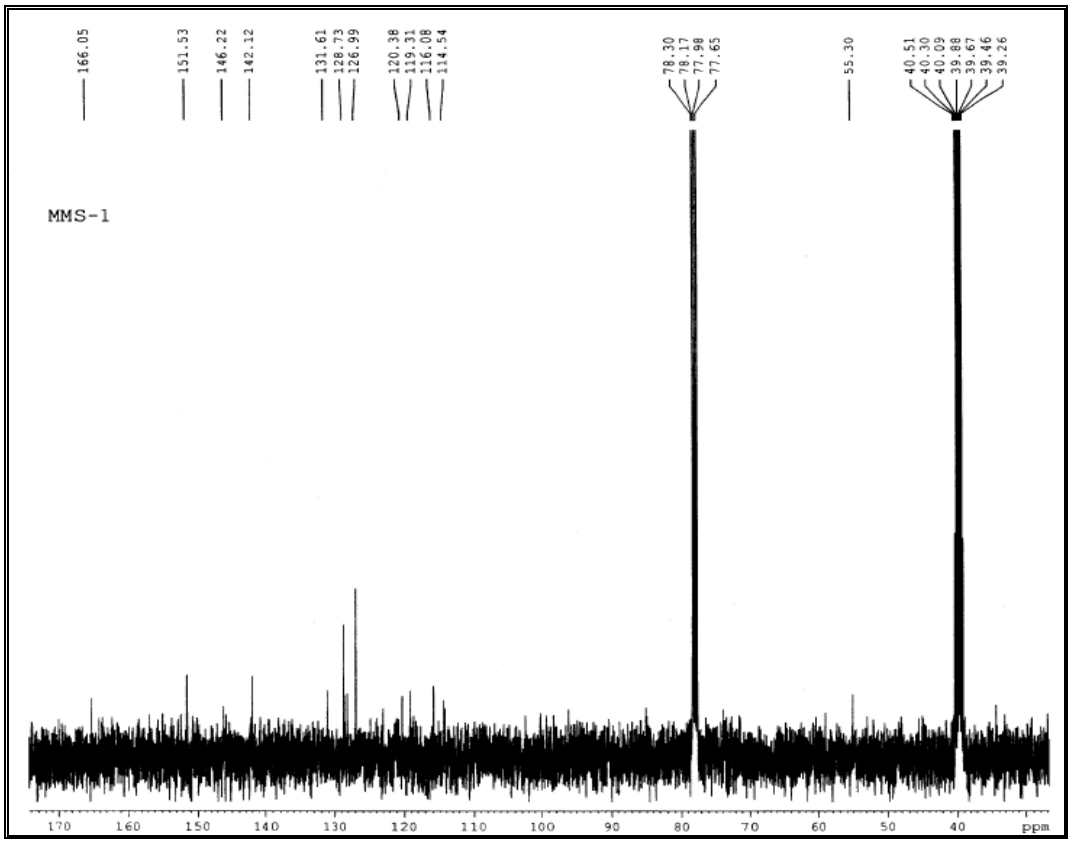
^{1}H NMR spectrum of compound MMS-7



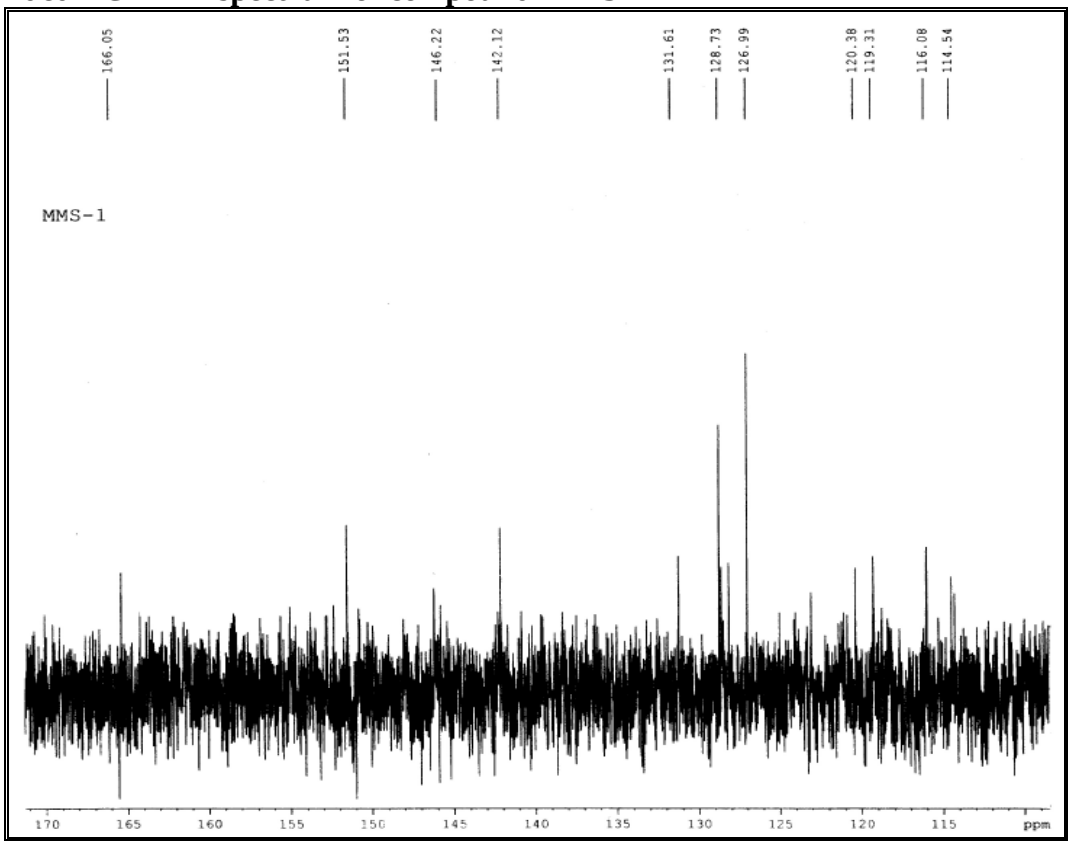
Expanded ^{1}H NMR spectrum of compound MMS-7



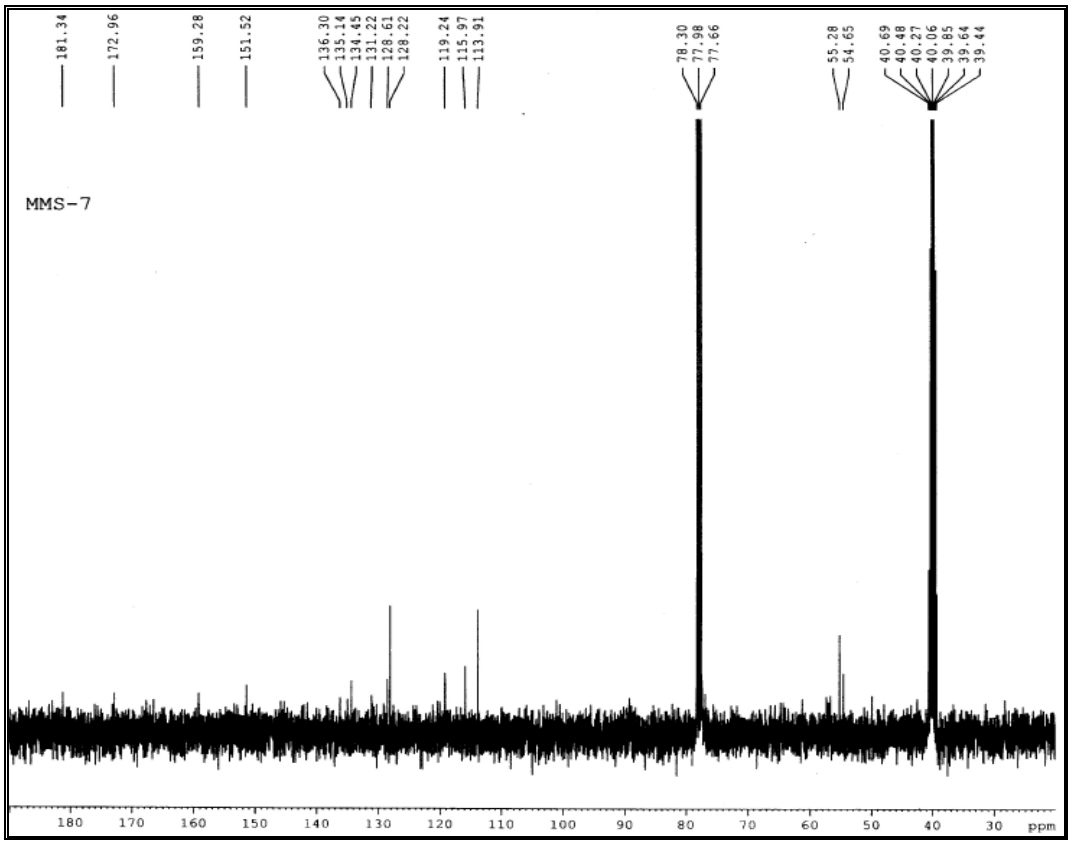
^{13}C NMR spectrum of compound MMS-1



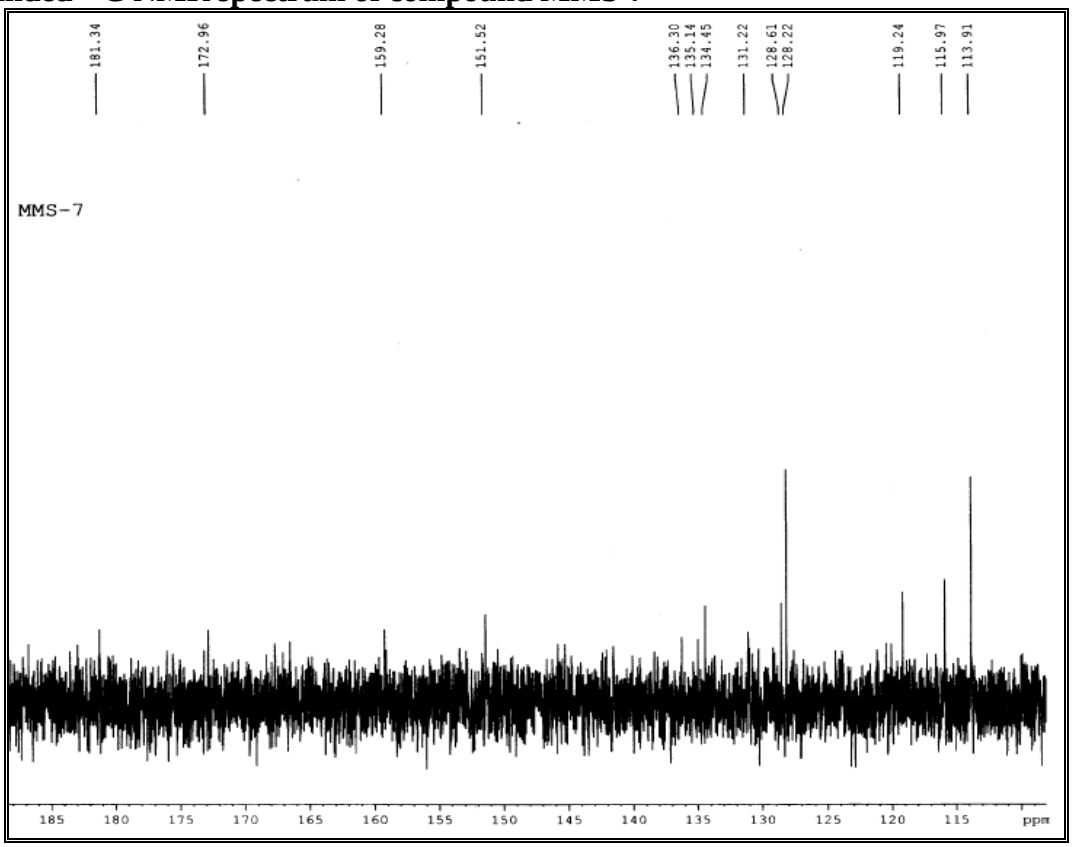
Expanded ^{13}C NMR spectrum of compound MMS-1



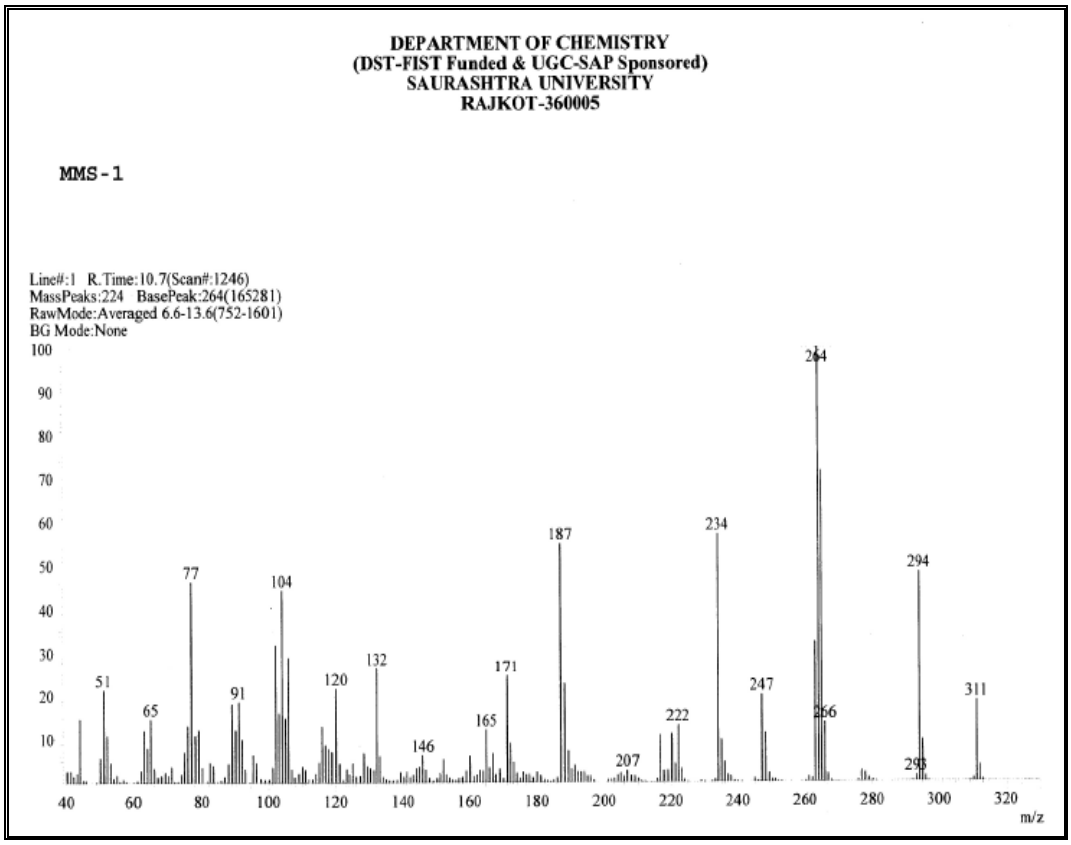
^{13}C NMR spectrum of compound MMS-7



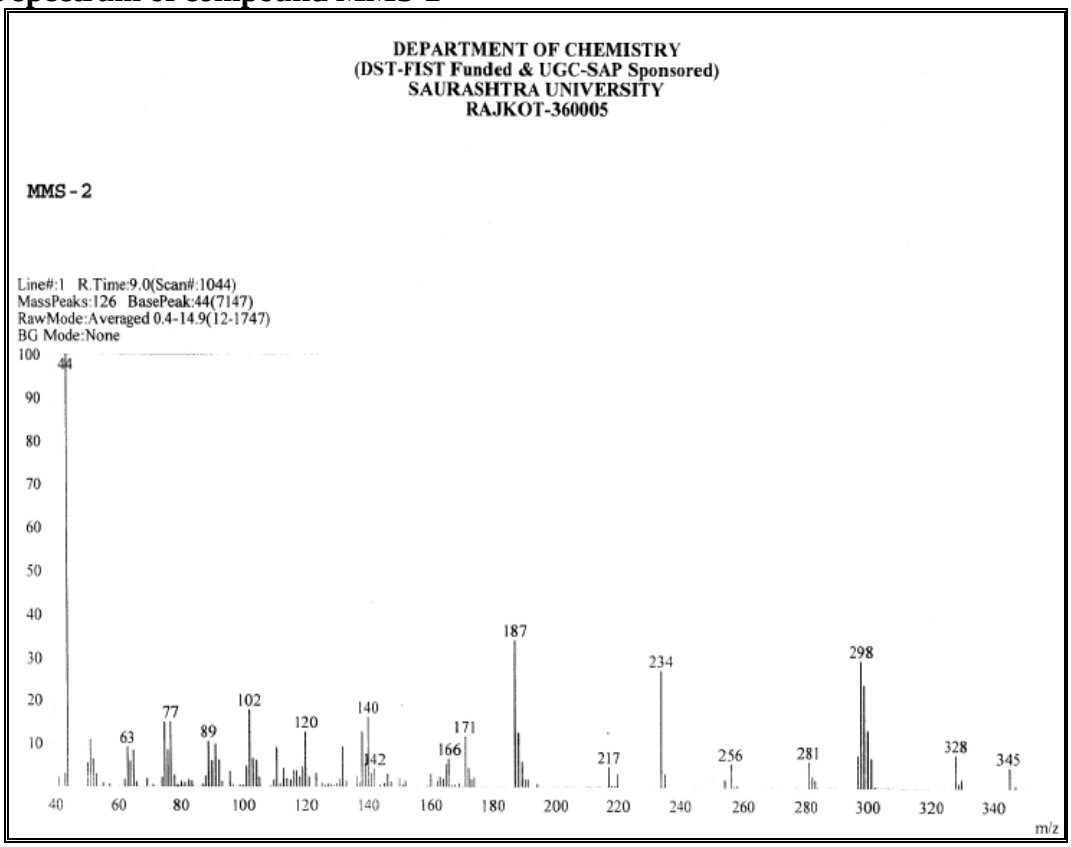
Expanded ^{13}C NMR spectrum of compound MMS-7



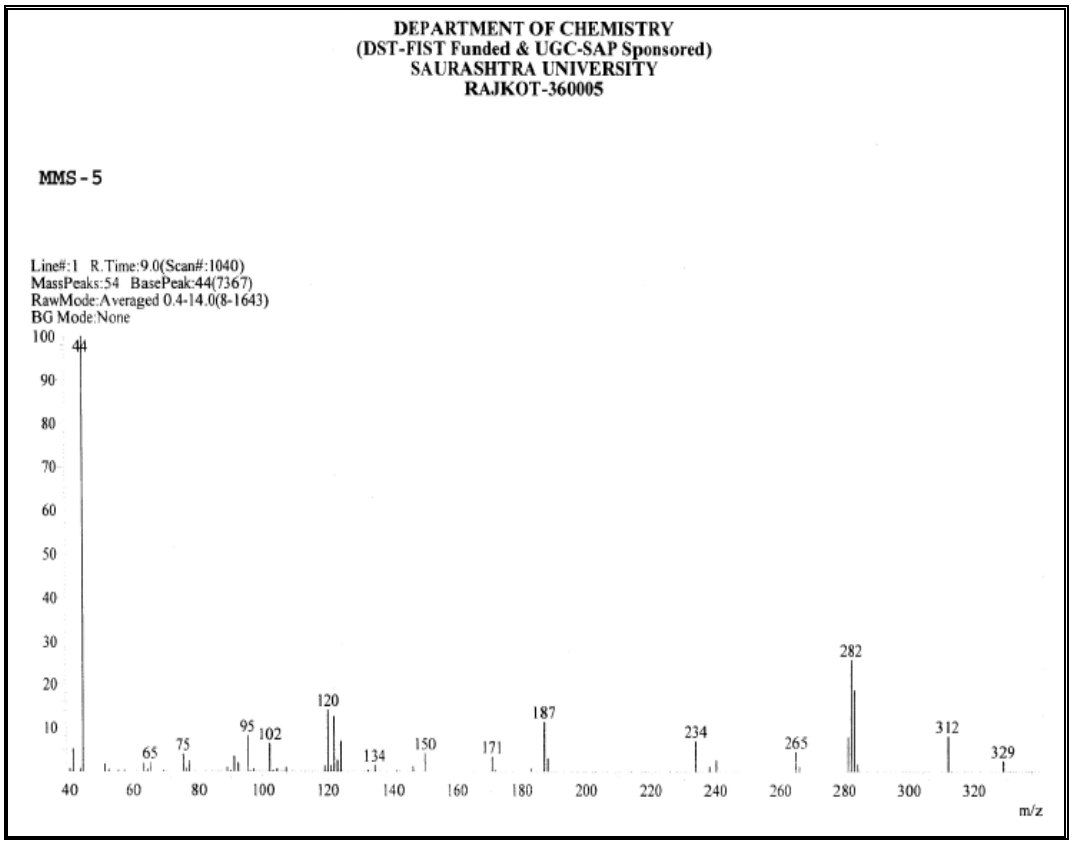
Mass spectrum of compound MMS-1



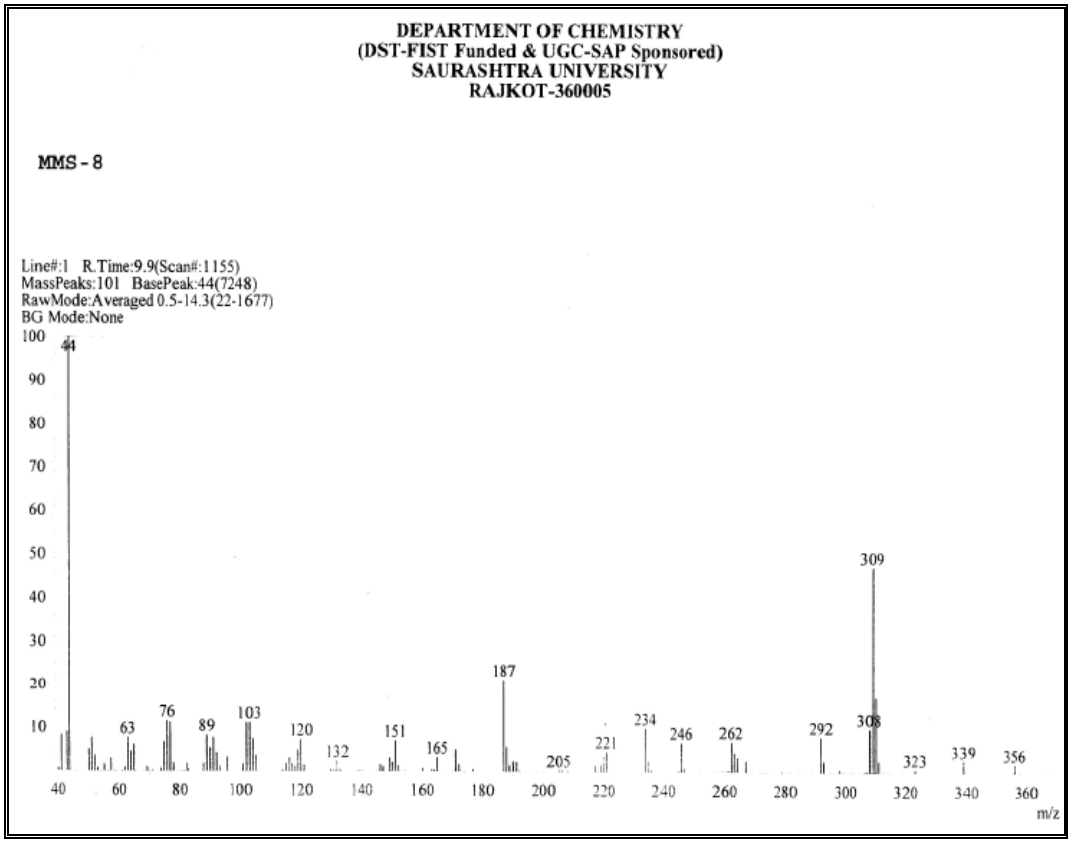
Mass spectrum of compound MMS-2



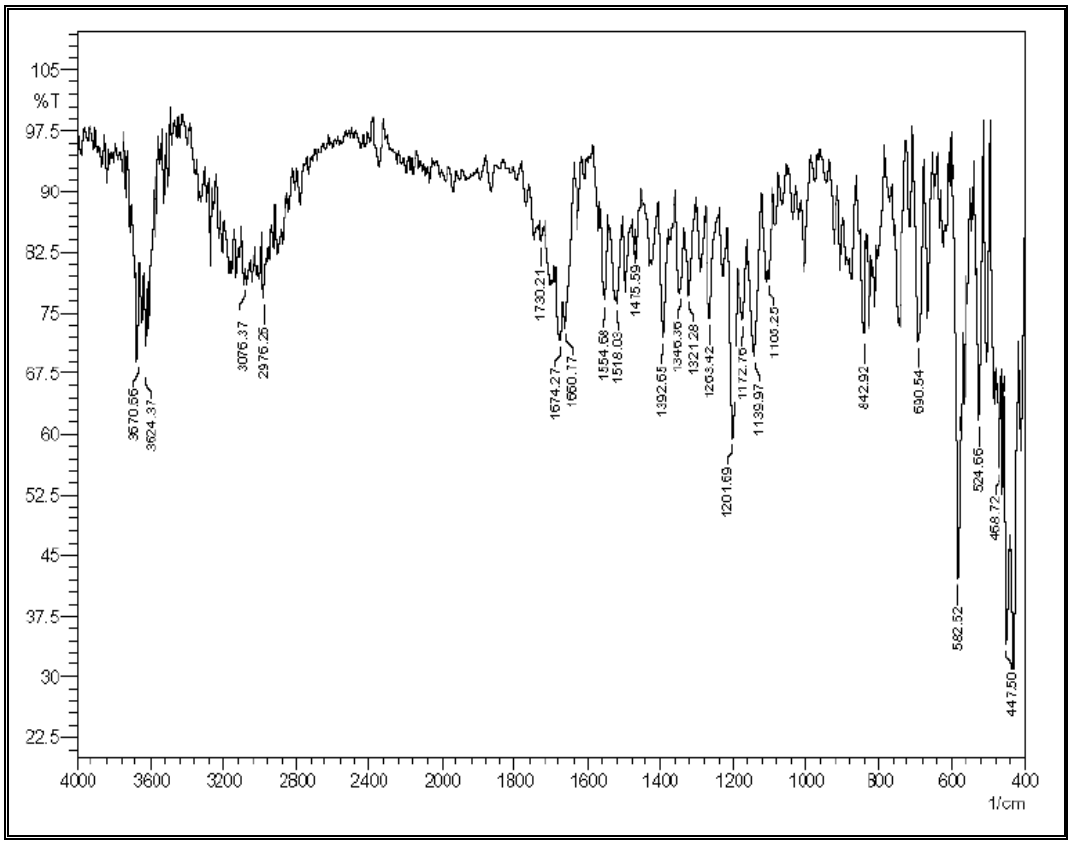
Mass spectrum of compound MMS-5



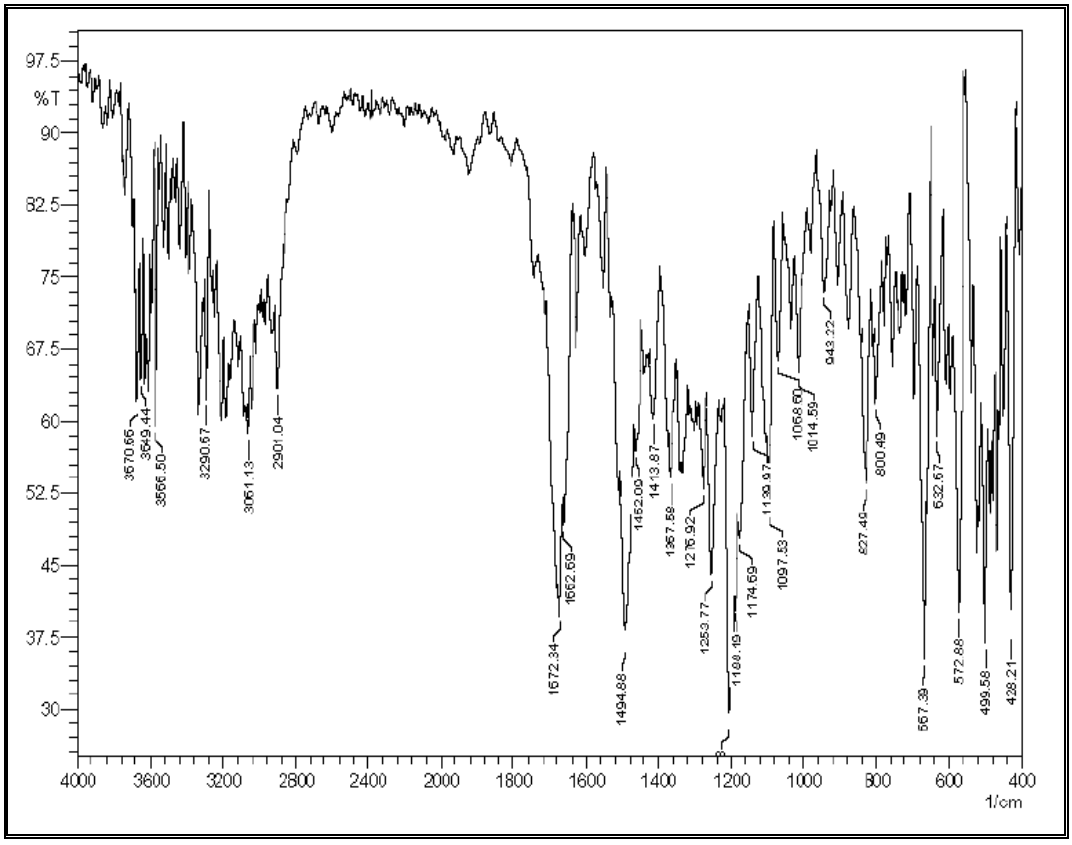
Mass spectrum of compound MMS-8



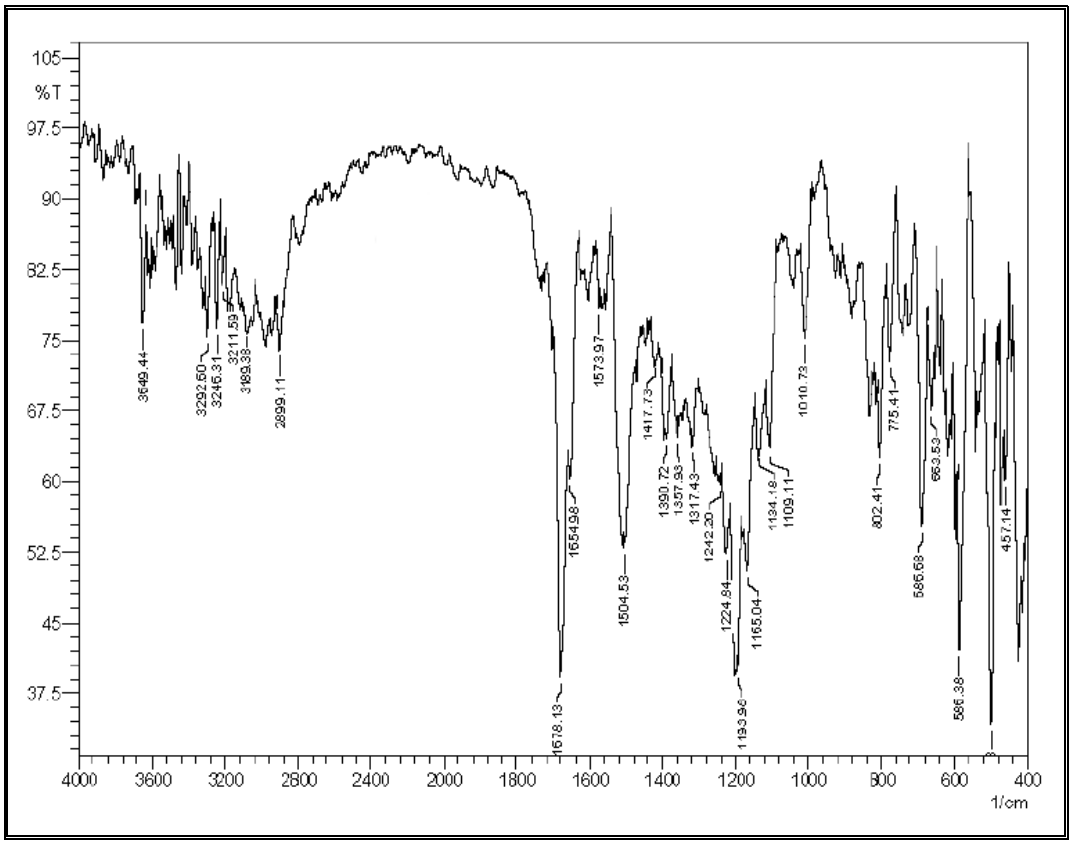
IR spectrum of compound MMS-1



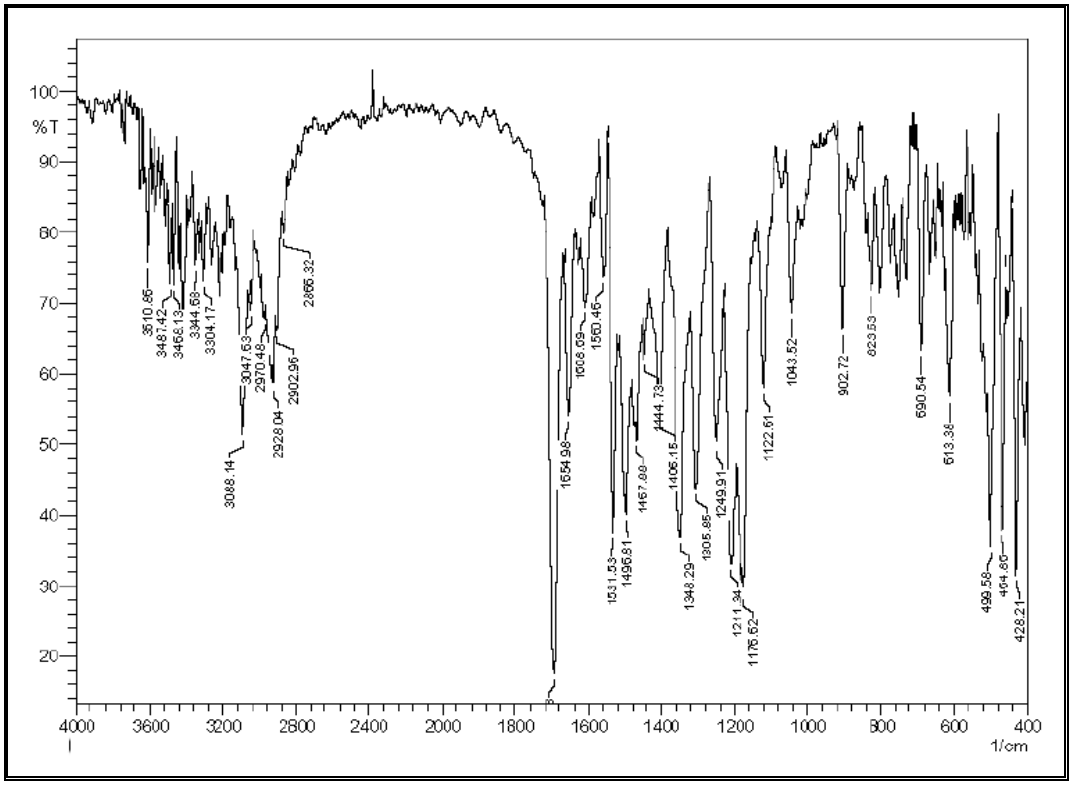
IR spectrum of compound MMS-2



IR spectrum of compound MMS-5



IR spectrum of compound MMS-7



1.10 REFERENCES

1. Mashkovskii, M. D.; Medicinals [in Russian], 12th ed., Meditsina, Moscow **1993**, Part 1, p. 736; **1993**, Part 2, p. 688.

2. Nikolaeva, N. B. (ed.), Medicinal Products from Foreign Firms in Russia [in Russian], Astrafarmservis, Moscow, **1993**, p. 720.

3. Biginelli, P. *Berichte*, **1891**, *24*, 1317.

4. Khanina, E. L.; Silinietse, G. O.; Ozol, Y. Y.; Dubur, G. Y.; Kimenis, A. A. *Khim. Farm. Zh*., **1978**, *10*, 72.

5. Cho, H.; Ueda, M.; Shima, K.; Mizuno, A.; Hayashimatsu, M.; Ohnaka, Y.; Nakeuchi, Y.; Hamaguchi, M.; Aisaka, K.; Hidaka, T.; Kawai, M.; Takeda, M.; Ishihara, T.; Funahashi, K.; Satoh, F.; Morita, M.; Noguchi, T. *J. Med. Chem*., **1989**, *32*, 2399.

6. Atwal, K. S.; Swanson, B. N.; Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; O'Reilly, B. C. *J. Med. Chem*., **1991**, *34*, 806.

7. Rovnyak, G. C.; Kimball, S. D.; Beyer, B.; Cucinotta, G.; DiMarco, J. D.; Gougoutas, J.; Hedberg, A.; Malley, M.; McCarthy, J. P.; Zhang, R.; Moreland, S.; *J. Med. Chem*., **1995**, *38*, 119.

8. Amine, M. S.; Nassar, S. A.; El-Hashash, M. A.; Essawy, S. A.; Hashish, A. A. *Ind.J. Chem*., Sect. B, **1996**, *35*, 388.

9. Desenko, S. M.; Lipson, V. V.; Gorbenko, N. I.; Livovarevich, L. P.; Ryndina, E. N.; Moroz, V. V.; Varavin, V. P. *Khim. Farm. Zh*., **1995**, *4*, 37.

10. Jelich, K.; Babczinski, P.; Sartel, H. J.; Smidt, R.; Strang, H.; German Patent No. 3,808,122; Ref. *Zh. Khim*., **1990**, 160433P.

11. Gsell, L. Eur. Pat. Appl. EP 375,613; *Chem. Abs*., **1990**, *113*, 231399.

12. Weis, A. L.; Van der Plas, H. C. *Heterocycles*, **1986**, *24*, 1433.

13. Weis, A. L.; Advances in Heterocyclic Chemistry, A. R. Katritzky (ed.), Academic Press, Orlando, etc., **1985**, Vol. 38, p. 3.

14. Evans, R. F.; The Pyrimidines, D. J. Brown (ed.), Wiley, New York, Chichester, Brisbane, Toronto, Singapore, **1994**, p. 737.

15. Rusinov, V. L.; Myasnikov, A. V.; Pilicheva, T. L.; Chupakhin, O. N.; Kiprianova, E. A.; Garagulya, A. D. *Khim. Farm. Zh*., **1990**, *1*, 39.

16. Pilicheva, T. L.; Rusinov, V. L.; Egorova, L. G.; Chupakhin, O. N.; Vladyko, G. V.; Korobchenko, L. V.; Boreko, E. I. *Khim. Farm. Zh*., **1990**, *1*, 41.

17. Vishnevskii, S. G.; Pirozhenko, V. V.; Chentsova, N. P.; Antonenko, S. V.; Barbasheva, E. V.; Grin, E. V.; Lyul'chuk, M. G.; Sorochinskii, A. E.; Remennikov, G. Y.; Luik, A. I.; Kukhar', V. P. *Khim. Farm. Zh*., **1994**, *12*, 44.

18. Tetko, I. V.; Tanchuk, V. Y.; Chentsova, N. P.; Antonenko, S. V.; Poda, G. I.; Kukhar, V. P.; Luik, A. I. *J. Med. Chem*., **1994**, *37*, 2520.

19. Rusinov, V. L.; Pilicheva, T. L.; Chupakhin, O. N.; Kovalev, G. V.; Komina, E. P.; *Khim. Farm. Zh*., **1986**, *8*, 947.

20. Remennikov, G. Y.; Shavaran, S. S.; Boldyrev, I. V.; Kapran, N. A.; Kurilenko, L. K.; Sevchuk, V. G.; Klebanov, B. M. *Pharm. Chem. Journal*, **1994**, *28*, 322.

21. Remennikov, G. Y.; Shavaran, S. S.; Boldyrev, I. V.; Kurilenko, Kapran, N. A.; B. M.; Shevchuk, V. G.; Klebanov, B. M. *Khim. Farm. Zh.*, **1994**, *5*, 25.

22. Remennikov, G. Y.; Shavaran, S. S.; Mokhort, M. A.; Slobodskoi, V. A.; Boldyrev, I. V.; Kurilenko, L. K.; Kapran, N. A.; Klebanov, B. M. *Physiologically Active Subs.*, **1993**, *25*, 69.

23. Remennikov, G. Y. *Chem. of Hetero. Comp.* **1997**, *33,* 1369.

24. Brown, D. J.; Comprehensive Org. Chem., Katritzky, A. R. and Rees, Ch. W (eds.), Pergamon, Oxford **1984**, Vol. 3, p. 117.

25. Sweet, F.; Fissekis, J. D. *J. Am. Chem. Soc*., **1973**, *95*, 8741.

26. Kappe, C. O. *Tetrahedron*, **1993**, *49*, 6937.

27. Mamaev, V. P.; Dubovenko, Z. D.; Izv. Sibirsk. Otd. Akad. Nauk SSSR. Ser. *Khim. Nauk*, **1972**, *3*, 101.

28. Remennikov, G. Y.; Shavaran, S. S.; Boldyrev, I. V.; Kapran, N. A.; Kurilenko, L. K. *Khim. Geterotsikl. Soedin*., **1993**, 3, 388.

29. Rusinov, V. L.; Chupakhin, O. N. Nitroazines, B. M. Vlasov (ed.), *Nauka, Novosibirsk,* **1991**, 347.

30. Rusinov, V. L.; Chupakhin, O. N.; Van der Plas, H. C. *Heterocycles*, **1995**, *40*, 441.

31. Makarov, V. A.; Sedov, A. L.; Nemeryuk, M. P.; Safonova, T. S. *Khim. Farm. Zh*., **1993**, *4*, 26.

32. Woznjak, M.; Van der Plas, H. C. *Acta Chem. Scand*., **1993**, *47*, 95.

33. Van der Plas, H. C.; Charushin, V. N.; Van Veldhuizen, B. *J. Org. Chem*., **1983**, *48*, 1354.

34. Janis, R. A.; Silver, P. J.; Triggle, D. J. *Adv. Drug Res*. **1987**, *16*, 309.

35. Bossert, F.; Vater, W. *Med. Res. Rev*. **1989**, *9*, 291.

36. Rovnyak, G. C.; Atwal, K. S.; Hedberg, A.; Kimball, S. D.; Moreland, S.; Gougoutas, J. Z.; O´Reilly, B. C.; Schwartz, J.; Malley, M. F. *J. Med. Chem*. **1992**, *35*, 3254.

37. Grover, G. J.; Dzwonczyk, S.; McMullen, D. M.; Normadinam, C. S.; Sleph, P. G.; Moreland, S. J. *J. Cardiovasc. Pharmacol*. **1995**, *26*, 289.

38. Triggle, D. J.; Padmanabhan, S. *Chemtracts: Org. Chem.* **1995**, *8*, 191.

39. Jauk, B.; Pernat, T.; Kappe, C. O. *Molecules,* **2000**, *5*, 227.

40. McConnell, J. D.; Bruskewitz, R.; Walsh, P.; Andriole, G.; Lieber, M.; Holtgrewe, H. L.; Albertsen, P.; Roehrborn, C. G.; Nickel, J. C.; Wang, D. Z.; Taylor, A. M.; Waldstreicher, J.; *N. Engl. J. Med.* **1998**, *338*, 557.

41. Roehrborn, C. G.; Oesterling, J. E.; Auerbach, S.; Kaplan, S. A.; Lloyd, L. K.; Milam, D. F.; Padley, R. J. *Urology* **1996**, *47*, 159.

42. Heible, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. *Pharmacol.Rev*. **1995**, *47*, 267-270.

43. Forray, C.; Bard, J. A.; Wetzel, J. M.; Chiu, G.; Shapiro, E.; Tang, R.; Lepor, H.; Hartig, P. R.; Weinshank, R. L.; Branchek, T. A.; Gluchowski, C.; *Mol. Pharmacol*. **1994**, *45*, 703.

44. Barrow, J. C.; Nantermet, P. G.; Selnick, H. G.; Glass, K. L.; Rittle, K. E.; Gilbert, K. F.; Steele, T. G.; Homnick, C. F.; Freidinger, R. M.; Ransom, R. W.; Kling, P.; Reiss, D.; Broten, T. P.; Schorn, T. W.; Chang, R. S. L.; O'Malley, S. S.; Olah, T. V.; Ellis, J. D.; Barrish, A.; Kassahun, K.; Leppert, P.; Nagarathnam, D.; Forray, C. *J. Med. Chem.* **2000,** *43,* 2703.

45. Nagarathnam, D.; Miao, S. W.; Lagu,, B.; Chiu, G.; Fang, J.; Murali Dhar, T. G.; Zhang, J.; Tyagarajan, S.; Marzabadi, M. R.; Zhang, F.; Wong, W. C.; Sun, W.; Tian, D.; Wetzel, J. M.; Forray,

C.; Chang, R. S. L.; Broten, T. P.; Ransom, R. W.; Schorn, T. W.; Chen, T. B.; O'Malley, S.; Kling, P.; Schneck, K.; Bendesky, R.; Harrell, C. M.; Vyas, K. P.; Gluchowski, C. *J. Med. Chem.* **1999,** *42,* 4764.

46. Atwal, K.; Rovnyak, G. C.; Schwartz, J.; Moreland, S.; Hedberg, A.; Gougoutas, J. Z.; Malley, M. F.; Floyd, D. M. *J. Med. Chem*. **1990**, *33*, 1510.

47. Atwal, K. S.; Rovnyak, G. C.; Kimball, S. D.; Floyd, D. M.; Moreland, S.; Swanson, B. N.; Gougoutas, J. Z.; Schwartz, J.; Smillie, K. M.; Malley, M. F. *J. Med. Chem*. **1990**, *33*, 2629.

48. Bryzgalov, A. O.; Dolgikh, M. P.; Sorokina, I. V.; Tolstikova, T. G.; Sedova, V. F.; Shkurko, O. P. *Bioorg. Med. Chem. Lett.,* **2006**, *16*, 1418.

49. Ma, Y.; Qian, C.; Wang, L.; Yang, M. *J. Org. Chem.,* **2000**, *65*, 3864.

50. Ranu, B. C.; Hajra, A.; Jana, U. *J. Org. Chem.* **2000**, *65,* 6270.

51. Heravi, M. M.; Derikvand, F.; Bamoharram, F. F. *J. Mol. Catal. A: Chem.,* **2005**, *242*, 173.

52. Heravi, M. M.; Bakhtiari, K.; Bamoharram, F. F. *Catal. Comm.,* **2006**, *7*, 373.

53. Zhou, H.; He, M.; Liu, C.; Luo, G. *Lett. In Org. Chem.***2006**, *3*, 225.

54. Jun, L.; Yinjuan B. *Synthesis,* **2002,** 466.

55. Jin, T. S.; Xiao, J. C.; Chen,Y. X.; Li, T. S. *J. of Chem.Res.,* **2004**, *3*, 190.

56. De, S. K.; Gibbs, R. A. *Synthesis,* **2005**, 1748.

57. Han, X.; Xu, F.; Luo, Y.; Shen , Q. *Eur. J. Org. Chem*., **2005**, *8*, 1500.

58. Badaoui, H.; Bazi, F.; Sokori, S.; Boulaajaj, S.; Lazrek, H. B.; Sebti, S; *Lett. in Org. Chem.,* **2005**, *2*, 561.

59. De, S. K.; Gibbs, R. A. *Syn Comm*, **2005** *35*, 2645.

60. Chen, X. H.; Xu, X. Y.; Liu, H.; Cun, L. F.; Gong, L. Z. *J. Am. Chem. Soc*. **2006**, *128*, 1480.

61. Sedova, V. F.; Krivopalov, V. P.; Shkurko, O. P. *Russ J. Org. Chem.*, **2007**, *43*, 90.

62. Bigdeli, M. A.; Jafari, S.; Mahdavinia, G. H.; Hazarkhani, H. *Catal. Comm.,* **2007**, *8*, 1641.

63. Yu, Y.; Liu, D.; Liu, C.; Luo, G. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 3508.

64. Chen, W. Y.; Qin, S. D.; Jin, J. R. *Catal.Comm.,* **2007**, *8*, 123.

65. Kumar, A.; Maurya, R. A. *J. Mol. Catal. A: Chem.,* **2007**, *272*, 53.

66. Wang, X.; Quan,, Z.; Zhang, Z. *Tetrahedron,* **2007**, *63*, 8227.

67. Ahmed, N.; Van Lier, J. E. *Tet. Lett.* **2007**, *48*, 5407.

68. Nandurkar, N. S.; Bhanushali, M. J.; Bhor, M. D.; Bhanage, B. M. *J. Mol. Catal. A:Chem*., **2007**, *271*, 14.

69. Shirini, F.; Marjani, K.; Nahzomi, H. T. *ARKIVOC,* **2007**, *1*, 51.

70. Nefzi, A.; Ostresh, J. M.; Houghton, R. A. *Chem. Rev.,* **1997**, *97*, 449.

71. Corbett, J. W. *Org. Prep. Proced. Int.,* **1998**, *30*, 489.

72. Ugi, I. *J. Prakt. Chem.,* **1997**, *339*, 499.

73. Domling, A. *Combi. Chem. High Throughput Scr.,* **1998**, *1*, 1.

74. Tietze, L. F.; Lieb, M. E. *Curr. Opin. Chem. Biol.,* **1998**, *2*, 363.

75. Dax, S. L.; McNally, J. J.; Youngman, M. A.; *Curr. Med. Chem.,* **1999**, *6*, 255.

76. Wipf, P.; Cunningham, A. *Tet. Lett.,* **1995**, *36*, 7819.

77. Li, W.; Lam, Y.; *J. Comb. Chem.,* **2005**, *7*, *721*.

78. Gross, G. A.; Wurziger, H.; Schober, A. *J. Comb. Chem.,* **2006**, *8*, 153.

79. Toy, P. H.; Janda, K. D. *Acc. Chem. Res.,* **2000**, *33*, 546.

80. (a) Gravert, D. J.; Janda, K. D. *Chem. Rev.,* **1997**, *97*, 489. (b) Wentworth, P.; Janda, K. D. *Chem. Comm.,* **1999**, *19*, 1917.

81. Fang, D.; Luo, J.; Zhou, X.; Ye, Z.; Liu, Z. *J. Mol. Catal. A: Chem.,* **2007**, *27*, 208.

82. Jean, C. L.; Jean, J. V. E.; Loic, T.; Jean, P. B. *ARKIVOC,* **2007**, *3*, 13.

83. Ming, L.; Si, G. W.; Rong, W. L.; Feng, L. Y.; Y. H. Zheng, *J. Mol. Catal. A: Chem.,* **2006**, *258*, 133.

84. (a) Caddick, S. *Tetrahedron,* **1995**, *51*, 10403. (b) Deshayes, S.; Liagre, M.; Loupy, A.; Luche, J.; Petit, A. *Tetrahedron,* **1999**, *55*, 10851. (c) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J.; *Tetrahedron,* **2001,** *57*, 9225. (d) Kirschning, A.; Monenschein, H.; Wittenberg, R. *Angew. Chem. Int. Ed.,* **2001**, *40*, 650. (e) Varma, R. S. *Pure Appl. Chem.,* **2001**, *73*, 193. (f) Loupy, A. *Microwaves in Organic Synthesis,* **2002,** pp. 499 Wiley-VCH: Weinheim.

85. Dandia, A.; Saha, M.; Taneja, H. *J. Fluorine Chem.,* **1998**, *90*, 17.

86. Diddams, P.; Butters, M. *Solid Supports and Catalysts in Organic Synthesis,* K. Smith; Ed.; Ellis Harwood and PTR Prentice Hall: New York and London, **1992**, pp. 3-39.

87. Gopalakrishnan, M.; Sureshkumar, P.; Kanagarajan, V.; Thanusu, J.; Govindaraju, R. *ARIKVOC,* 2006, *13***,** 130.

88. (a) Stefani, H. A.; Pereira, C. M. P.; Almeida, R. B.; Braga, R. C.; Guzen, K. P.; Cella, R. *Tet. Lett.,* **2005**, *46*, 6833. (b) Shen, Z. L.; Ji, S. J.; Wang, S. Y.; Zeng, X. F. *Tetrahedron,* **2005**, *61*, 10552.

89. Li, J. T.; Han, J. F.; Yang, J. H.; Li, T. S. *Ultrasonics Sonochem,* **2003**, *10*, 119.

90. Zhang, X.; Li, Y.; Liu, C.; Wang, J. *J. Mol. Catal. A: Chem.,* **2006**, *253*, 207.

91. Kumar, H.; Parmar, A. *Ultrasonics Sonochem.,* **2007**, *15*, 129.

92. Butler, A. R.; Brown, E. H. *ARKIVOC,* **2002**, 3, 166.

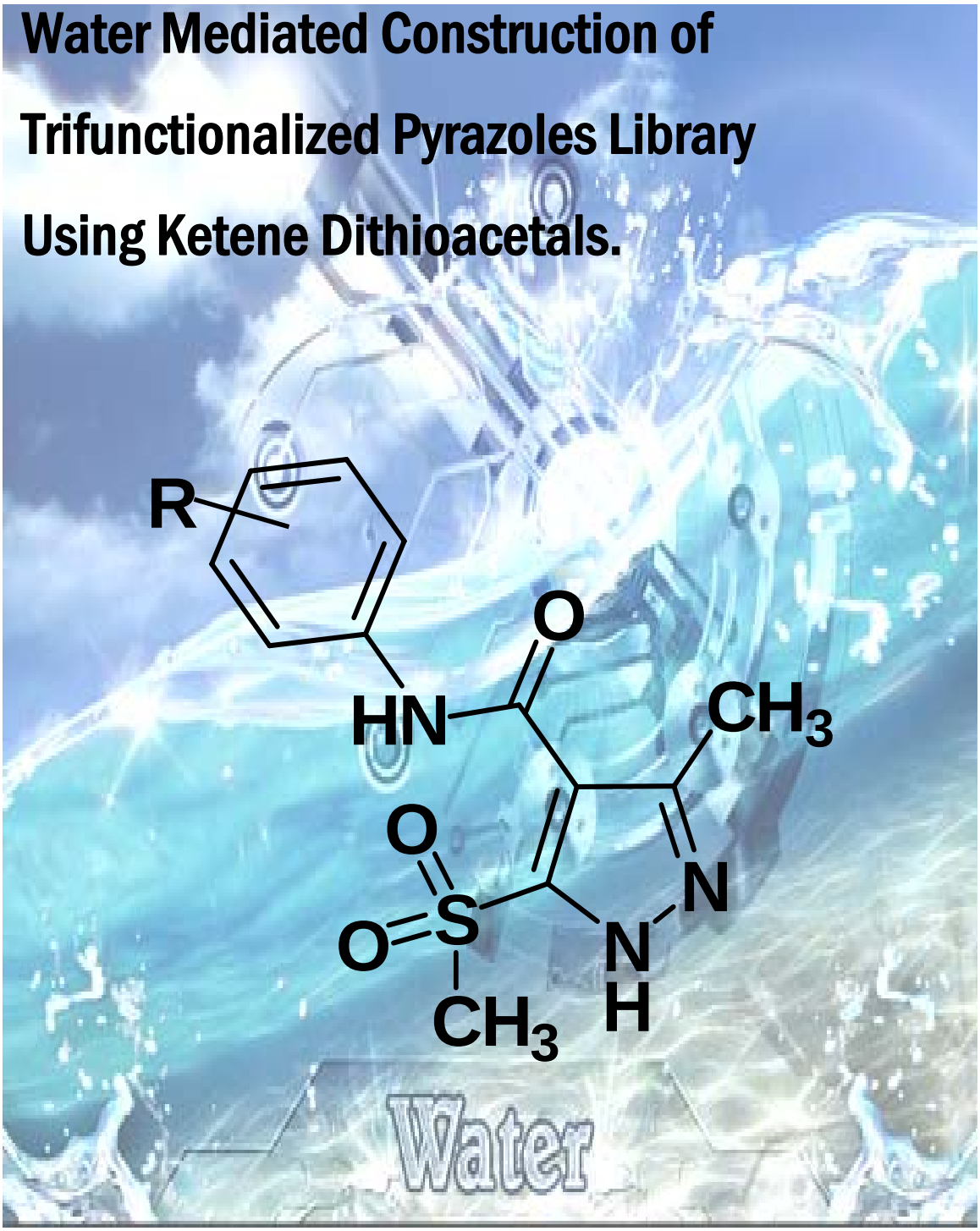
93. Pansuriya, A. M.; Savant, M. M.; Bhuva, C. V.; Singh, J.; Naliapara, Y. T.; *ARKIVOC*, **2009**, *7*, 79.

94. Merck Index, An encyclopedia of chemicals, drugs and biologicals, **2001**,13th ed, pp 683.

95. Pandey, S. C.; Haider, H.; Saxena, S.; Singh, M. K.; Thaper, R. K.; Dubey, S. K. **2006** WO134603 A1

96. (a) Folkers, K.; Johnson, T. B. *J. Am, Chem. Soc*., **1933**, *55*, 3784.(b) Kappe, C. O. *J. Org. Chem*., **1997**, *62*, 7201.

CHAPTER 2


Water Mediated Construction of
Trifunctionalized Pyrazoles Library
Using Ketene Dithioacetals.
R
O
HN
CH3
O
O
S
N
N
H
CH3
Water

2.1 INTRODUCTION

Pyrazoles are well known five member heterocyclic compounds and several procedures for its synthesis have been extensively studied (**Figure 1**). Such studies have been stimulated by various promising applications, especially in the case of highly substituted pyrazole derivatives. In fact, certain substituted pyrazoles are used as analgesic, anti-inflammatory, antipyretic, agrochemicals whereas some others are being studied for their medicinal interest. The pyrazole ring system consists of a doubly unsaturated five member ring containing two adjacent nitrogen atoms. The knowledge of such applications has pointed out that trisubstituted pyrazole are important target to be prepared to our interest on synthesis and molecular structure determination of some types of pyrazole.

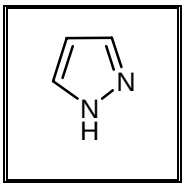


Figure 1

The discovery of pyrazole derivatives as antipyretic agents dates back to 1884, when the German chemist Ludwig Knorr[1] attempted to synthesize quinoline derivatives with antipyretic activity and accidentally obtained antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), which has analgesic, antipyretic and antirheumatic activity. Aminopyrine, a more potent analogue was synthesized there after and these drugs were widely used in market as antipyretics.

2.2 Biological activity of various substituted pyrazoles.

Pyrazole derivatives possessed diverse biological activities such as antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antibacterial, and sedative-hypnotic activity, cyclooxygenase-2 (Cox-2) inhibitors, IL-1 synthesis inhibitors, protein kinase inhibitors, as well as useful activities in conditions like schizophrenia, hypertension, and Alzheimer's disease.[2] In addition, they also have agrochemical properties including herbicidal and soil fungicidal activity; thus, they have been used as pesticides and insecticides[3] which are described briefly as follows.

Anti-inflammatory preparations are widely used in the modern clinic, as pathogenetic agents in the treatment of many illnesses and pathological processes, alone or more frequently in combination with other drugs. However many of the known anti-inflammatory agents cause a

range of side phenomena and complications in addition to the main effect. Consequently the search for and study of new more active anti-inflammatory agents of low toxicity is one of the urgent problems of contemporary science (**Figure 2**).[4]

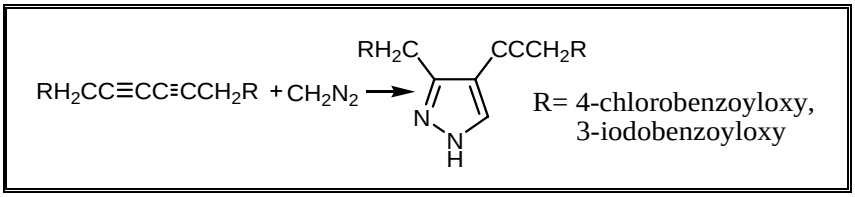


Figure 2

Tanitame A. et al[5] have synthesized pyrazole derivatives possesses antibacterial activity and inhibitory activity against DNA gyrase and topoisomerase IV. They have synthesized new pyrazole derivatives and found that 5-[(*E*)-2-(5-chloroindol-3-yl)vinyl]pyrazole (**Figure 3**) possesses potent antibacterial activity and selective inhibitory activity against bacterial topoisomerases. Many of the synthesized pyrazole derivatives were potent against clinically isolated quinolone coumarin-resistant Gram-positive strains and had minimal inhibitory concentration values against these strains equivalent to those against susceptible strains.

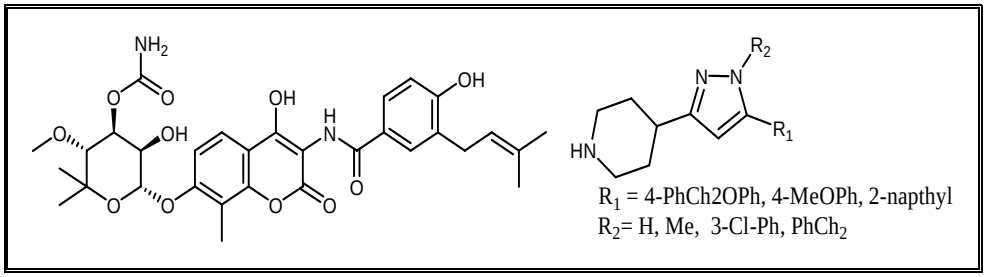


Figure 3

In 2004, Edwards P. J. et al[6] have synthesized numerous highly functionalized pyrazole derivatives (**Figure 4**) using various diketone and substituted hydrazine hydrate and screened for HIV mediated diseases. Among them such compounds were found to useful in the treatment of a variety of disorders including those in which the inhibition of reverse transcriptase is implicated. Disorders of interest include those caused by HIV and genetically related reteoviruses, such as AIDS.

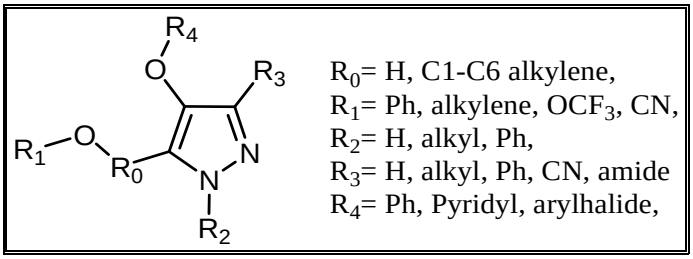


Figure 4

Bagley M. C. and co workers[7] have synthesized substituted *N*-pyrazole urea under the microwave irradiation. The reaction of substituted hydrazines and *β*-ketoesters afforded 5-aminopyrazoles in excellent yield, which can be transformed to the corresponding *N*-carbonyl derivatives by treatment with an isocyanate or chloroformate. Derivatization of 4-nitronaphth-1-ol using predominantly microwave heating methods and reaction with an *N*-pyrazole carbamate provides a rapid route to the *N*-pyrazole urea BIRB 796 (**Figure 5**) in high purity, as a potent and selective inhibitor of p38a mitogen-activated protein kinase for the study of accelerated ageing in Werner syndrome cells.

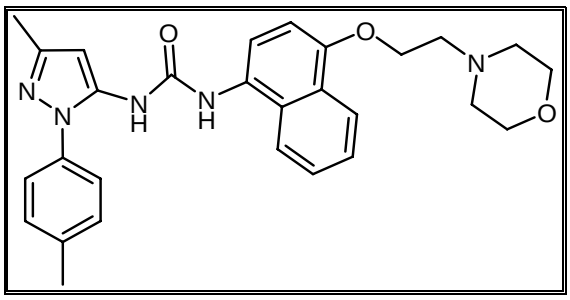


Figure 5

Stein R.G. et al[8] have combined the features of the pyrazole ring, a substituted quinoline, and an "antimalarial" side chain in one molecule for antimalarial testing. The key intermediate required was a 4-chloro-1*H*-pyrazolo [3,4-*b*]quinoline (**Figure 6**), in which the active C1 could be replaced with suitable amines expected to impart antimlalarial activity to the final products.

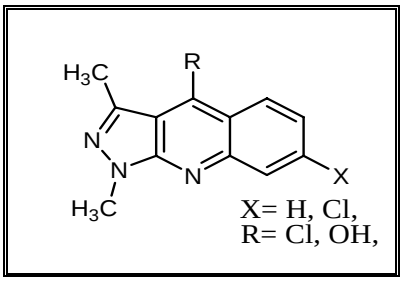


Figure 6

- **Biologically active molecules containing alkyl, sulfone and carboxamide functional groups**

Pyrazoles bearing sulfones and carboxamide moieties demonstrated to have significant pharmacological applications which are discussed as under.

The role of the cyclooxygenase-2 isoform in inflammation[9] and the attractiveness of COX-2 as a therapeutic target for the development of anti-inflammatory drugs are very well

recognized.[10] COX-2 selective inhibitors have proven to be effective anti-inflammatory and analgesic medicines with lower chronic gastrointestinal (GI) toxicity than traditional non-steroidal anti-inflammatory drugs (NSAIDs), which non-selectively inhibit COX-2 and COX-1. Prostaglandin (PG)-dependent and PG-independent factors are responsible for NSAID induced GI toxicity. Decreased PG production due to COX-1 inhibition may adversely affect mucus-bicarbonate secretion, acid secretion, and mucosal blood flow. COX inhibition may also elicit an increase in 5-lipoxygenase activity that would potentiate production of leukotriene-B4 and vasoconstrictor peptido-leukotrienes by the lipoxygenase pathway, and this may also contribute to the vascular and other mucosal damage induced by NSAIDs[11]Celecoxib (**Figure 7**) is one of the COX-2 selective inhibitors and are currently prescribed for the treatment of arthritis and inflammatory diseases. They show anti-inflammatory activity with reduced GI side effects.

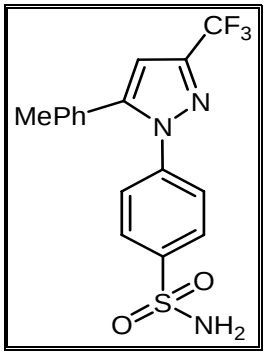


Figure 7

Sondhi S. M. et al[12] were carried out anti-inflammatory and analgesic activity of some amidine and hydrazone derivatives which possess sulfone group (**Figure 8**). The anti-inflammatory activity was carried out using carrageenin-induced paw edema assay and Analgesic activity evaluation was carried out using acetic acid writhing assay.

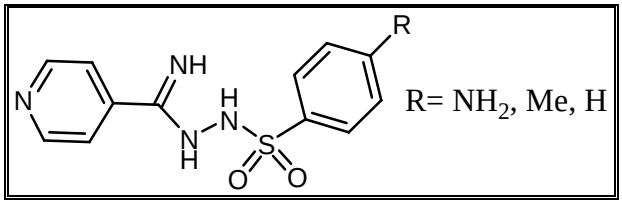


Figure 8

Moreover, Propargylic sulfones are known as pH-dependent DNA cleaving agents. In this context, Nishimoto S. et al[13] have designed a novel propargylic sulfone conjugated with an anthraquinone structure and evaluated its DNA binding and cleavage characteristics. The propargylic sulfone (**Figure 9**) showed high intercalating ability attributable to anthraquinone chromophore, leading to the efficient alkylation of DNA. The anthraquinone chromophore in

also acted as a photosensitizer and photoirradiation of this sulfone with DNA induced one-electron oxidation, resulting in the further DNA cleavage. Evaluation of the effect of propargylic sulfone against EMT6/KU cells revealed that it exhibited potent cytotoxicity, even without photoirradiation.

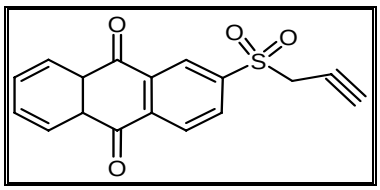


Figure 9

The synthesis of a series of novel pyrazoles containing a nitrate (ONO_2) moiety as a nitric oxide (NO)-donor functionality was reported by Ranatunge R. R. and coworkers.[14] Their COX-1 and COX-2 inhibitory activities in human whole blood were profiled and demonstrates that pyrazole ring substituents play an important role in COX-2 selective inhibition, such that a cycloalkyl containing pyrazole was found to be a potent and selective COX-2 inhibitor. Other modifications at the 3 position of the central pyrazole ring enhanced COX-2 inhibitory potency. Among the pyrazoles synthesized, the oxime (**Figure 10**) was identified as the most potent COX-2 selective inhibitor.

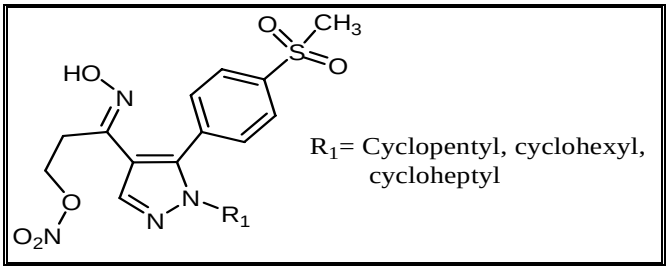


Figure 10

In addition, Bonacorso H. G. et al[15] have synthesized some novel *N*-substituted pyrazoles containing sulfone and trifluoromethyl groups at *N* and C5 position of pyrazole ring (**Figure 11**) and evaluated for antimicrobial activity. All the synthesized compounds were shown promising antimicrobial activity. The best activity was obtained when the structure possessed a 4-fluorophenyl substituent linked at the carbon-3 of the pyrazoline ring.

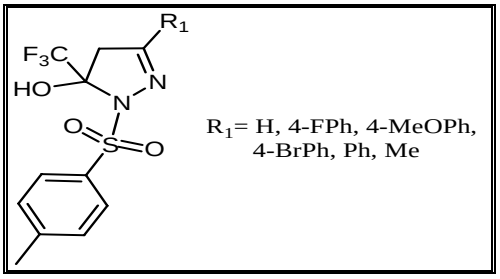


Figure 11

The identification of several potent pyrazole-based inhibitors of bacterial dihydroorotate dehydrogenase (DHODase) *via* a directed parallel synthetic approach is described below (**Figure 12**). The initial pyrazole-containing lead compounds were optimized for potency against Helicobacter pylori DHODase.[16] Using three successive focused libraries, inhibitors were rapidly identified with the following characteristics: *K*i<10 μM against H. pylori DHODase, sub-μg/mL H. pylori minimum inhibitory concentration activity, low molecular weight, and >10 000-fold selectivity over human DHODase.

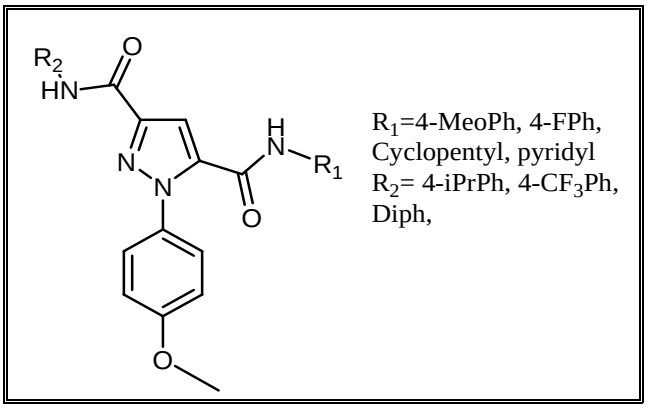


Figure 12

Helicobacter pylori is a Gram-negative microaerophilic bacterium that infects up to 50% of the world's human population.[17] *H.* pylori resides in the acidic surroundings of the stomach, utilizing a high urease enzyme activity to provide a locally alkaline environment. *H. pylori* has been implicated in numerous gastrointestinal disorders and is associated with gastric ulcers, gastritis, and gastric cancer.[18] The current treatment of *H. pylori* infections typically utilizes a multiple drug therapy involving at least one broad spectrum antibiotic (antimicrobial therapy) and a proton pump inhibitor (antisecretory therapy). However, a *H. pylori* specific antimicrobial would be very desirable; a specific agent should avoid many of the negative gastrointestinal side effects associated with a broad spectrum antibacterial resulting from eradication of the normal gastrointestinal flora.

2.3 Synthesis of functionalized pyrazole derivatives using various synthetic approaches

In 2005, Sakya S. M. et al[19] have offered fluoride-mediated nucleophilic substitution reactions of 1-(4-methylsulfonyl (or sulfonamido)-2-pyridyl)-5-chloro-4- cyano pyrazoles (**Figure 13**) with various amines and alcohols under mild conditions. The further reaction of novel pyrazoles provides the 5-alkyl amino and ether pyrazoles in moderate to high yields.

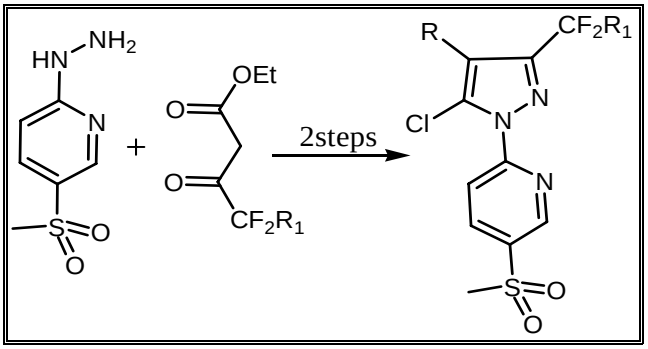


Figure 13

Recently, Dong D. and coworkers[20] have developed an efficient and divergent synthesis of fully substituted 1*H*-pyrazoles using cyclopropyl oximes (**Figure 14**). Under Vilsmeier conditions ($POCl_3$/DMF), substituted 1*H*-pyrazoles were synthesized from 1-carbamoyl, 1-oximyl cyclopropanes *via* sequential ring-opening, chlorovinylation, and intramolecular aza-cyclization.

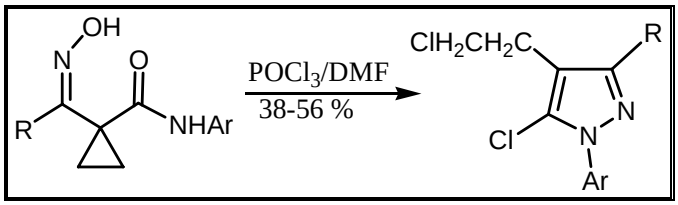


Figure 14

A novel approach to the synthesis of pyrazole derivatives from tosylhydrazones of *α,β*-unsaturated carbonyl compounds possessing a *β*-hydrogen was proposed by Rosa R. and coworkers (**Figure 15**),[21] exploiting microwave (MW) activation coupled with solvent free reaction conditions. The cycloaddition was studied on three ketones (*trans*-4-phenyl-3-buten-2-one, *β*-ionone and *trans*-chalcone). The corresponding 3,5-disubstitued-1*H*-pyrazoles were obtained in high yields and after short reaction times.

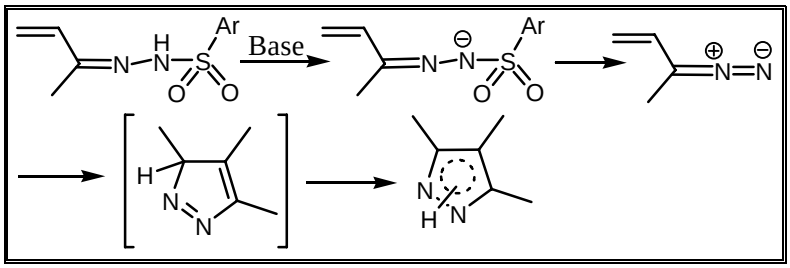


Figure 15

Tang L. et al[22] have synthesized the pyrazole analogs (**Figure 16**) from a common aryl isocyanide intermediate. The cyclization of isocyanide with the oxime or BOC-protected hydrazones of ethyl bromopyruvate furnished the pyrazole carboxy esters.

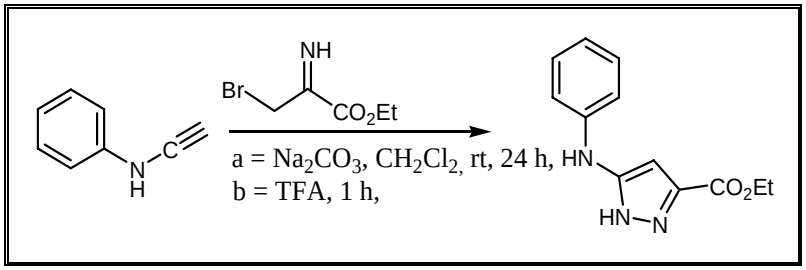


Figure 16

Kim J. N. et al[23] have reported the regio-selective synthesis of 1,3,4,5-tetrasubstituted pyrazole derivatives from the reaction of Baylis-Hillman adducts of alkyl vinyl ketone and hydrazine derivatives (**Figure 17**). During the continuous studies on the chemical transformations of Baylis-Hillman adducts including the synthesis of pyrazole.

Figure 17

Junjappa H. et al[24] have developed highly efficient and regioselective synthesis of 1-aryl-3,4-substituted/annulated-5-(methylthio)-pyrazoles and 1-aryl-3-(methylthio)-4,5-substituted/ annulated pyrazoles *via* cyclocondensation of arylhydrazines with either *α*-oxoketene dithioacetals or *α*-oxodithioesters (**Figure 18**).

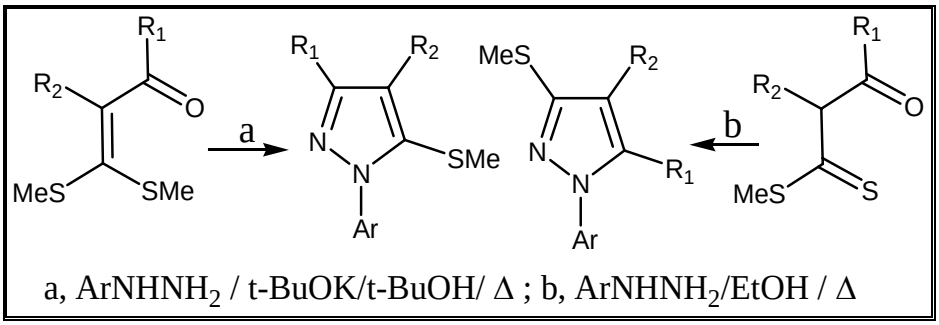


Figure 18

Elgemeie G. H. et al[25] were readily prepared novel ketene *N,S*-acetals by the reaction of cyanoacetamide or cyanothioacetamide with phenylisothiocyanate in the presence of potassium hydroxide, followed by alkylation of the produced salts with methyl iodide. Further, the reaction of ketene *N,S*-acetals with hydrazine afforded different substituted pyrazoles in excellent yields (**Figure 19**).

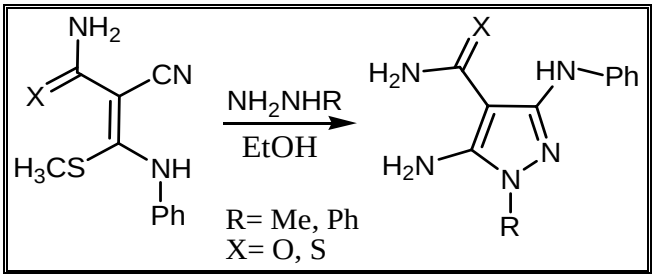


Figure 19

Junjappa H. et al[26] have demonstrated that 1-bis(methoxy)-4-bis(methylthio)-3-buten-2-one has been to a useful three carbon synthon for efficient regiospecific synthesis of a variety pyrazoles with mask or unmask aldehyde functionality by cyclocondensation with hydrazine hydrate in alcohol (**Figure 20**).

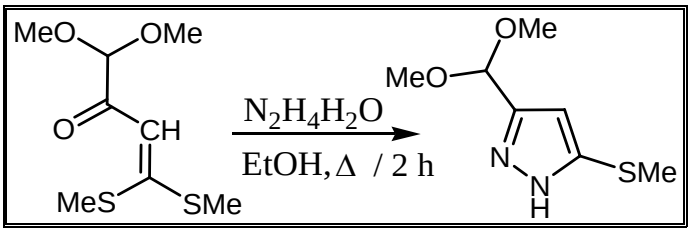


Figure 20

Kuettel S. et al[27] have synthesized 4-(3-phenylisoxazol-5-yl)morpholine derrivatives (**Figure 21**) using ketene dithioacetals. The reaction of substituted acetophenones with carbon disulfide in the presence of base and followed by alkylation with methyl iodide afforded 4-phenoxyphenyl-2,2- bis(methylthio)vinyl ketones, which were further reacts with hydrazine hydrate to give substituted pyrazoles through *in situ* cyclization of the resulting *N,S*-acetals.

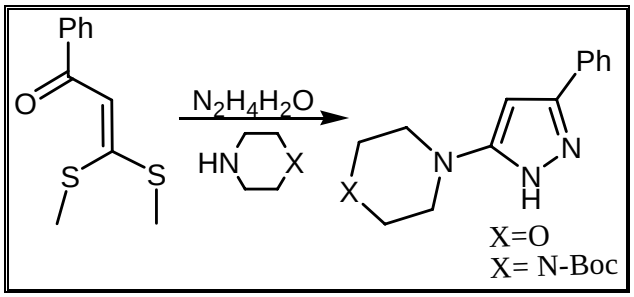


Figure 21

Kurz T. et al[28] have synthesized novel fluorinated ketene *N,S*-acetals by the reaction of fluorosubstituted cyanoacetamide derivatives with arylisothiocyanate in the presence of potassium hydroxide, followed by the alkylation with methyl iodide. The reaction of fluorinated ketene *N,S*-acetals with hydrazine afforded different fluorosubstituted pyrazole derivatives in good yield (**Figure 22**).

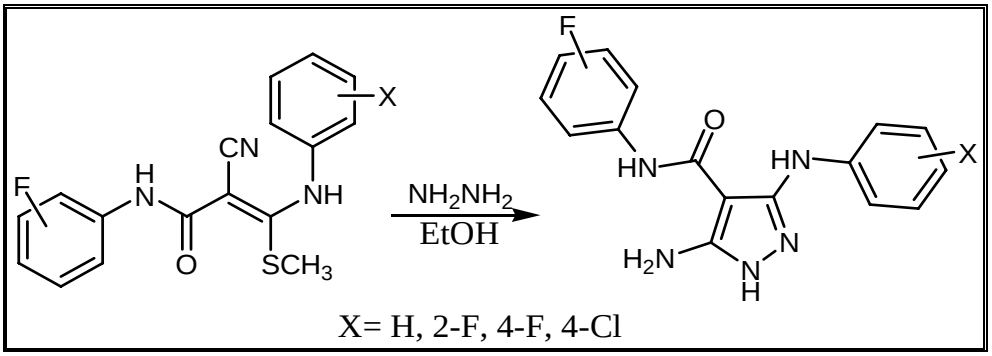


Figure 22

Elgemeie G. H. et al[29] synthesized variety of novel *α*-cyanoketene *S,S*-acetals, readily prepared by the reaction of cyanoacetanilides or cyanothioacetamide with carbon disulfide, followed by alkylation, react smoothly with nucleophile to afford variously substituted methylthio derivatives of pyrazole (**Figure 23**).

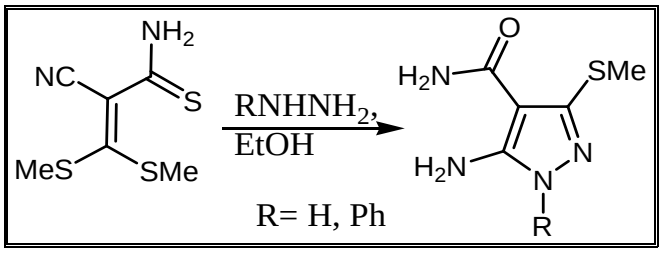


Figure 23

❖ **Synthesis of functionalized pyrazoles using combinatorial chemistry approach.**

In recent decades, combinatorial chemistry tools have enabled the rapid synthesis of a large number of heterocyclic small molecule libraries and it is recognized now as a key element of early drug discovery.[30] The main advantage of the combinatorial technique is the speed at which diverse types of organic compounds can be synthesized, formulated, and tested for a particular application. Moreover, in combinatorial study the quantity of required material is less in comparison to conventional methods, which makes it more suitable when the materials are expensive.[31]

In 2009, Laborde E. et al[32] have developed an efficient three-component, two-step "catch and release" solid-phase synthesis of 3,4,5-trisubstituted pyrazoles (**Figure 24)**. The reaction involves a base-promoted condensation of a 2-sulfonyl acetonitrile derivative **1** with an isothiocyanate **2** and *in situ* immobilization of the resulting thiolate anion **3** on Merrifield resin. Reaction of the resin-bound sulfonyl intermediate **4** with hydrazine, followed by release from the resin and intramoleculer cyclization, afforded 3,5-diamino-4-(arylsulfonyl)-

1*H*-pyrazoles **5**. However, this methodology has some drawback such as; long reaction time, isolation of product and high reaction temperature.

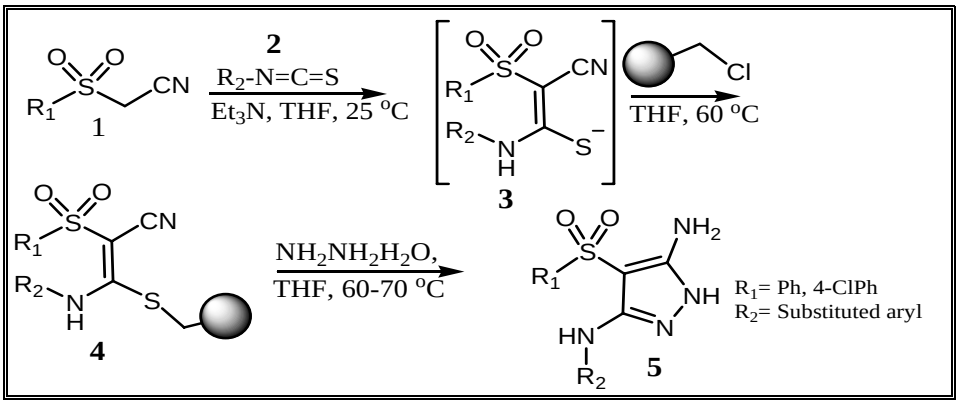


Figure 24

Recently, Laufer, S. et al[33] have synthesized structurally diverse and medicinally interesting series of 1, 4-dihydropyrano[2,3-*c*]pyrazoles *via* a three-component reaction using solution phase synthesis in excellent yields (**Figure 25**).

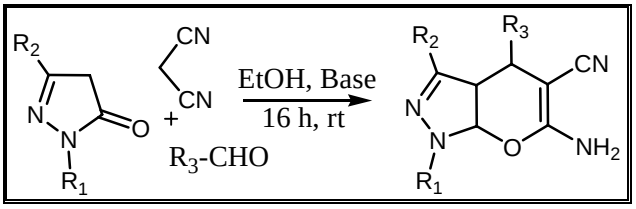


Figure 25

Ivachtchenko A. V. et al[34] have reported the parallel solution-phase approach of more than 2200 7-trifluoromethyl-substituted pyrazole[1,5-*a*]pyrimidine (**Figure 26**) and 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine carboxamides on a 50-100-mg scale. The reactions were include assembly of the pyrazole[1,5-*a*]pyrimidine ring by condensation of 5-aminopyrazole derivatives with the corresponding trifluoromethyl-*α*-diketones. The libraries from libraries were then obtained in good yields and purities using solution-phase acylation and reduction methodologies. Simple manual techniques for parallel reactions using special CombiSyn synthesizers were coupled with easy purification procedures (crystallization from the reaction mixtures) to give high-purity final products.

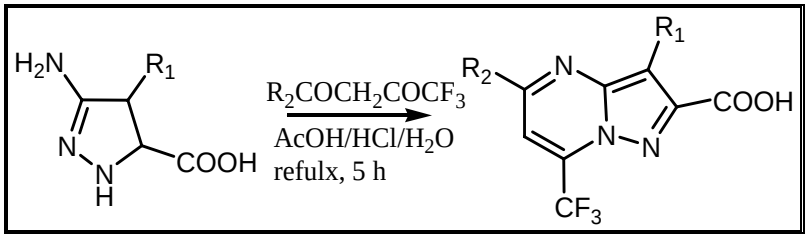


Figure 26

Yang H. Z. et al[35] have developed a small combinatorial library containing pyrazolyl-pyrazoles and pyrazole[1,5-a]pyrimidines (**Figure 27**) by traditional organic synthesis and parallel-liquid-phase combinatorial synthesis using *α-S,S*-acetal of ethyl cyanoacetate as key synthon and hydrazine hydrate.

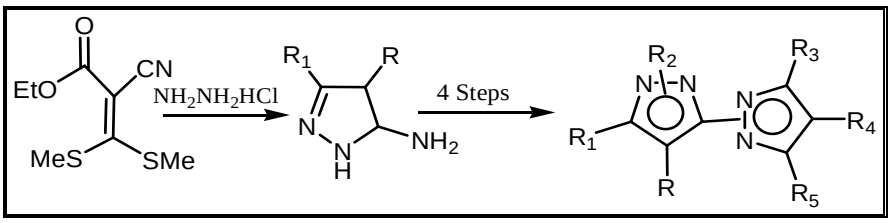


Figure 27

Organ M. G. et al[36] have developed a library of 4-(5-Iodo-3-Methylpyrazolyl)Phenyl-sulfonamide derivatives (**Figure 28**) *via* solution-phase Suzuki coupling using Pd/C as a solid-supported catalyst.

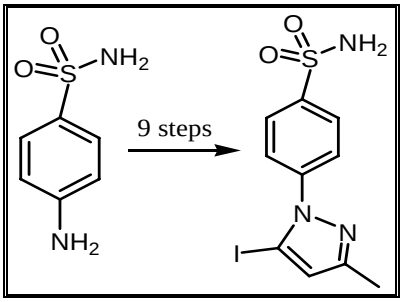


Figure 28

Taddei M. et al[37] have developed the libraries of substituted pyrazole through *in situ* generation of polymer-bound enaminones (**Figure 29**). The synthetic protocol makes use of commercially available aniline cellulose, a low-cost and versatile biopolymer, under very mild conditions. This new support allowed carrying out reactions in polar solvents under both conventional heating and MW irradiation without degradation of the polymer. The reaction between cellulose-bound enaminones and hydrazine to afford the target heterocycles in high yields directly in solution is the key step. The support can be conveniently recycled.

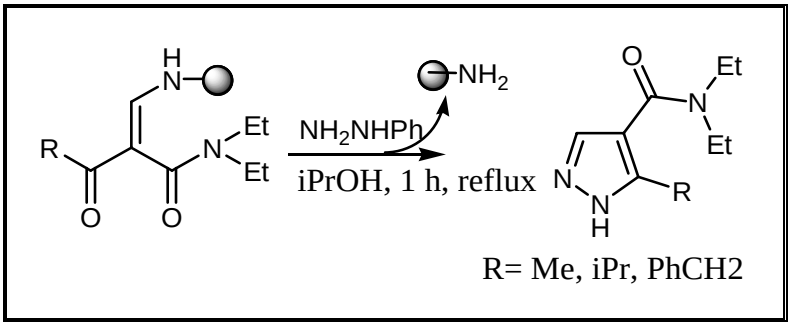


Figure 29

2.4 Various oxidizing agent for oxidation of sulfide to sulfones.

Functional group oxidation is a fundamental process in organic synthesis, and an enormous range of reagents, reagent combinations and conditions is available for almost every conceivable type of oxidative transformation. The search for new, modified and improved procedures continues unabated, however, driven largely by the need for higher efficiency and cleaner selectivity and, to an increasingly significant extent, by economic and environmental constraints. Exquisite selectivity can be achieved for some functional group oxidations,[38] especially for small scale laboratory operations, but for most transformations, and for larger scale applications in particular, the ultimate objective of cheap, safe oxidation under catalytic conditions has not been reached. The use of stoichiometric amounts of oxidizing agents will therefore necessarily continue until that goal is attained.

The cheapest oxidizing agents are air, chlorine and nitric acid, all of which have important uses. Each also has associated disadvantages and/or limitations:

(i)Air: the need for heterogeneous catalysts or biocatalysts; often rather poor selectivity; and high capital plant costs.

(ii)Chlorine: side reactions due to chlorination; environmental problems, especially in effluent disposal.

(iii)Nitric acid: limited selectivity in oxidation; side reactions; generation of nitrogen oxides.

The fourth cheapest oxidant is hydrogen peroxide, which is environmentally friendly and relatively easy to handle. However, it is quite a weak oxidizing agent which often requires specific activation towards the functional group to be transformed. The persalts such as; sodium percarbonate or sodium perborate one, means of providing such activation; they are also granular solids which can be an additional handling advantage in small to medium scale synthetic work.

In this context recently, Habibi D. et al[39] have developed a process for chemoselective catalytic oxidation of sulfides. A variety of aliphatic and aromatic sulfides were subjected to the sulfoxidation reaction by treatment of SPB and/or SPC, silica sulfuric acid (SSA) and catalytic amounts of KBr in the presence of wet SiO2 (50% w/w) in dichloromethane at room temperature with moderate to good yields (**Figure 30**).

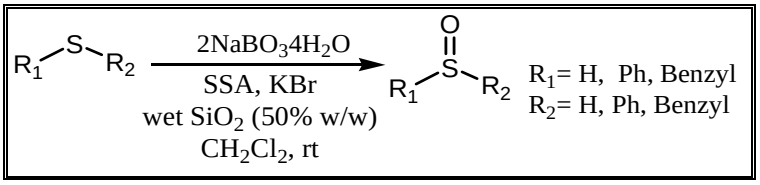


Figure 30

In addition, Kappe O. et al[40] were synthesized 2-substituted pyrimidines *via* sequential oxidation of 2- methylthiodihydropyrimidines with manganese dioxide and then with oxone to provide 2-methylsulfonyl-pyrimidines (**Figure 31**) which on nucleophilic displacement of the reactive sulfonyl group with nitrogen, oxygen, sulfur, and carbon nucleophiles afforded substituted pyrimidines.

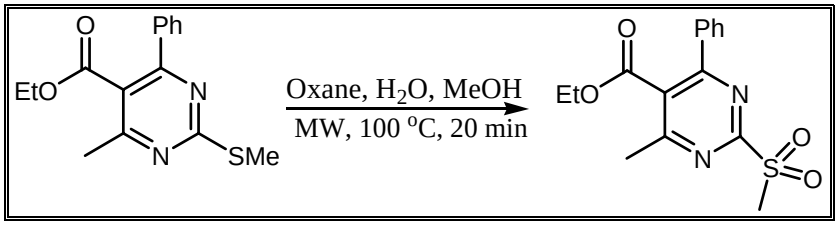
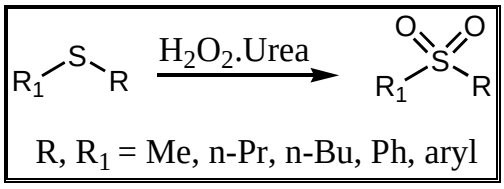


Figure 31

A survey of the literature[41] revealed that a adduct between H_2O_2 and urea (UHP) is a cheap, safe and stable source of pure H_2O_2 for oxidation. Several systems containing UHP with some acid anhydrides were used as efficient oxidizing agents for sulfides (**Figure 32**).



(**Figure 32**)

Moreover, a review[42] of literature reveled that the SPB (**Figure 33**) has been proved an efficient and excellent reagent for the oxidation of thiols and selenols to disulfides and diselenides, and of sulfides to sulfones.

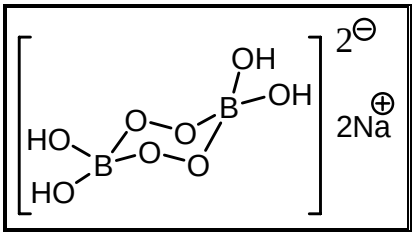


Figure 33

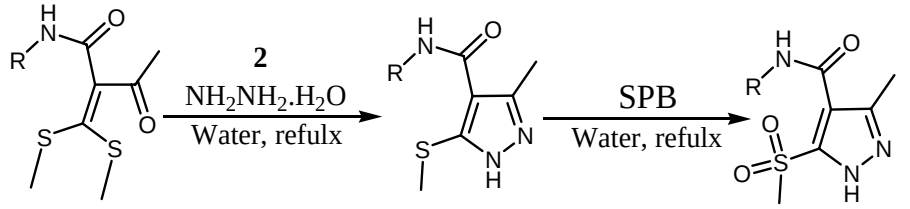
2.5 CURRENT RESEARCH WORK

The pyrazole nucleus is present in a wide variety of biologically interesting compounds, which exhibit antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antibacterial, hypoglycemic, sedative-hypnotic activity.[1-5] As we described, the tremendous biological potential of the sulfone group and carboxamide group bearing pyrazole scaffolds have attracted many chemists to synthesize this class of molecules. Thus, continuous efforts have been devoted to the development of general and versatile synthetic methodologies to this class of compounds. Many research groups have been synthesized pyrazole derivatives using various methods. However, the existing methods suffered with some drawbacks such as; long reaction time, product isolation, etc. Thus, the practical synthesis of structurally diverse pyrazole based small molecules is of great significance.

Nowadays, a great deal of effort has been focused on the field of green chemistry in adopting methods and processes. As a part of this “green” concept, toxic and/or flammable organic solvents are replaced by alternative non-toxic and nonflammable media. In this context, many efforts have been made to use aqueous media. Among alternative green solvents, water has been the solvent of choice for a variety of transformations.[21] On the other hand; functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the construction of substituted heterocycles. Given the importance of sulfone and carboxamide groups containing pyrazoles, and our ongoing interest on the synthesis various heterocycles using novel ketene dithioacetals starting from acetoacetanilides, encouraged us to utilize the novel ketene dithioacetals for the construction of small molecule library of 3-methyl-5-(methylsulfonyl)-*N*-aryl-1*H*-pyrazole-4-carboxamide derivatives in aqueous medium. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. The biological screening of the synthesized compounds is under process. Chemical purity of all the newly synthesized compounds was examined by UPLC.

2.6 RESULTS AND DISCUSSION

Scheme 1: Water mediated synthesis of pyrazoles containing methyl, sulfone and carboxamide groups.



1a-w **3a-w** **HPMS 1-23**

A series of various *α*-AKDTAs **1a-w** was prepared by some modification in reported procedure.[43] Initially, condensation of *α* -AKDTA **1a** with hydrazine hydrate **2** took place smoothly in isopropyl alcohol reflux to afford the 3-methyl-5-(methylsulfonyl)-*N*-phenyl-1*H*-pyrazole-4-carboxamide **3a** in good yield (Scheme 1; Table 1, entry 1). The condensation of **1a** with **2** to generate pyrazole **3a** was investigated using a variety of solvents, as a part of the "green chemistry" concept and to optimize the yield, and the results are summarized in Table 1.

Table 1: Synthesis of 3-methyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide using variety of solvents.

Entry	Solvents	Time h	Yield %
1	*i*PrOH	2.8	85
2	MeOH	4.0	81
3	EtOH	3.5	83
4	THF	4.5	79
5	CH_3CN	4.0	75
6	Dioxane	3.5	80
7	Water	3.0	97

The condensation reaction was clean in water and the yield of desired product was higher (Entry 7, Table 1). On the other hand, the reaction was relatively fast when *i*PrOH was used as a solvent with 12% lower yield (Entry 1, Table 1). The yield of desired product was reasonable when MeOH, EtOH and dioxane were used as a solvent (Entry 2,3,6, Table 1). The other solvents THF and acetonitrile gave lower yield with higher reaction time (Entry 4,5, Table 1). Thus, it is clear from the aforementioned experiments that the best yield of pyrazoles **3a** could be obtained by employing water as a solvent.

To test generality of the condensation and to realize synthesis of a small combinatorial library of substituted pyrazoles various α-AKDTAs were reacted with hydrazine hydrate to furnish pyrazoles **3a-w** in excellent yield using water as a solvent (Scheme 1, Table 2). The synthesized compounds were characterized by spectral data. The ^{1}H NMR spectra of compound **3c** displayed characteristic singlet for methyl, mehtylthio and methoxy hydrogen, respectively, at δ 2.54, 2.64 and 3.92. The two singlets appeared for pyrazole *NH* at δ 10.12 and amide hydrogen at δ 9.62 which revealed the formation of pyrazole ring.

Table 2: 3-mehtyl, 5-methylthio, 4-carboxamide substituted pyrazoles.

Entry	R	Time h	Yield %	mp ºC
3a	Ph	3.0	97	120-122
3b	4-CH_3Ph	3.5	95	125-127
3c	4-OCH_3Ph	3.0	96	118-120
3d	4-FPh	2.8	94	132-134
3e	2-OCH_3Ph	2.9	92	126-128
3f	2-CH_3Ph	3.2	93	122-124
3g	4-ClPh	3.8	94	128-130
3h	4-EtPh	3.5	95	130-132
3i	4-NO_2Ph	2.5	91	135-137
3j	3-Cl,4-FPh	3.2	90	128-130
3k	5-Cl,2-MeOPh	3.0	93	136-138
3l	2,5-diClPh	3.4	89	126-128
3m	2,5-diCH_3Ph	3.2	91	122-124
3n	4-Cl,2-CH_3Ph	2.9	94	121-123

3o	3,4-diFPh	3.2	93	130-132
3p	2-ClPh	3.5	94	142-144
3q	2-FPh	3.5	96	133-135
3r	4-BrPh	4.0	95	121-123
3s	3,4-diClPh	4.0	90	151-152
3t	3-NO_2Ph	3.2	88	115-117
3u	3-CH_3Ph	3.5	94	165-167
3v	2,3-diCH_3Ph	3.8	85	158-160
3w	2-OCH_3,4-NO_2Ph	4.0	96	148-150

Since, the remarkable utility of sulfone group in pharmaceutical and to develop library of pyrazole and isoxazole functionalized with alkyl, carboxamide and sulfone, we next planned to oxidize the sulfides to sulfones. Although sulfides can be easily oxidized by a wide variety of oxidizing reagents, but unfortunately, some of these reagents are not satisfactory for the oxidation of sulfide to sulfone due to low yields of products, toxicity and expensive reagents or catalysts.[44] the reaction condition for oxidation of sulfide to sulfone was optimized with variety of oxidizing agent in various solvent (Table 3).

Table 3: Optimization of the reaction condition for oxidation of 3a to its sulfone.

Entry[a]	Oxidant[b]	Solvent	Yield[c] %	Time min
1	mCPBA	CH_2Cl_2	74	125
2	mCPBA	Acetone	56	95
3	mCPBA	Water	65	75
4	SPB	CH_2Cl_2	79	110
5	SPB	Acetone	62	95
6	SPB	Water	91	60
7	SPC	CH_2Cl_2	59	120
8	SPC	Acetone	52	90
9	SPC	Water	75	60

[a]All solution-phase reactions were heated at reflux temperature of the solvent used. [b]Oxidant: *m*CPBA-2 equiv, SPB-3 equiv and SPC-3 equiv. [c]Isolated yield after purification.

The results gathered in table 3, indicate that when dichloromethane was used as a solvent the yield of sulfone was higher with *m*CPBA as compared to SPC and SPB, but it required high reaction time (Entry 1,4,7, Table 3). The yields of desired products were very poor when acetone was used as solvent and the products were isolated using column chromatography (Entry 2,5,8, Table 3). The best results were obtained when water was used as solvent with the SPB and the sulfide underwent oxidation to the corresponding sulfone in 45 min with excellent yield (Entry 6, Table 3). However, an excess amount of SPC did not improve yield. When the amount of SPB was reduced, the yield of desired product was lower. The above results indicate, the cheap, environmentally friendly and effective oxidizing agent in water was SPB and gave quantitatively yield of product without use of any activator. With this oxidizing system, all the synthesized pyrazoles were oxidized to generate sulfone containing pyrazoles and isoxazole based small molecule library using solution phase synthesis and the results are gathered in table 4.

Table 4: 3-mehtyl, 5-sulfone, 4-carboxamide functionalized library of pyrazoles.

Entry	R	Time min	Yield %	mp ºC
HPMS-1	Ph	60	91	168-170
HPMS-2	4-CH_3Ph	45	92	172-174
HPMS-3	4-OCH_3Ph	55	89	166-168
HPMS-4	4-FPh	50	88	175-177
HPMS-5	2-OCH_3Ph	60	90	170-172
HPMS-6	2-CH_3	50	94	165-167
HPMS-7	4-ClPh	55	89	173-175
HPMS-8	4-EtPh	65	92	177-179
HPMS-9	4-NO_2Ph	55	92	181-183
HPMS-10	3-Cl,4-FPh	50	91	176-178
HPMS-11	5-Cl,2-OCH_3Ph	50	93	186-188
HPMS-12	2,5-diClPh	65	88	176-178
HPMS-13	2,5-diCH_3Ph	60	87	170-172
HPMS-14	4-Cl,2-CH_3Ph	55	89	171-173
HPMS-15	3,4-diFPh	50	85	181-183

HPMS-16	2-ClPh	60	91	186-188
HPMS-17	2-FPh	50	88	172-174
HPMS-18	4-BrPh	60	89	181-182
HPMS-19	3,4-diClPh	55	92	167-168
HPMS-20	3-NO_2Ph	65	78	154-156
HPMS-21	3-CH_3Ph	55	86	169-171
HPMS-22	2,3-diCH_3Ph	60	90	184-186
HPMS-23	2-OCH_3,4-NO_2Ph	65	92	154-156

The chemical purity of all the newly synthesized compounds was examined using UPLC. Among all the final compounds, compound **HPMS-11** has shown less than 95% chemical purity and other shown more than 95% chemical purity (**Figure 34**). The ^{1}H NMR spectrum of pyrazole **HPMS-10** displayed two characteristic singlets for methyl and methylthio proton, respectively, at δ 2.53 and 3.64. However, two singlets appeared for pyrazole *NH* at δ 12.97 and amide hydrogen at δ 9.61.

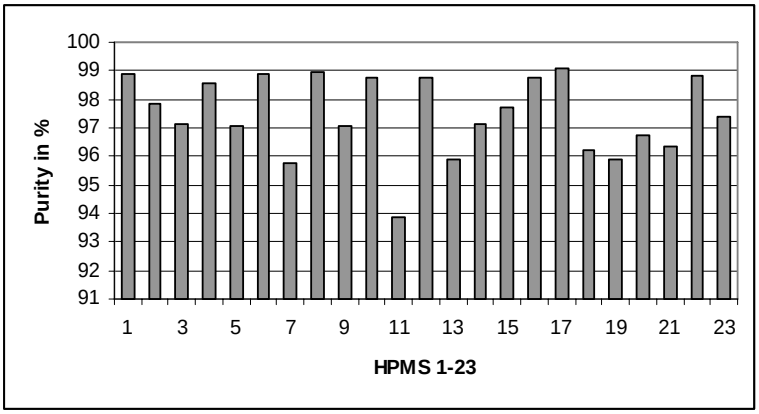


Figure 34: Chemical purity of trifunctionalized pyrazoles using UPLC.

The mechanism (**Figure 35**), in ketene dithioacetal system the carbonyl carbon and *β*-carbon atoms regarded as hard and soft electrophilic centers, since the carbonyl carbon is adjacent to the hard-base oxygen while the *β*-carbon is flanked by the soft-base methylthio groups. Thus, the binucleophile hydrazine hydrate attack on *β*-carbon of systems and formed heterocyclic product by removal of methylthio and group as good leaving group and water molecule. The pyrazoles on oxidation by sodium perborate afford sulfone containing pyrazoles.

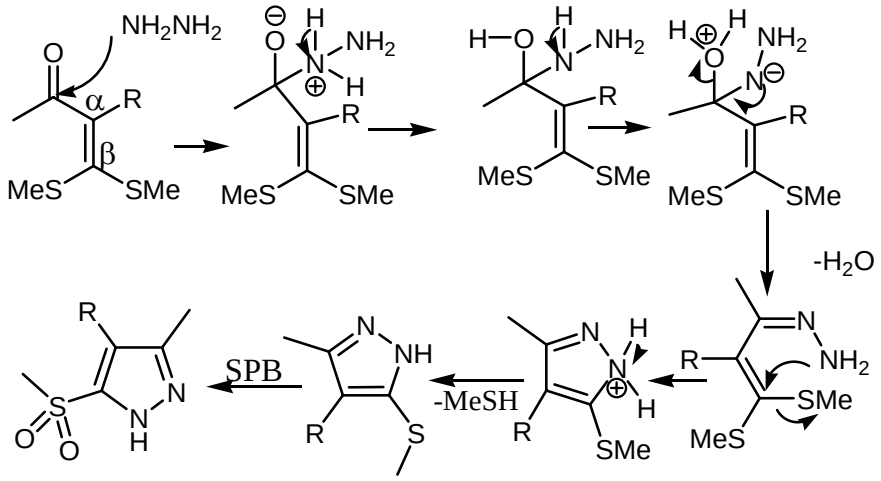


Figure 35: Proposed mechanism for the formation of pyrazole.

2.7 CONCLUSION

In Summary, we have synthesized solution-phase library of pyrazoles functionalized with methyl, sulfone and carboxamide groups in two steps with excellent yield and chemical purity for biological interest. Water was emerged as an efficient and green solvent in the condensation reaction of various ketene dithioacetals with hydrazine hydrate. Further, the facile synthesis of sulfone containing pyrazoles was achieved *via* oxidation of sulfide to sulfone. A comparative study of various oxidants has been performed, and revealed that SPB is more efficient and effective for oxidation of sulfide to sulfone in aqueous medium. This procedure offers a good scope for the synthesis of a wide variety of pyrazoles containing caboxamide and sulfone in two steps with excellent yield, purity and simple isolation of products.

2.8 EXPERIMENTAL SECTION

Melting points were determined in open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F_{254} (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Chemical purity was determined on Waters Acquity UPLC with PDA Detector using Acquity BEH C18, 50 × 2.1, 1.7µm column at 210-400nm.

❖ **Gradient Program for UPLC**

Mobile Phase: A - 10 mM Ammonium dihydrogenphosphate pH = 2.5,

B – Acetonitrile

Entry	Time	Flow	%A	%B
1	Initial	0.2 mL	95	05
2	4.0	0.2 mL	05	95
3	4.1	0.2 mL	95	05
4	5.0	0.2 mL	95	05

❖ **General procedure for the synthesis of various *α*-acylketene dithioacetals 1a-w.**

To a well-stirred suspension of sodium *tert*-butoxide (30 mmol) in THF (15 mL) at 0-5 ºC was added CS_2 (15 mmol) diluted with 10 mL THF along with *N*-(aryl)-3-oxobutanamide (15 mmol) over a period of 30 min. After completion of the addition, the reaction mixture was stirred at 0-5 ºC for 1.0 h. Appearance of reddish solid in the reaction medium indicated the formation of disodium salt. To this reaction, a solution of methyl iodide (30 mmol) in THF (5 mL) was added dropwise within 15 min at 0-5 ºC. The mixture was allowed to warm to room temperature and stirred for 5 h, and then poured onto crushed ice under stirring. The separated solid was collected by filtration, washed with water (2 × 100 mL), dried in *vacuo* and crystallized from chloroform to furnish the analytically pure products in excellent yield.

❖ **General procedure for the synthesis of trisubstituted pyrazoles 4a-w.**

To a suspension of various *α*-acylketene dithioacetals **1a-w** (10 mmol) in water (25 mL), hydrazine hydrate 80% (20 mmol) was added and the reaction mixture was refluxed for appropriate time (Table 2) with constant stirring. After completion of the reaction, the reaction mixtures were cooled to room temperature and add cold water (50 mL). The separated solid was filtered, washed with water (2 × 50 mL), dried and crystallized from methanol to afford analytically pure products which were used for next step without further purification.

❖ **General procedure for the oxidation of sulfide to sulfones HPMS 1-23.**

The appropriate compound **3a-w** (6 mmol) in water (15 mL) was added sodium per borate (18 mmol) and the resulting mixture was then heated to reflux for appropriate time (Table 2). The mixture was cooled to room temperature and extracted with ethyl acetate (2 × 15 mL). The organic layer was washed with water (2× 20 mL) and dried over magnesium sulphate. The solvent was evaporated at room temperature and product was isolated by crystallization technique in excellent yield.

3-methyl-5-(methylsulfonyl)-*N*-phenyl-1*H*-pyrazole-4-carboxamide (HPMS-1): White solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3250, 3230, 3032, 2926, 1647, 1548, 1281 cm^{-1}; ^1H NMR: δ 2.54 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 7.11-7.15 (t, 1H, ArH), 7.68-7.73 (m, 2H, ArH), 7.83 (d, *J*=8.1 Hz, 2H, ArH), 9.60 (s, 1H, NH), 12.86 (s, 1H, NH); ^{13}C NMR: δ 12.32, 46.92, 111.51, 121.38, 132.86, 138.57, 143.02, 154.13, 162.55; MS (*m/z*): 279 (M^+); Anal. Calcd for $C_{12}H_{13}N_3O_3S$: C, 51.60; H, 4.69; N, 15.05; Found: C, 51.48; H, 4.56; N, 15.12.

***N*-(4-methylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-2):** White solid; R_f 0.35 (6:4 hexane-EtOAc); IR (KBr): 3242, 3211, 3029, 1647, 1596, 1435, 1247 cm^{-1}; ^1H NMR: δ 2.37 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 7.22-7.28 (t, 2H, ArH), 7.61 (d, *J*=7.5 Hz, 1H, ArH), 7.63 (*J*=7.8 Hz. 1H, ArH), 9.79 (s, 1H, NH), 12.97 (s, 1H, NH); ^{13}C NMR: δ 12.37, 18.26, 46.89, 114.64, 120.03, 121.60, 129.51, 134.27, 136.27, 138.93, 146.66, 158.36, 161.09; MS (*m/z*): 293 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32; Found: C, 53.08; H, 5.13; N, 14.19.

***N*-(4-methoxyphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-3):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3252, 3214, 2985, 1675, 1598, 1548, 1124 cm^{-1}; MS (*m/z*): 309 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_4S$: C, 50.48; H, 4.89; N, 13.58; Found: C, 50.36; H, 4.73; N, 13.45.

***N*-(4-fluorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-4):** White solid; R_f 0.37 (6:4 hexane-EtOAc); IR (KBr): 3242, 3124, 2985, 1658, 1518, 1489, 1214 cm^{-1}; MS (*m/z*): 297 (M^+); Anal. Calcd for $C_{12}H_{12}FN_3O_3S$: C, 48.48; H, 4.07; N, 14.13; Found: C, 48.34; H, 3.93; N, 14.03.

***N*-(2-methoxyphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-5):** White solid; R_f 0.37 (7:3 hexane-EtOAc); IR (KBr): 3272, 3164, 2885, 1695, 1527, 1448, 1264 cm^{-1}; ^{13}C NMR: δ 12.50, 46.98, 55.87, 110.27, 114.64. 120.43, 121.10, 123.60, 128.13, 138.03, 148.62, 153.12, 161.19; MS (*m/z*): 309 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_4S$: C, 50.48; H, 4.89; N, 13.58; Found: C, 50.38; H, 4.79; N, 13.44.

***N*-(2-methylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-6):** White solid; R_f 0.38 (6:4 hexane-EtOAc); IR (KBr): 3233, 3114, 2945, 1675, 1588, 1448, 1164 cm^{-1}; MS (*m/z*): 293 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32; Found: C, 53.11; H, 5.09; N, 14.21.

***N*-(4-chlorophenyl)-3-methyl-5-(methysulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-7):** White solid; R_f 0.35 (6:4 hexane-EtOAc); IR (KBr): 3252, 3214, 2985, 1675, 1598, 1548, 1124 cm^{-1}; MS (*m/z*): 313 (M^+); Anal. Calcd for $C_{12}H_{12}ClN_3O_3S$: C, 45.94; H, 3.85; N, 13.39; Found: C, 45.83; H, 3.74; N, 13.27.

***N*-(4-ethylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-8):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3242, 3224, 2985, 1676, 1578, 1518, 1124 cm^{-1}; MS (*m/z*): 307 (M^+); Anal. Calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67; Found: C, 54.64; H, 5.46; N, 13.54.

***N*-(4-nitrophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-9):** White solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3256, 3224, 2975, 1685, 1578, 1498, 1227 cm^{-1}; MS (*m/z*): 324 (M^+); Anal. Calcd for $C_{12}H_{12}N_4O_5S$: C, 44.44; H, 3.73; N, 17.28; Found: C, 44.32; H, 3.34; N, 17.20.

***N*-(3-chloro-4-fluorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-10):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3274, 3104, 2958, 1656, 1558, 1468, 1224 cm^{-1}; ^1H NMR: δ 2.53 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 7.12-7.16 (t, 1H, ArH), 7.46-7.49 (m, 1H, ArH), 7.96-7.98 (q, 1H, ArH), 9.61 (s, 1H, NH), 12.97 (s, 1H, NH);

MS (*m/z*): 331 (M^+); Anal. Calcd for $C_{12}H_{11}ClFN_3O_3S$: C, 43.45; H, 3.34; N, 12.67; Found: C, 43.34; H, 3.29; N, 12.58.

***N*-(5-chloro-2-methoxyphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-11):** White solid; R_f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3282, 3243, 2985, 1686, 1554, 1427, 1124 cm^{-1}; MS (*m/z*): 343 (M^+); Anal. Calcd for $C_{13}H_{14}ClN_3O_4S$: C, 45.42; H, 4.10; N, 12.22; Found: C, 45.34; H, 4.03; N, 12.13.

***N*-(2,5-dichlorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-12):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3255, 3218, 3085, 1685, 1598, 1588, 1224, 1147 cm^{-1}; MS (*m/z*): 348 (M^+); Anal. Calcd for $C_{12}H_{11}Cl_2N_3O_3S$: C, 41.39; H, 3.18; N, 12.07; Found: C, 41.26; H, 3.04; N, 11.95.

***N*-(2,5-dimethylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS- 13):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3251, 3171, 2817, 1735, 1675, 1448, 1164 cm^{-1}; ^1H NMR: δ 2.33 (d, J = 7.6 Hz, 6H), 2.58 (s, 3H), 3.84 (s, 3H), 6.84-6.87 (q, 1H), 7.08 (d, J = 7.68 Hz, 1H), 7.68 (d, J = 1.28 Hz, 1H), 9.03 (s, 1H), 12.94 (s, 1H); IR (KBr): 3254, 3211, 3014, 1715, 1657, 1588, 1467, 1124 cm^{-1}; MS (*m/z*): 307 (M^+); Anal. Calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67; Found: C, 54.64; H, 5.44; N, 13.58.

***N*-(4-chloro-2-methylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-14):** White solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3252, 3214, 2985, 1675, 1598, 1548, 1124 cm^{-1}; MS (*m/z*): 327 (M^+); Anal. Calcd for $C_{13}H_{14}ClN_3O_3S$: C, 47.64; H, 4.30; N, 12.82; Found: C, 47.51; H, 4.17; N, 12.69.

***N*-(3,4-difluorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-15):** White solid; R_f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3242, 3194, 3085, 1695, 1587, 1471, 1224 cm^{-1}; MS (*m/z*): 315 (M^+); Anal. Calcd for $C_{12}H_{11}F_2N_3O_3S$: C, 45.71; H, 3.52; N, 13.33; Found: C, 45.59; H, 3.38; N, 13.21.

***N*-(2-chlorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-16):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3232, 3094, 2865, 1795, 1687, 1471, 1124 cm^{-1}; MS (*m/z*): 313 (M^+); Anal. Calcd for $C_{12}H_{12}ClN_3O_3S$: 45.94; H, 3.85; N, 13.39; Found: C, 45.84; H, 3.73; N, 13.25.

***N*-(2-fluorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-17):** White solid; R_f 0.36 (6:4 hexane-EtOAc); IR (KBr): 3244, 3114, 2955, 1678, 1528, 1499, 1114 cm^{-1}; MS (*m/z*): 297 (M^+); Anal. Calcd for $C_{12}H_{12}FN_3O_3S$: C, 48.48; H, 4.07; N, 14.13; Found: C, 48.32; H, 3.91; N, 14.05.

***N*-(4-bromophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-18):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3265, 3234, 2915, 1685, 1588, 1448, 1127 cm^{-1}; MS (*m/z*): 356 (M^+); Anal. Calcd for $C_{12}H_{12}BrN_3O_3S$: C, 40.24; H, 3.38; N, 11.73; Found: C, 40.13; H, 3.27; N, 17.62.

***N*-(3,4-dichlorophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-19):** White solid; R_f 0.30 (6:4 hexane-EtOAc); IR (KBr): 3275, 3118, 3085, 1675, 1558, 1488, 1224, 1047 cm^{-1}; MS (*m/z*): 348 (M^+); Anal. Calcd for $C_{12}H_{11}Cl_2N_3O_3S$: C, 41.39; H, 3.18; N, 12.07; Found: C, 41.34; H, 3.04; N, 11.91.

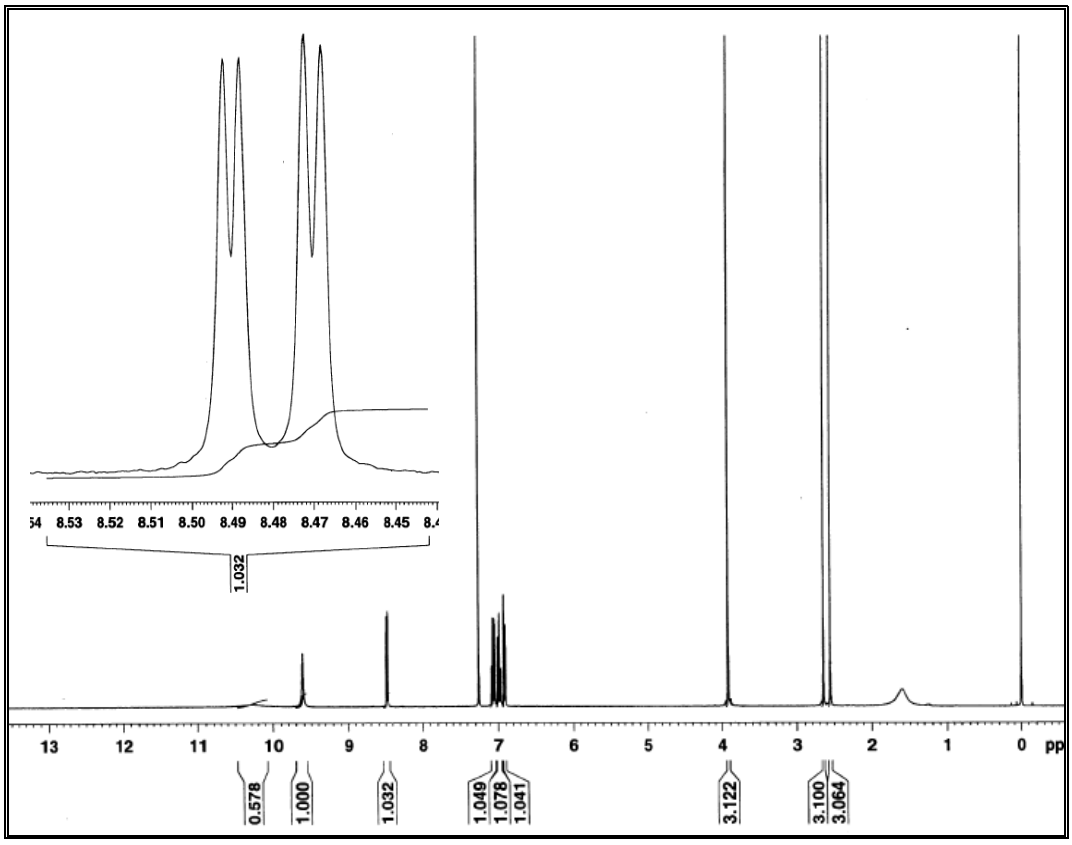
***N*-(3-nitrophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-20):** White solid; R_f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3255, 3244, 2975, 1665, 1548, 1498, 1127 cm^{-1}; MS (*m/z*): 324 (M^+); Anal. Calcd for $C_{12}H_{12}N_4O_5S$: C, 44.44; H, 3.73; N, 17.28; Found: C, 44.33; H, 3.31; N, 17.22.

***N*-(3-methylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-21):** White solid; R_f 0.37 (6:4 hexane-EtOAc); IR (KBr): 3243, 3154, 2915, 1695, 1578, 1348, 1364 cm^{-1}; MS (*m/z*): 293 (M^+); Anal. Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32; Found: C, 53.10; H, 5.04; N, 14.20.

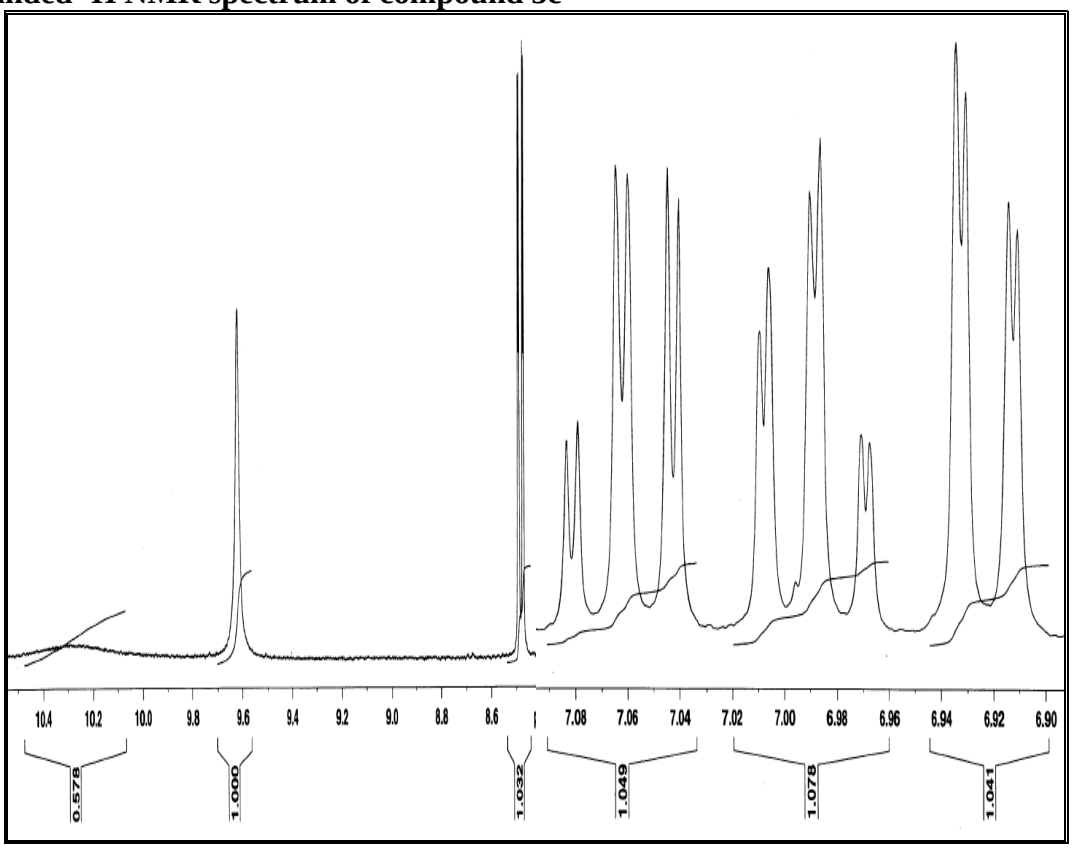
***N*-(2,3-dimethylphenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-22):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3253, 3174, 2815, 1725, 1678, 1448, 1164 cm^{-1}; MS (*m/z*): 307 (M^+); Anal. Calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67; Found: C, 54.65; H, 5.43; N, 13.60.

***N*-(2-methoxy,4-nitrophenyl)-3-methyl-5-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (HPMS-23):** White solid; R_f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3253, 3175, 2805, 1765, 1678, 1348, 1264, 1174 cm^{-1}; MS (*m/z*): 354 (M^+); Anal. Calcd for $C_{13}H_{14}N_4O_6S$: C, 44.06; H, 3.98; N, 15.81; Found: C, 44.05; H, 3.83; N, 15.66.

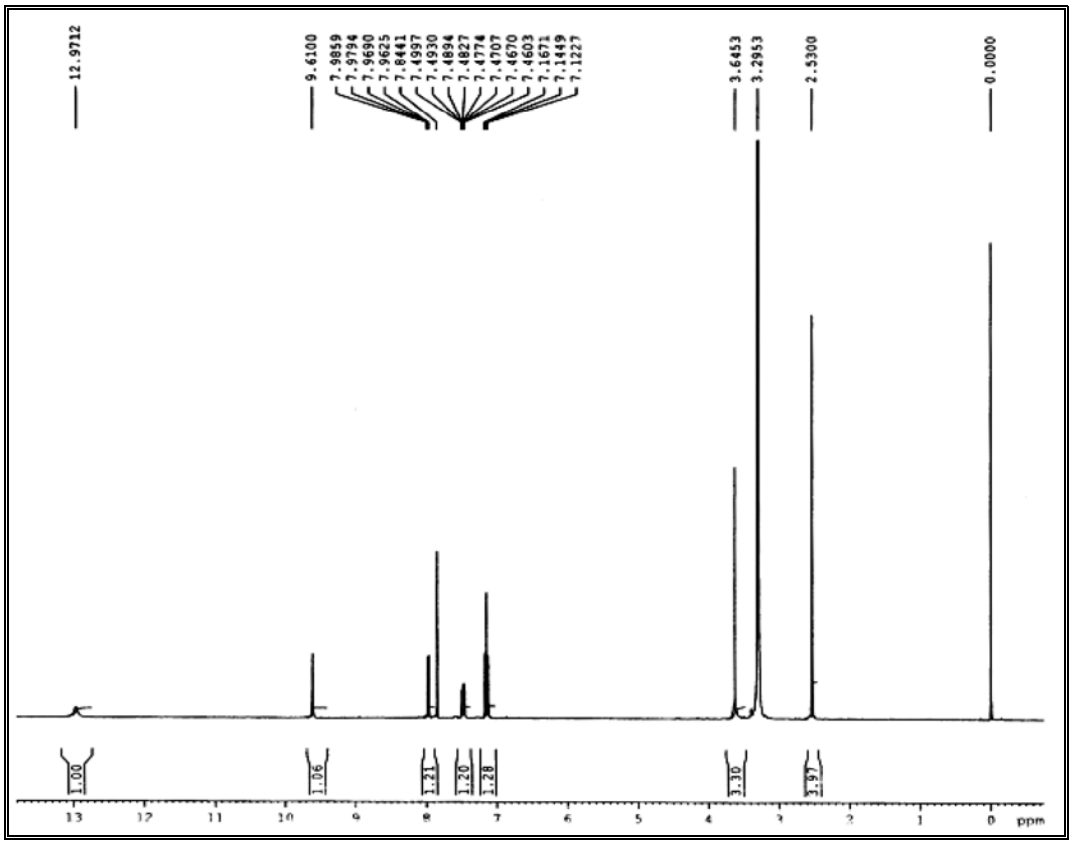
^{1}H NMR spectrum of compound 3c



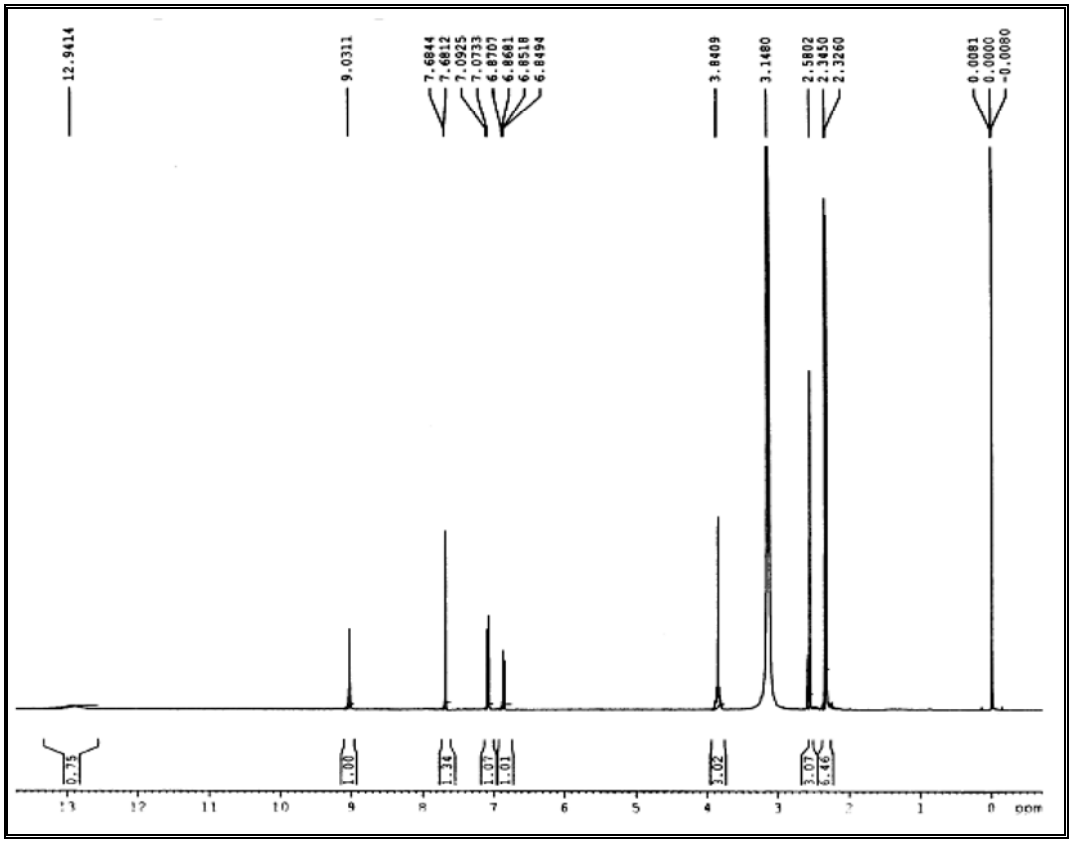
Expanded ^{1}H NMR spectrum of compound 3c



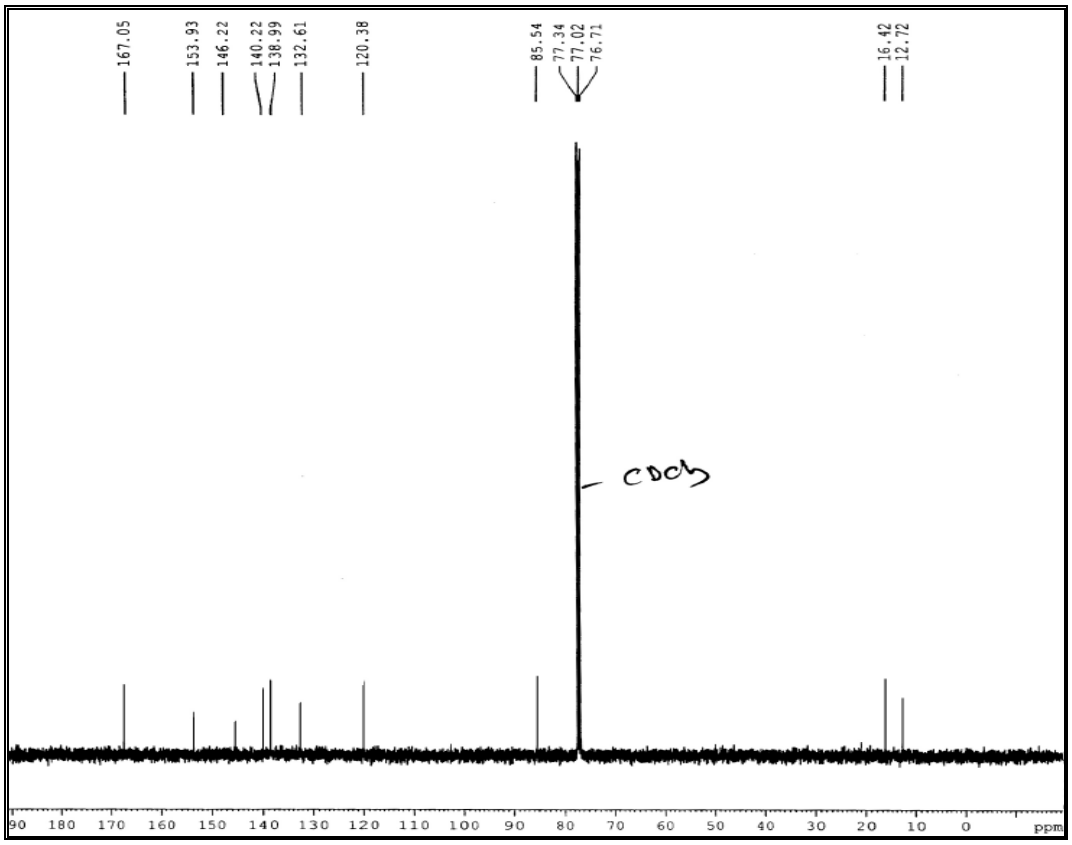
^{1}H NMR spectrum of compound HPMS-10



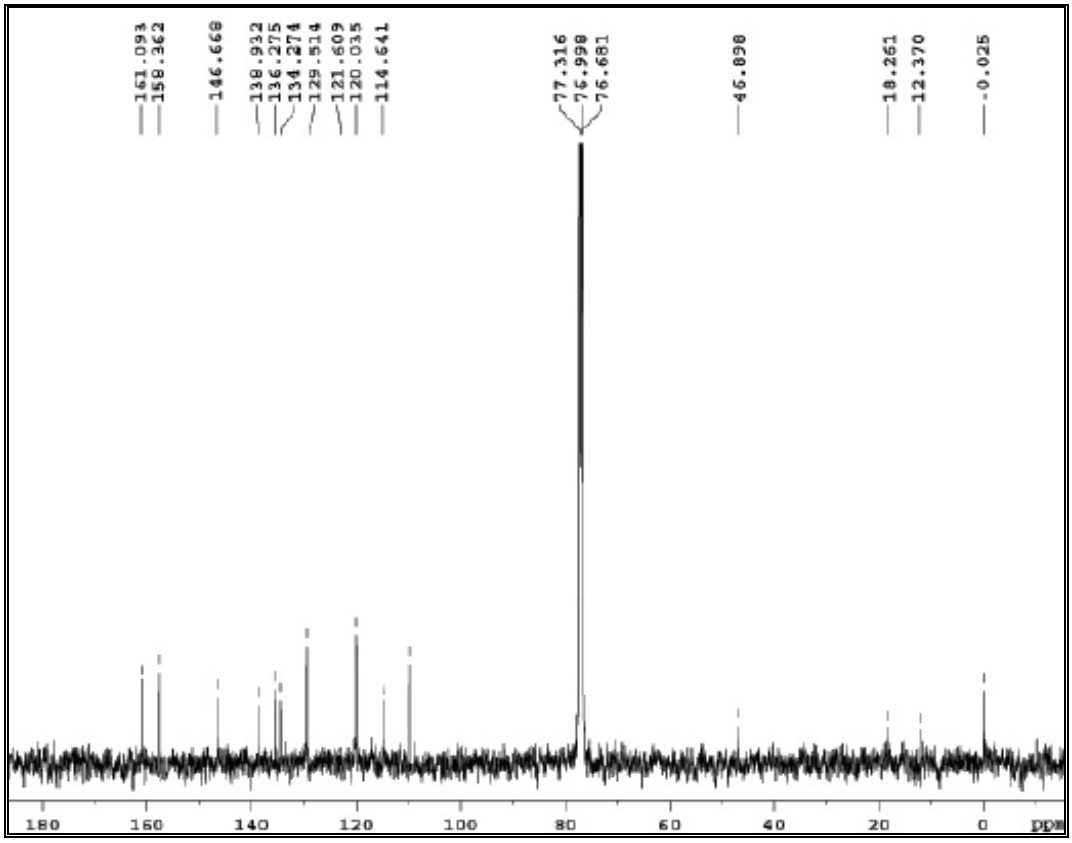
^{1}H NMR spectrum of compound HPMS-13



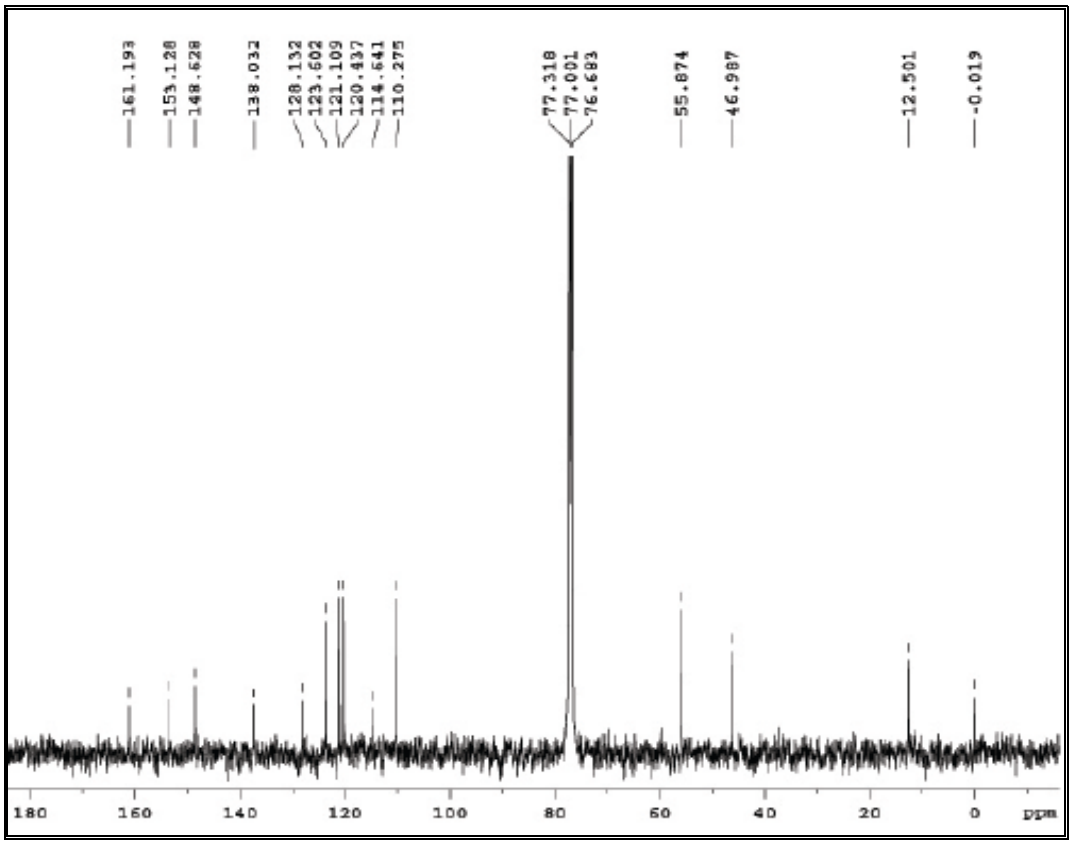
^{13}C NMR spectrum of compound-3c



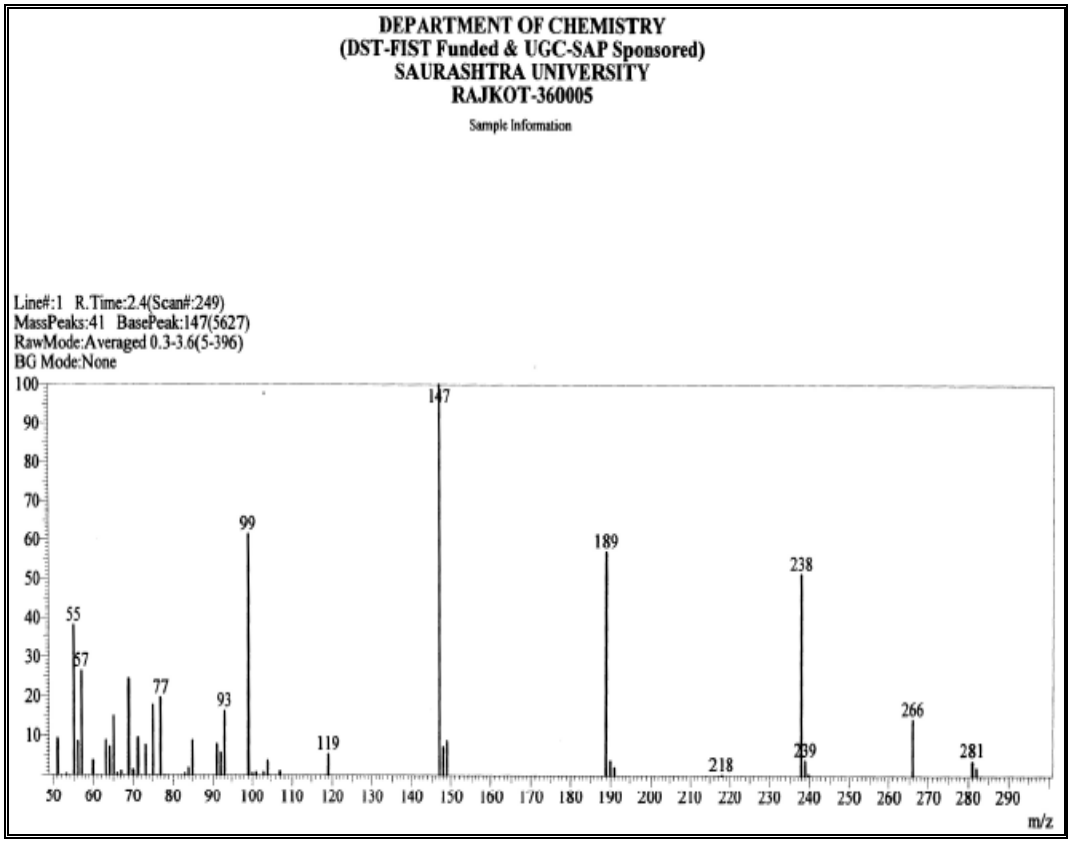
^{13}C NMR spectrum of HPMS-2



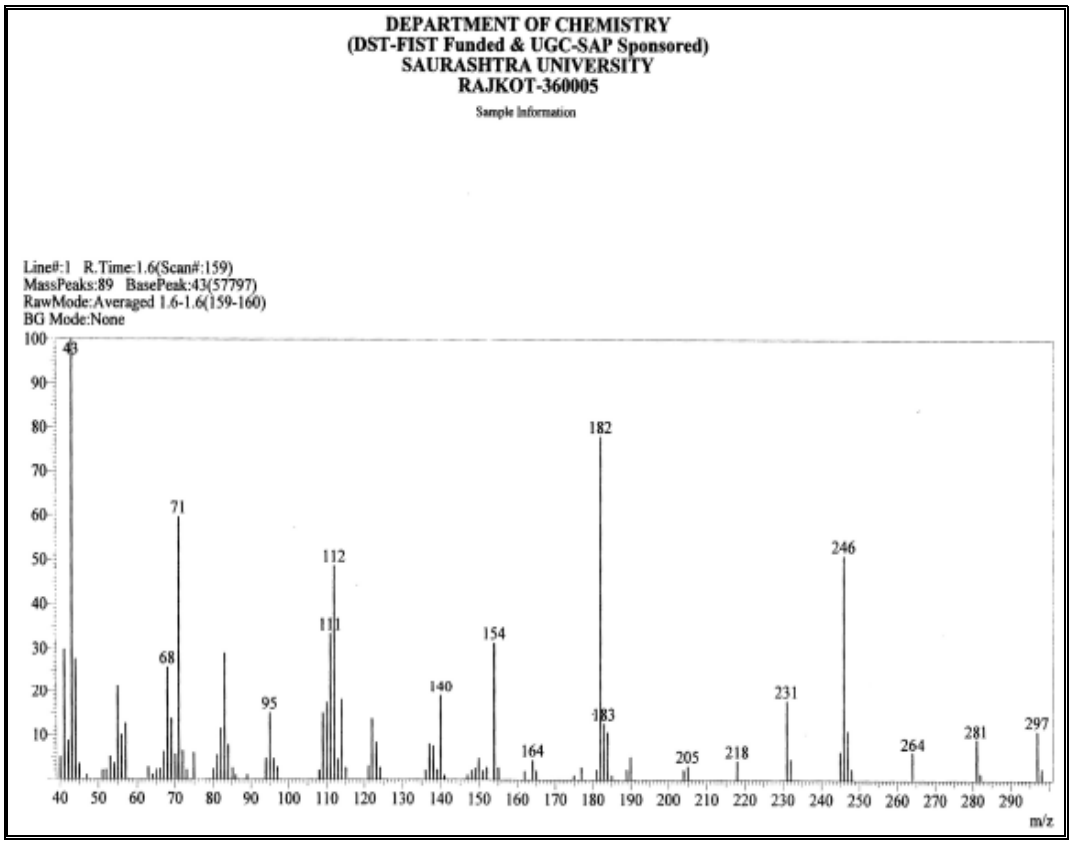
^{13}C NMR spectrum of HPMS-5



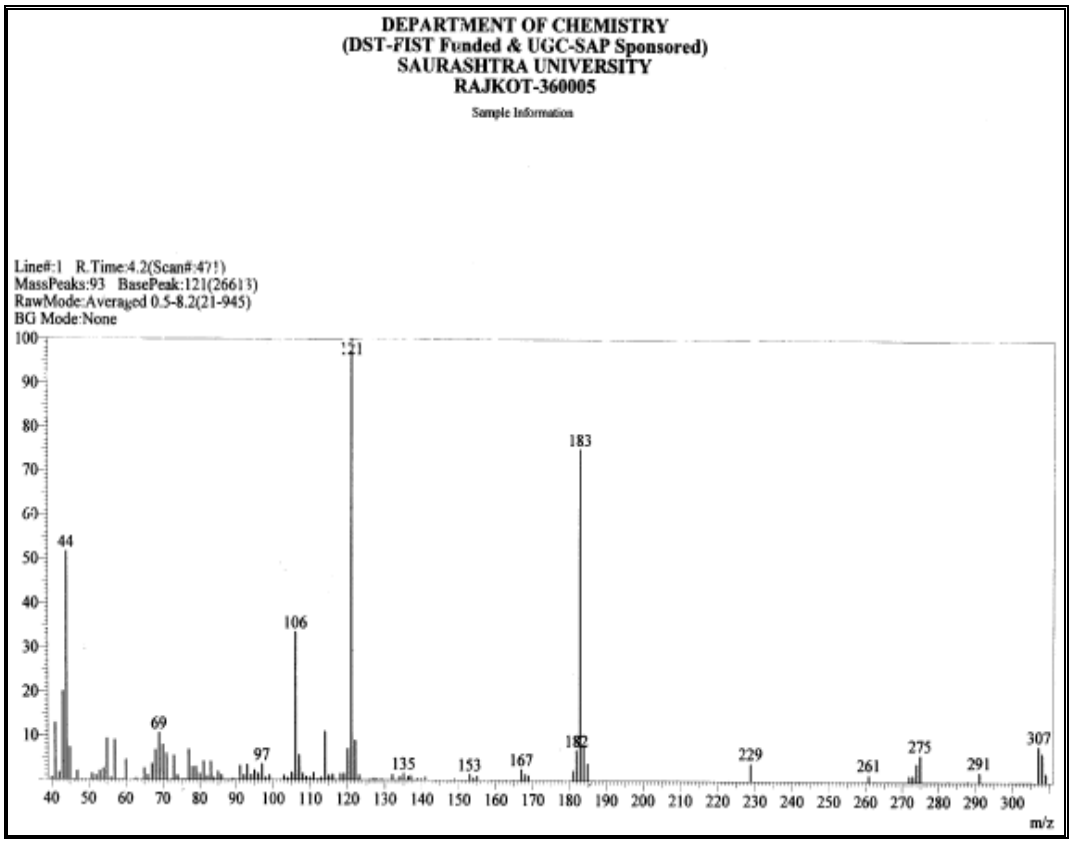
Mass spectrum of compound-1a



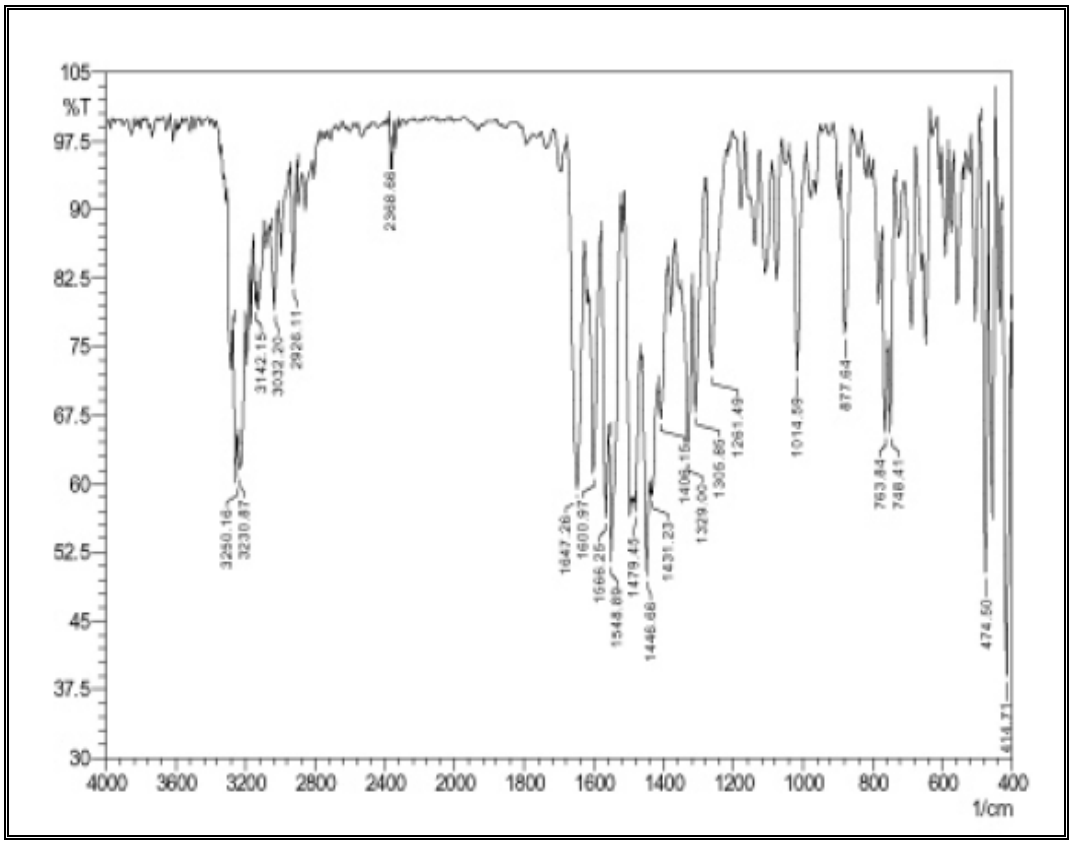
Mass spectrum of HPMS-4



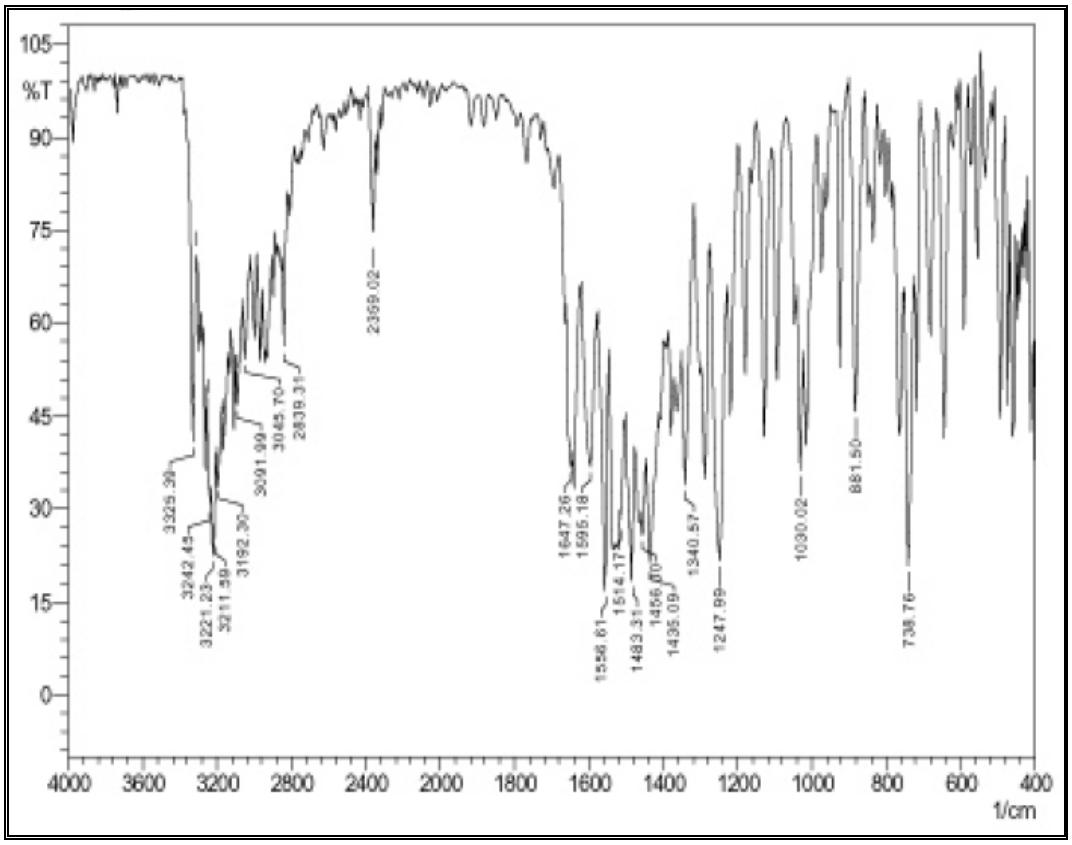
Mass spectrum of HPMS-8



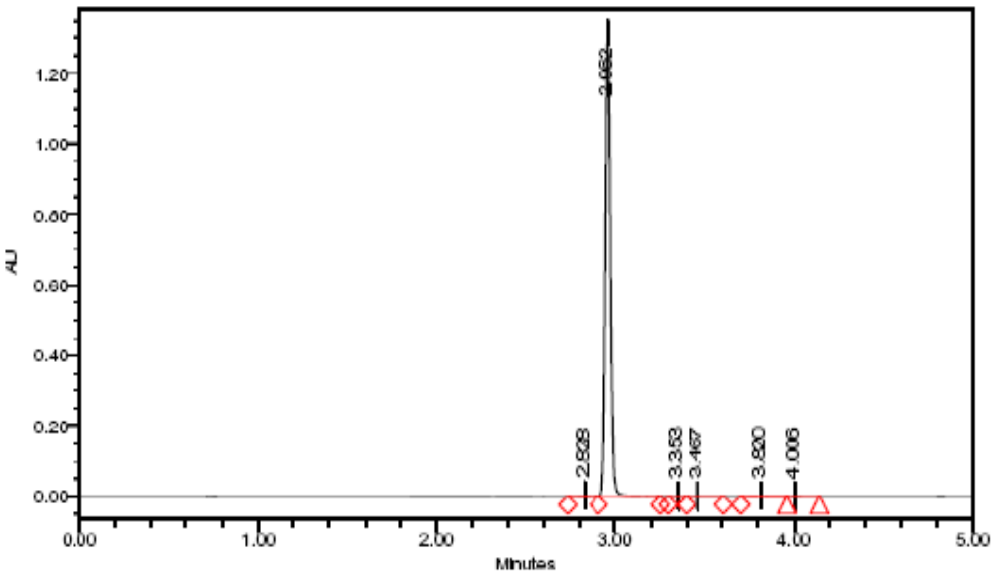
IR Spectrum of HPMS-1



IR spectrum HPMS-2

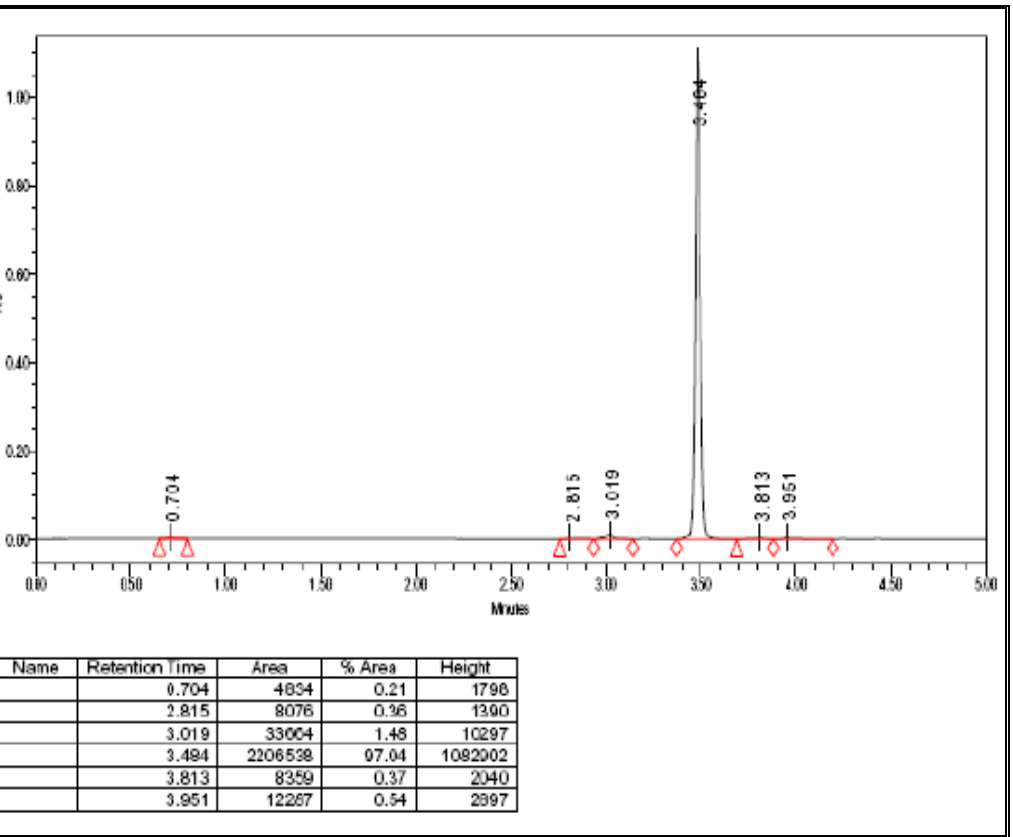


Chemical purity of compound HPMS-1



	Name	Retention Time	Area	% Area	Height
1		2.828	10982	0.41	1867
2		2.962	2626764	98.92	1318353
3		3.353	2527	0.10	614
4		3.467	4445	0.17	701
5		3.820	7021	0.29	1108
6		4.006	3109	0.12	761

Chemical purity of compound HPMS-9



	Name	Retention Time	Area	% Area	Height
1		0.704	4634	0.21	1798
2		2.815	8076	0.36	1390
3		3.019	33004	1.48	10297
4		3.484	2206538	97.04	1092002
5		3.813	8359	0.37	2040
6		3.951	12267	0.54	2997

2.9 REFERENCES

1. Knorr, L. J. *Ann. Chem*., **1894**, *379*, 236.

2. (a) Rowley, M.; Broughton, H. B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Ragan, C. I.; Freedman, S. B.; Leeson, P. D. *J. Med. Chem.* **1996**, *39*, 1943. (b) Wittenberger, S. J. *J. Org. Chem.* **1996**, *61*, 356. (c) Dannhardt, G.; Dominiak, P.; Laufer, S. *Arznei-Forschung.* **1993**, *43*, 441.

3. (a) Pinho, E. M.; Teresa, M. V. D. *Curr. Org. Chem.* **2005**, *9*, 925. (b) Colliot, F.; Kukorowski, K. A.; Hawkins, D. W.; Roberts, D. A. *Brighton Crop Prot. Conf. Pests Dis.* **1992**, *1*, 29. (c) Chen, H. S.; Li, Z. M.; Han, Y. F. *J. Agric. Food.Chem.* **2000**, *48*, 5312. (d) Vicentini, C. B.; Romagnoli, C.; Reotti, E.; Mares, D. *J. Agric. Food Chem.* **2007**, *55*, 10331. (e) Vicentini, C. B.; Mares, D.; Tartari, A.; Manfrini, M.; Forlani, G. *J. Agric. Food Chem.* **2004**, *52*, 1898.

4. Makhsumov, A. G.; Dzhuraev, A. D.; Kilichov, G.; Nikbaev, A. T.; Tashkent Medical institute. Translated from *Khim. Farm. Zh.*, **1986**, Vol. 20, No. 3, pp. 289.

5. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Terauchi, H.; Kawasaki, M.; Nagai, K. *J. Med. Chem.* **2004,** *47,* 3693.

6. Edwards, P. J.; Jones, L. H.; Mowbray, C. E.; Stupple, P. A.; Tran, I. Pfizer limited, US, New York, WO 2004/031156 A1.

7. Bagley, M. C.; Davis, T.; Dix, M. C.; Widdowson, C. S; Kipling, D. *Org. Biomol. Chem.*, **2006**, *4*, 4158.

8. Stein, R. G.; Biel, J. H.; Singh, T. *J. Med. Chem*. **1970**, *13*, 153.

9. Xie, W.; Chipman, J. G.; Robertson, D. L.; Erickson, R. L.; Simmons, D. L. *Proceedings of the National Academy of Sciences U. S. A.* **1991**, *88*, 2692.

10. (a) Turini, M. E.; DuBois, R. N. *Annual Reviews in Medicine* **2002**, *53*, 35-57. (b) Vane, J. R.; Botting, R. M. *Therapeutic roles of selective COX-2 inhibitors*; William Harvey Press: London, UK, **2001**.

11. (a) Dubois, A. *Prostaglandins & Other Lipid Mediators* **2000**, Special Issue: Guest Editor, A. Dubois, 1-174. (b) Khanapure, S. P.; Letts, L. G. In *The Eicosanoids* ; Curtis-Prior, P. B., Ed.; John Wiely & Sons: London, **2003**, p 131.

12. Sondhi, S. M.; Dinodia, M.; Kumar, A. *Bioorg. & Med. Chem.* **2006**, *14,* 4657.

13. Haruna, K.; Kanezaki, H.; Tanabe, K.; Daib, W. M.; Nishimotoa, S. *Bioorg. Med. Chem.* **2006**, *14*, 4427.

14. Ranatunge, R. R.; Augustyniak, M.; Bandarage, U. K.; Earl, R. A.; Ellis, J. L.; Garvey, D. S.; Janero, D. R.; Letts, L. G.; Martino, A. M.; Murty, M. G.; Richardson, S. K.; Schroeder, J. D.; Shumway, M. J.; Tam, S. M.; Trocha, A. M.; Young, D. V. *J. Med. Chem.* **2004**, *47*, 2180.

15. Bonacorso, H. G.; Wentz, A. P.; Lourega, R. V.; Cechinel, C. A.; Moraes, T. S.; Coelho, H. S.; Zanatta, N.; Martins, A. P.; Hoerner, M.; Alves, S. H. *J. Fluorine Chem.* **2006**, *127*, 1066.

16. Haque, T. S.; Tadesse, S.; Marcinkeviciene, J.; Rogers, M. J.; Kopcho, L. M.; Amsler, K. *J. Med. Chem.* **2002,** 45*,* 4669.

17. Covacci, A.; Telford, J. L.; Giudice, G. D.; Parsonnet, J.; Rappuoli, R.; Helicobacter pylori Virulence and Genetic Geography. *Science,* **1999**, *284*, 1328.

18. McGowan, C. C.; Cover, T. L.; Blaser, M. J.; Helicobacter pylori and Gastric Acid: Biological and Therapeutic Implications. *Gastroenterology,* **1996**, *110*, 426.

19. Shavnya, A.; Sakya, S. M.; Minich, M. L.; Rast, B.; DeMello, K. L.; Jaynes B. H. *Tet. Lett.* **2005**, *46,* 6887.

20. Wang, K.; Xiang, D.; Liu, J.; Pan, W.; Dong, D. *Org. Lett.*, **2008**, *10,* 1691.

21. Corradi, A.; Leonelli, C.; Rizzuti, A.; Rosa, R.; Veronesi, P.; Grandi, R.; Baldassari, S.; Villa, C. *Molecules,* **2007***, 12,* 1482.

22. Wang, X.; Xu, F.; Xu, Q.; Mahmud, H.; Houze, J.; Zhu, L.; Akerman, M.; Tonn, G.; Tang, L. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2800.

23. Kim, H. S.; Kim, J. N. *Bull. Korean Chem. Soc*. **2007**, *28*, 1841.

24. Peruncheralathan, S.; Khan, T. A.; Ila, H.; Junjappa, H. *J. Org. Chem*. **2005**, *70*, 10030.

25. Elgemeie, G. H.; Elghandour, A. H.; Elaziz, G. W. *Synth. Comm.* **2007**, *37*, 2827.

26. Mahata, P. K.; SyamKumar, U. K.; Sriram, V.; Ila, H.; Junjappa, H. *Tetra.* **2003**, *59*, 2631.

27. Kuettel, S.; Zambon, A.; Kaiser, M.; Brun, R.; Scapozza, L.; Perozzo, R. *J. Med. Chem*. **2007**, *50*, 5833.

28. Kurz, T.; Widyan, K.; Elgemeie, G. H. *Phosphorus Sulfur and Silicon* **2006**, *181*, 299.

29. Elgemeie, G. H.; Elghandour, A. H; Elaziz, G. W.; Ahmed, S. A. *J. Chem. Soc. Perkin Trans.* **1997***, 1,* 3285.

30. (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (b) Booth, S.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1998**, *54*, 15385.

31. (a) Devlin, J. P. *High Throughput Screening: The Discovery of Bioactive Substances*; Marcel Dekker: New York, **1997**.(b) Gordon, E. M.; Kerwin, J. F., Jr. *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Wiley: New York, **1998**.

32. Wenli, M.; Peterson, B.; Kelson, A.; Laborde, E. *J. Comb. Chem.* **2009**, *11*, 697.

33. Lehmann, F.; Holm, M.; Laufer, S. *J. Comb. Chem.* **2008,** *10,* 364.

34. Dalinger, I. L.; Vatsadse, I. A.; Shevelev, S. A.; Ivachtchenko, A. V. *J. Comb. Chem.* **2005,** *7,* 236.

35. Rena, X. L.; Lib, H. B.; Wub, C.; Yang, H. Z. *Arkivoc,* **2005**, *15*, 59.

36. Organ, M. G.; Mayer, S. *J. Comb. Chem.* **2003,** *5,* 118.

37. Luca, L. D.; Giacomelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *J. Comb. Chem*. **2003**, 5, 465.

38. See, e.g. Ley, S. V.; Norman, J.; Oriffith, W. P.; Marsden, S. P. *Synth.,* **1994**, 639.

39. Habibi, D.; Zolfigol, M. A.; Safaiee, M.; Shamsian, A.; Choghamarani, A. G.; *Cat. Comm.* **2009**, *10*, 1257.

40. Matloobi, M.; Kappe, C. O. *J. Comb. Chem.* **2007,** *9,* 275.

41. Balicki, R.; *J. Prakt. Chem.* **1999**, *341*, 184.

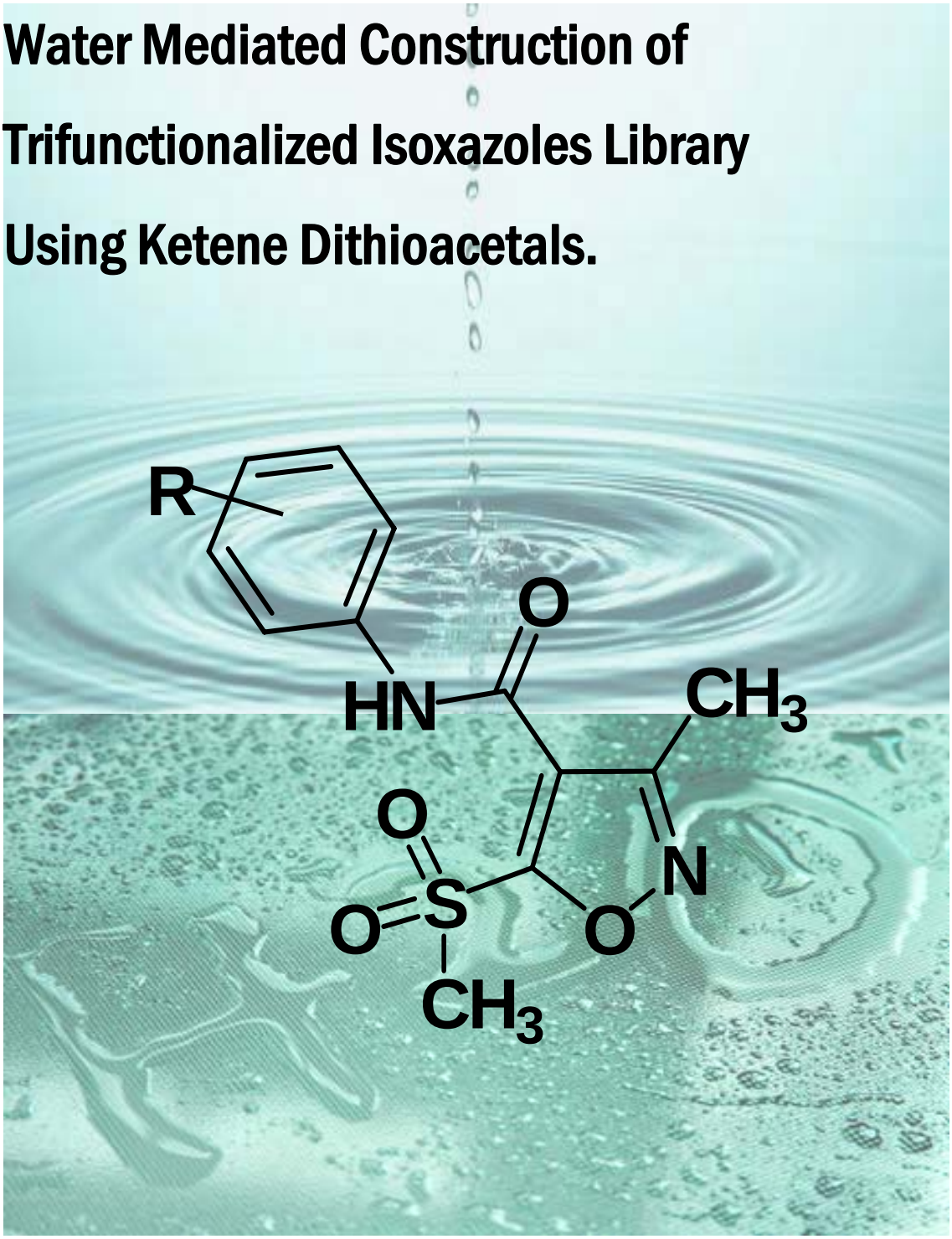
42. McKillop, A.; Sanderson, W. R. *Tet.* 1995, *51*, 6145.

43. Bi, X.; Dong, D.; Liu, Q.; Pan, W.; Zhao, L.; Li, B. *J. Am. Chem. Soc.* **2005**, *127*, 4578.

44. (a) Al-Ajlouni, A. M.; Daiafla, T. M.; El-Khateeb, M. *J. Mol. Catal. A: Chem.* **2007**, *275*, 139. (b) Egami, H.; Katsuki, T. *J. Am. Chem. Soc.* **2007**, *129*, 8940. (c) Shirini, F.; Zolfigol, M. A.; Abri, A. R. *Chin. Chem. Lett.* **2008**, *19*, 51. (d) Shaabani, A.; Rezayan, A. H. *Catal. Commun.* **2007**, *8*, 1112.

Chapter 3

Water Mediated Construction of Trifunctionalized Isoxazoles Library Using Ketene Dithioacetals.



3.1 INTRODUCTION

Isoxazole is a five member heterocyclic compound having two hetero atoms oxygen at position 1 and nitrogen at position 2. In 1888, Claisen et al have first synthesized an isoxazole molecule (**Figure 1**) with the reaction of 1,3-diketone with hydroxylamine.[1] Subsequently a solid foundation for the chemistry of isoxazole was laid down by Claisen and his students. It was shown to possess typical properties of an aromatic system but under certain reaction conditions. Particularly in reducing or basic media, it becomes very highly labile.

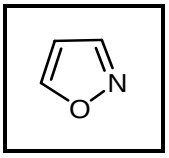


Figure 1

The next important contribution to the chemistry of isoxazoles was made by Quelico.[2] In 1945, when he began to study the formation of isoxazoles from nitrile *N*-oxide and unsaturated compounds.

3.2 Biological activity of various substituted isoxazole derivatives

The biological activity of substituted isoxazoles[3] has made them a focus of medicinal chemistry over the years. Isoxazoles are potent, selective agonists at human cloned dopamine D4 receptors[4] and exhibit $GABA_A$ antagonist,[5] analgesic,[6] antiinflammatory,[6] ulcerogenic,[6] antimicrobial,[7] antifungal,[7] COX-2 inhibitory,[8] antinociceptive,[9] and anticancer[10] activity.

Cushman M. et al[11] have designed benzo[*d*]isoxazole and oxazolidine-2-one derivatives and evaluated as a new series of potent HIV-1 non-nucleoside reverse transcriptase inhibitors with anti-HIV activity. The most promising compound in this series was ADAM (**Figure 2**), with EC_{50} values of 40 μM (vs HIV-1_{RF}) and 20 μM(vs HIV-1_{IIIB}). Methyl 5-((*Z*)-5-(methoxycarbonyl)-1- (3-methoxy-7-methylbenzo [d] isoxazole-5-yl) pent-1-enyl-2-methoxy-3-methylbenzoate also inhibited HIV-1 reverse transcriptase with an IC_{50} of 0.91 μM. ADAM 4 has an antiviral EC_{50} of 0.6 μM in CEM-SS cells and a plasma half-life of 51.4 min.

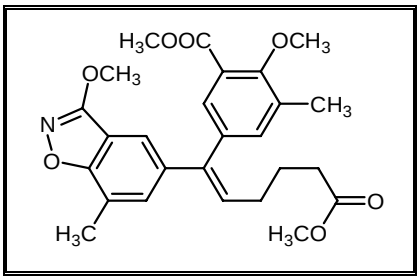


Figure 2

HIV-1 is the etiological agent of AIDS, one of the world's most serious health problems with about 33 million people infected worldwide in 2007. The reverse transcriptase (RT) of HIV-1 is an essential enzyme in HIV replication and has been a key target in anti-AIDS drug discovery. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine, and efavirenz have been approved by the Food and Drug Administration (FDA) for the treatment of AIDS.[12] They are very useful drugs in combination therapy with nucleoside analogues (NRTIs) and protease inhibitors (PIs)[13-14] for the treatment of AIDS. Recently, the NNRTI etravirine was approved by the FDA for treatment of antiretroviral drug-resistant HIV infections. The cytotoxicities of the newly synthesized ADAMs were determined along with their abilities to inhibit the cytopathic effect of HIV-1 in cell culture. The inhibition of HIV-1 RT by the ADAMs, and their metabolic stabilities in rat plasma were also investigated.

Liljefors T. et al[15] have synthesized a series of 4-aryl-5-(4-piperidyl)-3-isoxazololes and evaluated for $GABA_A$ antagonists. The *meta*-phenyl-substituted compounds and the *para*-phenoxy-substituted compound (**Figure 3**) all display high affinities (K_i = 10-70 nM) and antagonist potencies in the low nanomolar range (K_i = 9-10 nM).

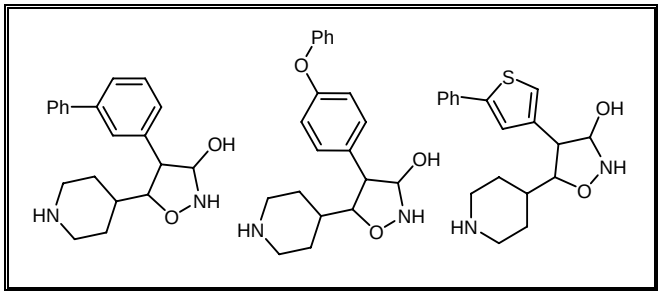


Figure 3

Shvets N. et al[16] were demonstrated that the 2,3,5-substituted perhydropyrrolo[3,4-*d*]isoxazole-4,6-dione (**Figure 4**) derivatives have potent antibacterial activity. The reaction involved the cycloaddition reaction of *N*-methyl-*C*-arylnitrones with *N*-substituted

maleimides. Most of the compounds exhibited high activity against *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923).

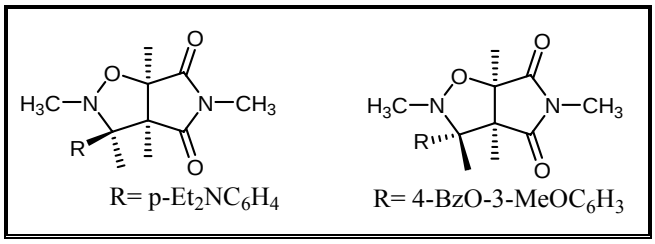


Figure 4

Due to the exceptional anticonvulsant activity displayed by substituted aniline enaminones, related pyridine derivatives[17] and phenothiazines, the further investigation of various aromatic heterocycles was undertaken and found that the isoxazoles are important heterocycles for the treatment of convulsant diseases. The reaction of cyclic 1,3-diketo esters with aminoisoxazole derivatives led to a series of potent anti-maximal electroshock analogues. Sodium channel binding studies, as well as evaluations against pentylenetetrazol, bicuculline, and picrotoxin on isoxazole were all negative, leading to an unknown mechanism of action. X-ray diffraction patterns of a representative of the 3-amino series (**Figure 5**) unequivocally display the existence of intramolecular hydrogen bonding of the nitrogen to the vinylic proton in the cyclohexene ring, providing a pseudo three ring structure.

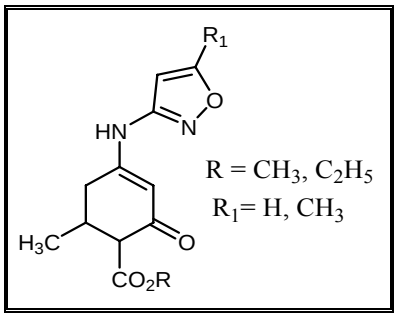


Figure 5

Giovannoni M. P. et al[18] have synthesized a number of arylpiperazinylalkylpyridazinones and tested for their analgesic activity. They were observed that many of the tested molecules, at the dose of 20 mg kg^{-1} p.o., showed high antinociceptive activity, in particular, substituted lead (**Figure 6**) a compound which was able to reduce the number of abdominal constrictions by more than 50% in writhing test. They were investigated the mechanism of action of this compound, which shown that it carries out its analgesic action through the inhibition of reuptake of noradrenaline.

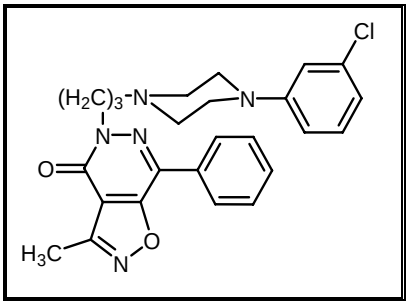


Figure 6

Christian Peifer et al[19] have reported the discovery of isoxazole (**Figure 7**) as a potent dual inhibitor of *p*38*α* (IC_{50}= 0.45 μM) and CK1*δ* (IC_{50}= 0.23 μM). Among the synthesized isoxazoles, selected compounds were profiled over 76 kinases and evaluation of their cellular efficacy showed 18 (CKP138) to be a highly potent and dual-specific inhibitor of CK1δ and p38α.

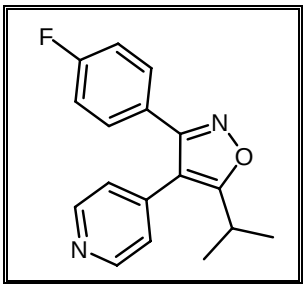


Figure 7

In 2006, Johnson & Johnson Pharmaceutical Research & Development[20], has been reported the synthesis of a series of 7-amino-3a,4-dihydro-3*H*-[1]benzopyrano[4,3-c]isoxazole (**Figure 8**) derivatives using substituted salisaldehydes and ethyl 4-bromocrotonate. Among the synthesized isoxazoles, some of which proved to be the most potent α_2-adrenoceptor blockers with potent serotonin (5-HT) reuptake inhibiting activity. Serotonin is one of the important monoamine for human body and the deficit of 5-HT mainly lead to the depression

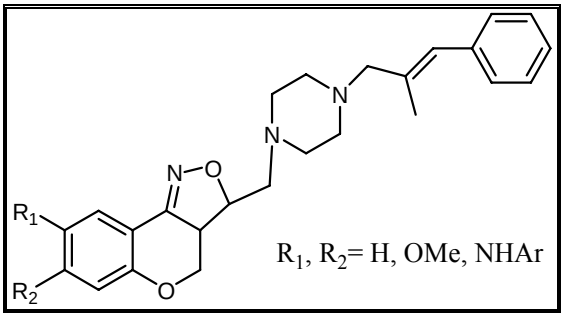


Figure 8

Carr J. B. et al[21] have been synthesized a series of highly substituted isoxazoles (**Figure 9**) and screened for anthelmintic activity at doses ranging from 16 to 500 mg/kg orally against the rat roundworm, *Nlppostrongjlus Erazikensis*. They were found that the newly synthesized isoxazole derivatives have potent anthelmintic activity.

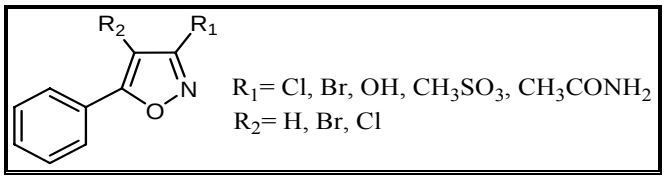


Figure 9

❖ **Biologically active isoxazoles containing alkyl, amide and sulfone groups**

Isoxazoles bearing sulfones and carboxamide moieties demonstrated to have significant pharmacological applications. For examples, COX-2 selective inhibitor, valdecoxib (**Figure 10**)[22] are currently prescribed for the treatment of arthritis and inflammatory diseases. These COX-2 inhibitors exhibited anti-inflammatory activity with reduced gastrointestinal side effects. Moreover, oxacillin (**Figure 10**)[23] and its derivatives are useful compounds because of their narrow spectrum anti biotic properties. [23]

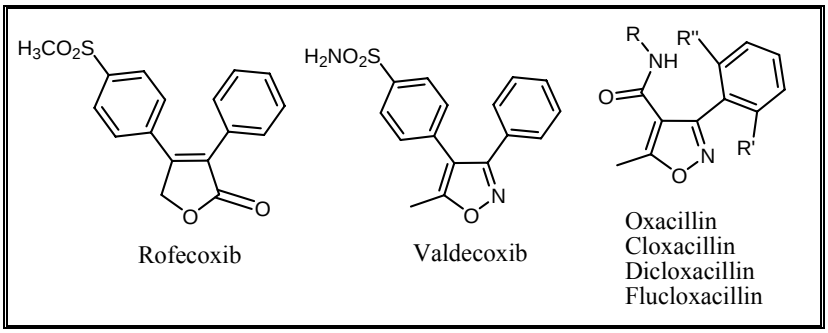


Figure 10

Some *N*-phenyl and *N*-benzyl-substituted amido (**Figure 11**) analogs of COX-2 selective tricyclic non-steroidal anti-inflammatory drugs have been synthesized by Balsamo A. and coworkers[24] with the aim to obtain information on the structural requirements for the COX-inhibitory activity. The newly synthesized compounds were tested *in vitro* for their inhibitory properties only towards COX-2 enzyme by measuring prostaglandin E2 (PGE2) production on activated J774.2 macrophages. Some of the new compounds showed a modest activity, with percentage inhibition values near 30% at a concentration of 10 μM. The biological data was indicates that the *N*-phenyl-substituted amides present in isoxazole moiety with steric hindrances may prevent a good interaction with COX-2 active site.

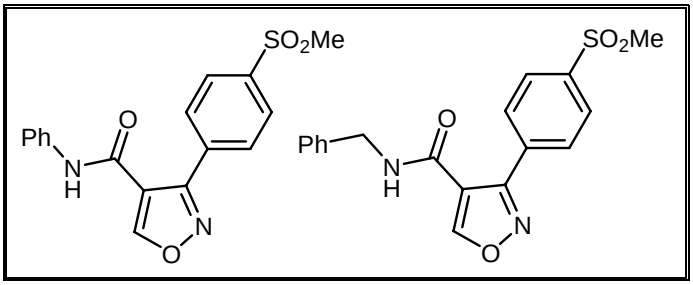


Figure 11

Aldo Balsamo et al[24] have reported several heteroaromatic analogs of (2-aryl-1-cyclopentenyl-1-alkylidene)-(arylmethyloxy)amine COX-2 inhibitors (**Figure 12**), in which the cyclopentene moiety was replaced by pyrazole, thiophene or isoxazole ring, were synthesized, in order to verify the influence of the different nature of the central core on the COX inhibitory properties of these kinds of molecules. Among the compounds tested, only the 3-(*p*-methylsulfonylphenyl) substituted thiophene derivatives, showed a certain COX-2 inhibitory activity, accompanied by an appreciable COX-2 versus COX-1 selectivity. Only one of the 1-(*p*-methylsulfonylphenyl) pyrazole compounds displayed a modest inhibitory activity towards both type of isoenzymes, while the pyrazole 1-(*p*-aminosulfonylphenyl) substituted proved to be significantly active only towards COX-1. All the isoxazole derivatives were inactive on both COX isoforms.

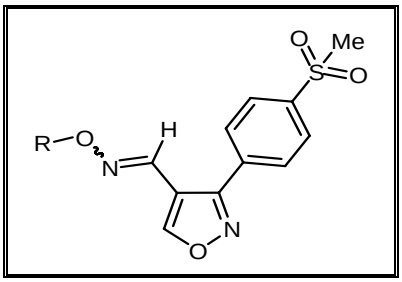


Figure 12

Habeeb A. G. et al[25] were also reported the synthesis of 4,5-diphenyl-4-isoxazolines **(Figure 13)** possessing a variety of substituents (H, F, MeS, $MeSO_2$) at the *para*-position of one of the phenyl rings and evaluated as analgesic and selective COX-2 inhibitory anti-inflammatory agents. Although the 4,5-phenyl-4-isoxazolines **(Figure 13)**, which do not have a methyl at C3, exhibited potent analgesic and anti-inflammatroy activities, those compounds evaluated were not selective inhibitors of COX-2. In contrast, 2,3-dimethyl-5-(4-methylsulfonylphenyl)-4-phenyl-4-isoxazoline exhibited excellent analgesic and anti-inflammatory activities, and it was a potent and selective COX-2 inhibitor (COX-1, IC_{50}) 258 μM; COX-2, IC_{50}) 0.004 μM).

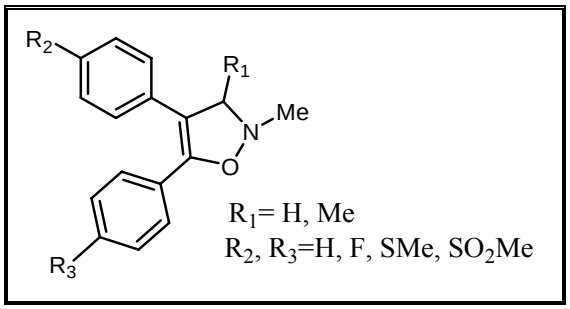


Figure 13

A fluoro substituent at the para position of the 4-phenyl ring was also a selective (SI =3162) but less potent (IC_{50} =0.0316 μM) inhibitor of COX-2 than 2,3-dihydro-2,3-dimethyl-5-(4-(methylsulfonyl)phenyl)-4-phenylisoxazole. A molecular modeling for 4-(4-fluorophenyl)-2,3-dihydro-2,3-dimethyl-5-(4-(methylsulfonyl)phenyl)-4-phenylisoxazole showed that the S atom of the $MeSO_2$ substituent is positioned about 6.46 Å inside the entrance to the COX-2 secondary pocket (Val^{523}) and that a C3 Me (2,3-dihydro-2,3-dimethyl-5-(4-(methylsulfonyl)phenyl)-4-phenylisoxazole, 4-(4-fluorophenyl)-2,3-dihydro-2,3dimethyl-5-(4-(methylsulfonyl)phenyl)-4-phenylisoxazole) central isoxazoline ring substituent is crucial to selective inhibition of COX-2 for this class of compounds.

Talley J. J. et al[26] have synthesized sodium salt of *N*-[[(5-Methyl-3-phenylisoxazol-4-yl)-phenyl]sulfonyl]propanamide, parecoxib sodium (**Figure 14**) and evaluated as a potent and selective inhibitor of COX-2 for parenteral administration.

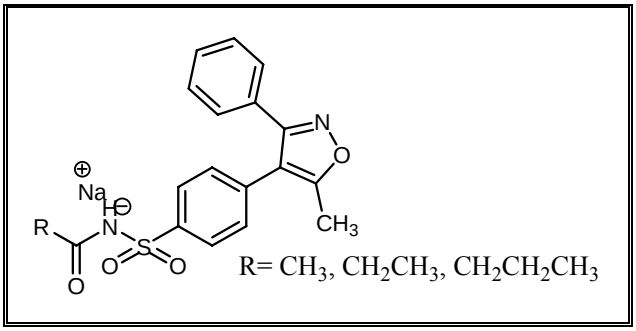


Figure 14

Among the most potent and selective COX-2 inhibitors that have been identified is the isoxazole sulfonamide valdecoxib (**Figure 10**). In addition, sulfonamide valdecoxib possesses exceptional anti-inflammatory activity *in vivo*.[27] Talley et al have developed an injectable COX-2 inhibitor with a water-soluble prodrug of sulfonamide valdecoxib that would undergo biotransformation *in vivo*.

Moreover, the 3-substituted phenyl-5-isoxazolecarboxaldehydes[28] has been identified as activated aldehydes for the generation of isoxazole-based combinatorial libraries on solid phase through automation. Three highly functionalized isoxazole-based libraries (**Figure 15**) comprising of compounds each have been synthesized in parallel format using Baylis Hillman reaction, Michael addition, reductive amination and alkylation reactions. With an objective of lead generation all the three libraries were evaluated for their antithrombin activity *in vivo*. All the compounds obtained were evaluated for their antithrombotic activity *in vivo*. Swiss mice (20–25 g, from CDRI animal colony) were used in a group of at least 10 animals each. Thrombosis was induced by infusion of a mixture of 15 mg collagen and 5 mg adrenaline in a volume of 100 mL into the tail vein of each mouse. The compounds were administered at 30 μmol/kg by oral route 1 h prior to the thrombotic challenge. The antithrombotic effects of these compounds were assessed by the percentage protection offered by these agents to mice from death or paralysis following thrombotic challenge using aspirin as a standard.

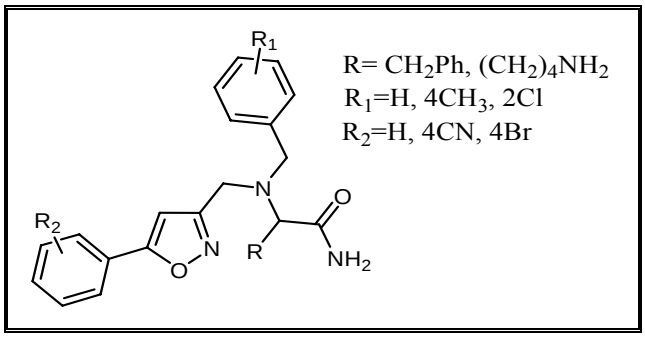


Figure 15

Pruitt J. R. et al[29] have evaluated trisubstituted isoxazoles for their *in vitro* and *in vivo* antithrombotic efficacy. They were compared to trisubstituted isoxazolines (**Figure 16**) for Factor Xa selectivity and potency. They were also compared in an arterio-venous (A-V) shunt model of thrombosis. Factor Xa (fXa) catalyzes the production of thrombin from prothrombin and sits at the junction of the intrinsic and extrinsic pathways of the coagulation cascade. It has recently been suggested that fXa inhibitors may be more effective as antithrombotic agents than direct inhibitors of thrombin and may have less bleeding risk, leading to a better safety-efficacy ratio.

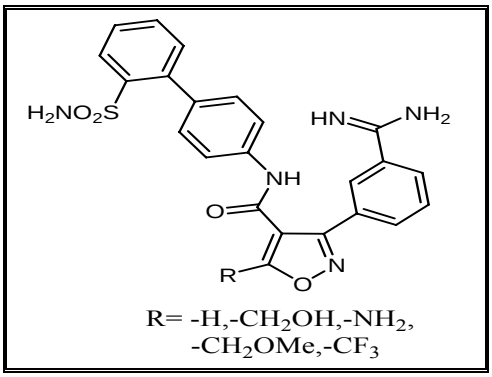


Figure 16

Some of the biological activities described to isoxazole derivatives includes PAF antagonist, hypolipidemic, nootropic, immunomodulator, antiobesity and CNS modulation.[30] The substituted isoxazoles, are also considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates such as 1,3-dicarbonyl, 1,3-iminocarbonyl and γ-amino alcohols. The significance of this class of molecules gets further impetus due to their involvement as intermediates in the synthesis of various natural products.[31]

3.3 Synthesis of functionalized isoxazoles using various synthetic approaches.

Isoxazoles can be synthesized by various methods, which are described as under.

Tweedie S. R. et al.[32] have synthesized a palladium-catalyzed couplings of heteroaryl amines with aryl halides using sodium phenolate as the stoichiometric base (**Figure 17**).

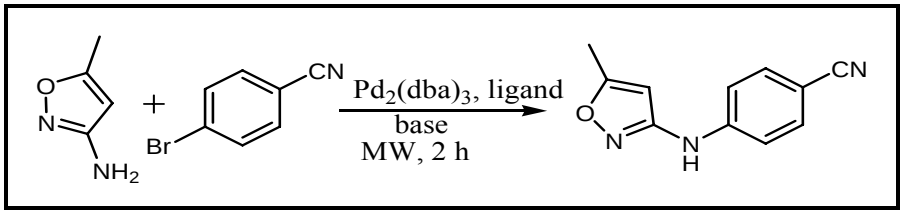


Figure 17

Yavari I. et al.[33] were synthesized isoxazole derivatives (**Figure 18**) through the reaction of activated acetylenes and alkyl 2-nitroethanoates in the presence of triphenylphosphine.

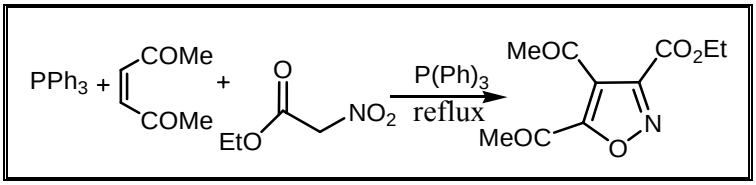


Figure 18

Burkhart D. J. et al.[34] have synthesized the 4-acetyl-5-methyl-3-isoxazoyl carboxylate (**Figure 19**) by the reaction of *α*-halo oxime with 1,4-diketone in presence of base. Further, the isoxazole smoothly lithiated at the 5-methyl position and followed by quenched the anion with a variety of electrophiles such as alkyl halides, aldehyde, TMSCl and Me_3SnCl in good to excellent yields.

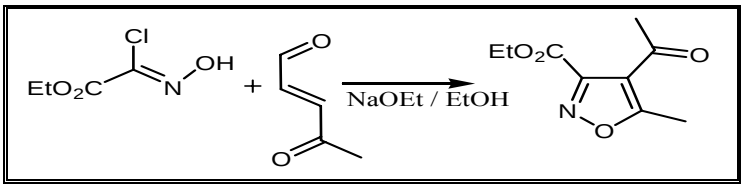


Figure 19

Suzuki K. et al.[35] have synthesized functionalized isoxazole derivatives (**Figure 20**) by cyclocondensation of *C*-chlorooximes with cyclic 1,3-diketones in the presence of base.

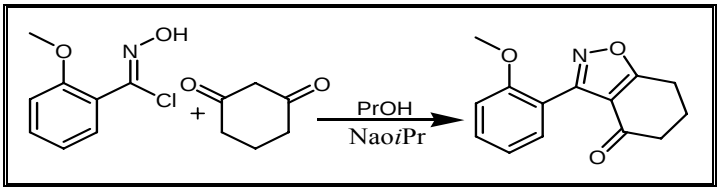


Figure 20

Lauten M. et al.[36] have synthesized highly substituted isoxazole derivatives (**Figure 21**) by the reaction of *N*-acetoacetyl derivatives and hydroxyl amine hydrochloride in methanol using sodium acetate.

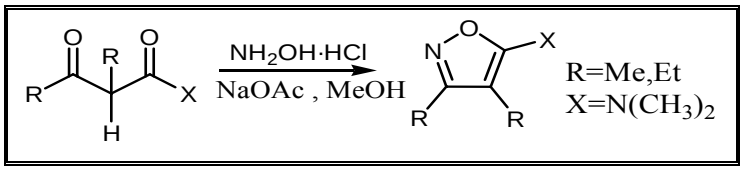


Figure 21

Kislyi V. P. et al.[37] were prepared 4-amino-5-benzoyl (acetyl) isoxazole-3-carboxamides (**Figure 22**) by the cyclization of *α*-hydroxyimino nitriles *o*-alkylated with bromo acetophenones (bromoacetone) in presence of lithium salt.

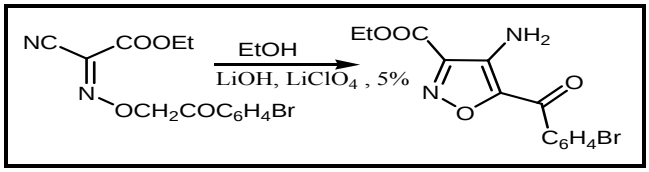


Figure 22

Holzer W. et al.[38] have synthesized 1,3-disubstituted 4-benzoyl-5-hydroxypyrazoles with phosphorusoxytrichloride affords the corresponding 4-benzoyl-5-chloropyrazoles. Reaction of the latter with hydroxylamine leads to oximes, which can be cyclized to novel 3-phenyl-6*H*-pyrazolo [4, 3-*d*] isoxazoles (**Figure 23**) by treatment with sodium hydride in dimethylformamide.

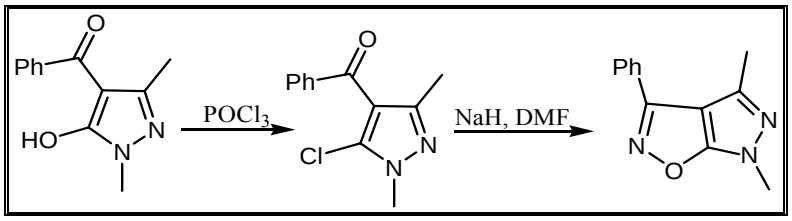


Figure 23

Bourbeau M. P. et al.[39] have synthesized series of 4-alkyl-5-aminoisoxazoles (**Figure 24**) in high yield by nucleophilic addition of lithiated alkyl nitriles to *α*-chlorooximes. The scope and limitations of this reaction were examined by varying the nature of the nitrile and chloride oxime.

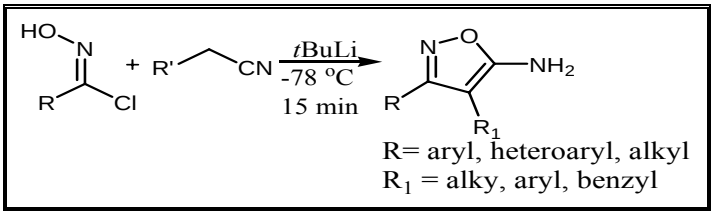


Figure 24

Recentyl, Dong D. and coworkers[40] have developed an efficient and divergent synthesis of highly substituted isoxzoles using cyclopropyl oximes (**Figure 25**). Under Vilsmeier conditions ($POCl_3/CH_2Cl_2$), substituted isoxazoles were synthesized from 1-carbamoyl, 1-oximyl cyclopropanes *via* sequential ring-opening, chlorovinylation, and intramolecular aza-cyclization.

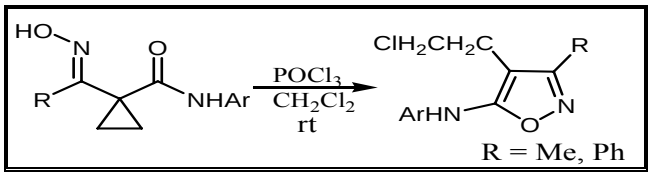


Figure 25

Duan H. et al.[41] have reported a catalytic cascade synthesis of isoxazoline-*N*-oxide (**Figure 26**) through proline-catalyzed nitroalkene activation. A large substrate scope was obtained

with good to excellent yields. Mechanistic studies were revealed that intramolecular cyclization as the rate-determining step, giving only *trans* isomers in all cases.

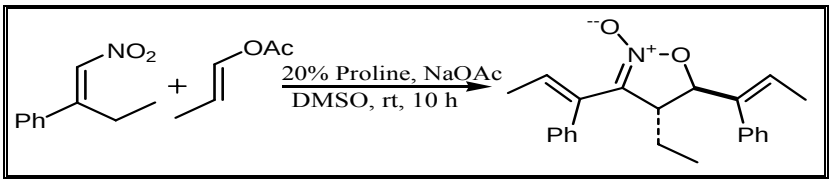


Figure 26

Daidone G. et al.[42] were obtained *N*-(5-methylisoxazol-3-yl)-2-aminobenzamide derivatives (**Figure 27**) starting from the 2-nitroaroyl chlorides and 3-amino-5-methylisoxazole in presence of stannous chloride and hydrochloric acid.

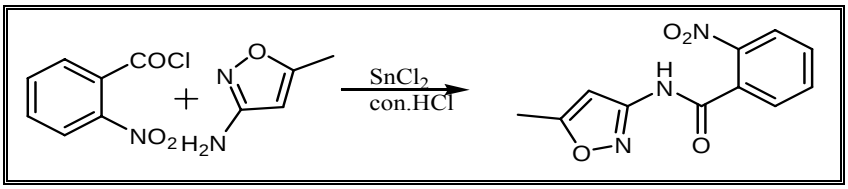


Figure 27

In 1995, Junjuppa H. et al[43] have synthesized some novel isoxazole fused estrone (**Figure 28**) derivatives *via* α-oxoketene dithioacetal.

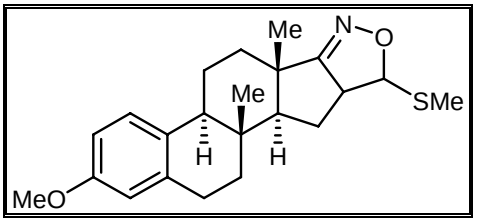


Figure 28

Moreover, 10,11-Dihydro-11-[bis(methylthio)methylene]dibenzoxepin-10-one (**I**) has been utilized as a useful three carbon synthon for the efficient regiospecific annulation of a isoxazole derivatives (**Figure 29**) by Junjappa H. and coworkers.[44] The reaction involves cyclocondensation of **I** with hydroxylamine in EtOH/NaOEt gave 72% fused isoxazoles.

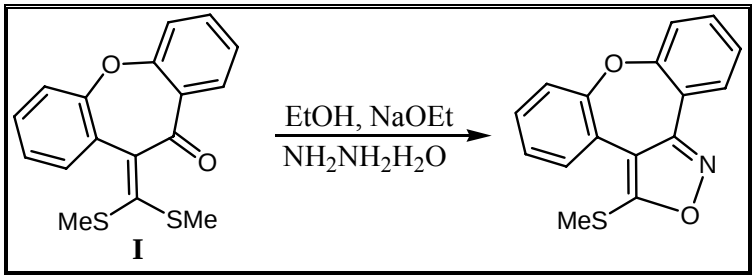


Figure 29

Kuettel S. et al[45] have synthesized 4-(3-phenylisoxazol-5-yl)morpholine derivatives (**Figure 30**) by two synthetic routes, in which substituted acetophenones were reacted with carbon disulfide and methyl iodide in the presence of sodium hydride to give 4-phenoxyphenyl-2,2-bis(methylthio)vinylketones, followed by *in situ* cyclization of the resulting *N*,*S*-acetals with hydroxylamine.

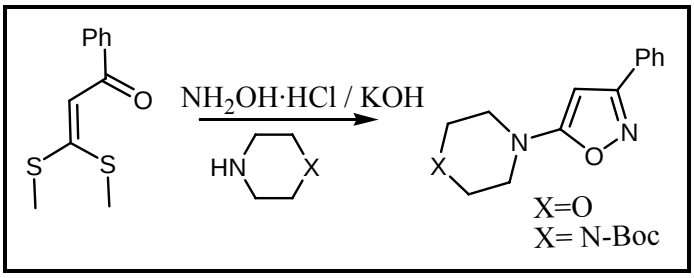


Figure 30

Mahata P. K. et al[46] have synthesized 3-dimethoxymethyl-5-(methylthio) isoxazole derivatives (**Figure 31**) with mask or unmask aldehyde functionality by cyclocondensation reaction of 1,1-dimethoxy-4,4-bis(methylthio)but-3-en-2-one as three carbon synthon with hydroxylamine in alcohol.

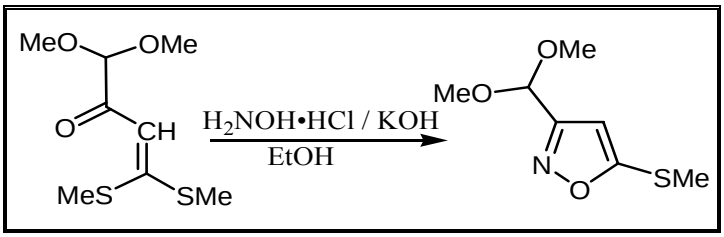


Figure 31

Dieter R. K. et al[47] have demonstrated that *α*-oxoketene dithioacetals derived from various aliphatic or aromatic ketones afforded oximes**,** upon treatment with hydroxylamine in ethanol at reflux. They were further converted the oximes into isoxazoles upon treatment with Amberlyst 15 ion exchange resin (**Figure 32**).

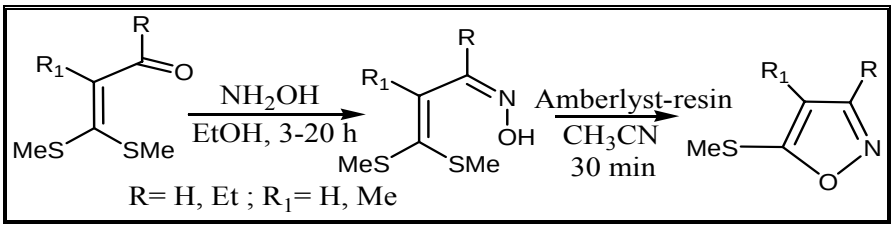


Figure 32

❖ **Synthesis of functionalized isoxazoles using combinatorial chemistry approach.**

Recently, Tu S. J. et al[48] have developed a series of new polycyclic-fused isoxazole [5,4-*b*]pyridines (**Figure 33**) through a one-pot tandem reaction under microwave irradiation in water without any use of additional reagent or catalyst. The synthetic protocol represents a green one and makes this methodology suitable for library synthesis in drug discovery efforts.

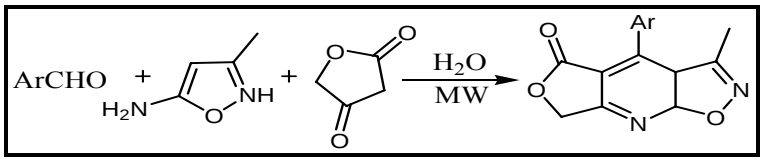


Figure 33

Laborde E. et al[49] have developed an efficient three-component, two-step "catch and release" solid-phase synthesis of 3,4,5-trisubstituted isoxazoles (**Figure 34)**. The reaction involves a base-promoted condensation of a 2-sulfonyl acetonitrile derivative **1** with an isothiocyanate **2** and *in situ* immobilization of the resulting thiolate anion **3** on Merrifield resin. Reaction of the resin-bound sulfonyl intermediate **4** with hydroxylamine, followed by release from the resin and intramoleculer cyclization, afforded 3,5-diamino-4-(arylsulfonyl)-isoxazoles **5**. However, this methodology has some drawback such as; long reaction time, isolation of product and high reaction temperature.

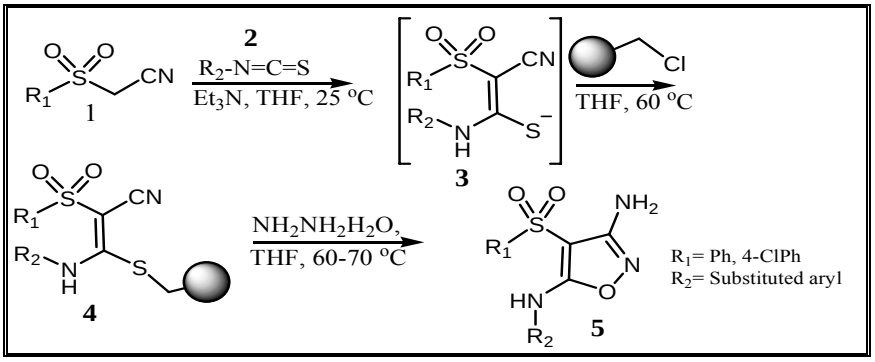


Figure 34

In 2008, Waldo J. P. et al[50] have reported solution phase synthesis of a diverse library of highly substituted isoxazoles (**Figure 35**). The reaction involved iodocyclization of *o*-methyloximes of 2-alkyn-1-ones affords 4-iodoisoxazoles, which undergo various palladium-catalyzed reactions to yielded 3,4,5-trisubstituted isoxazoles. The palladium-catalyzed processes have been adapted to parallel synthesis utilizing commercially available boronic acid, acetylene, styrene, and amine sublibraries. Accordingly, a diverse 51-member library of 3,4,5-trisubstituted isoxazoles has been generated.

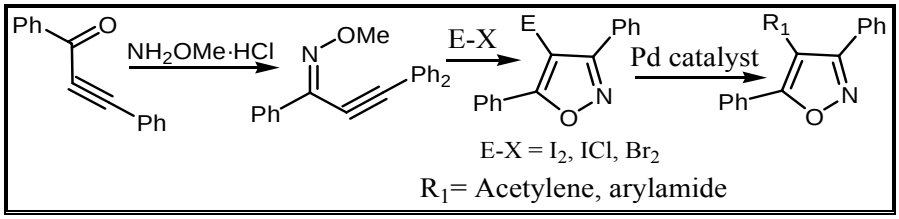


Figure 35

Kurth M. J. et al[51] have constructed a library of 3-Aryl-4,5-dihydroisoxazole-5-carboxamides (**Figure 36**). They were investigated the reaction order (nitrile oxide 1,3-dipolar cycloaddition followed by amide formation, or vice versa) both experimentally and computationally to determine which route would result in the highest yields, minimize purification efforts, and give higher 1,3-dipolar cycloaddition regioselectivity.

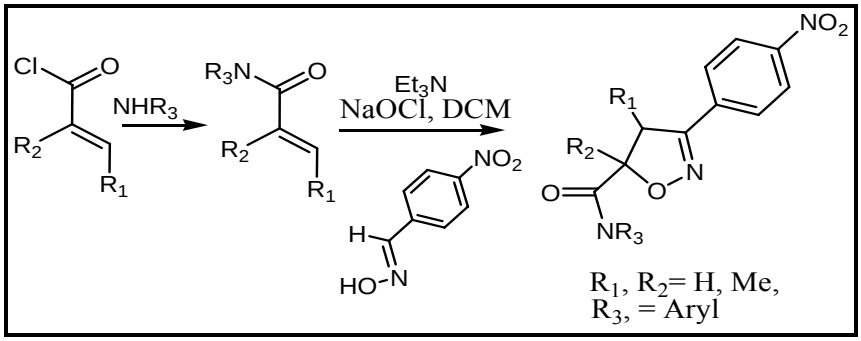


Figure 36

In addition, Taddei M. et al.[52] have developed libraries of substituted isoxazole through *in-situ* generation of polymer-bound enaminones (**Figure 37**). The synthetic protocol makes use of commercially available aniline cellulose, a low-cost and versatile biopolymer, under very mild conditions. This new support allowed carrying out reactions in polar solvents under both conventional heating and MW irradiation without degradation of the polymer. The reaction between cellulose-bound enaminones and hydroxylamine to afford the target heterocycles in high yields directly in solution is the key step. The support can be conveniently recycled.

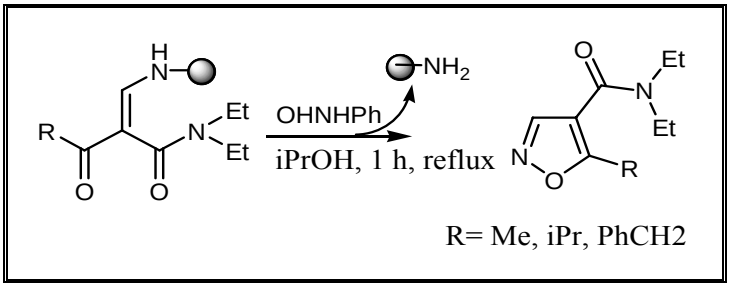


Figure 37

3.4 CURRENT RESEARCH WORK

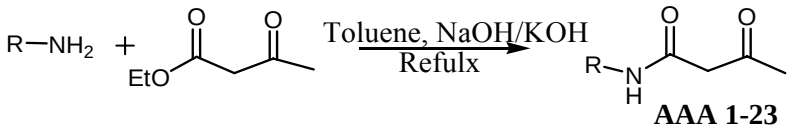
The isoxazole nucleus is present in a wide range of biologically interesting molecules, which show anti HIV, analgesic, anti-inflammatory, antibacterial, hypoglycemic, sedative-hypnotic activities.[3-21] As we discussed, the remarkable biological potential of the sulfone group and carboxamide group bearing isoxazole scaffolds have attracted many chemists to synthesize this class of molecules. Thus, continuous efforts have been devoted to the development of more general and versatile synthetic methodologies for this class of compounds. Many research groups have been synthesized isoxazole derivatives using various methods. However, the existing methods suffered with some drawbacks such as; long reaction time, product isolation, etc. Thus, the practical synthesis of structurally diverse isoxazole based small molecules is of great significance.

Nowadays, a great deal of effort has been focused on the field of green chemistry in adopting methods and processes. As a part of this "green" concept, toxic and/or flammable organic solvents are replaced by alternative non-toxic and nonflammable media. In this context, many efforts have been made to use aqueous media. Among alternative green solvents, water has been the solvent of choice for a variety of transformations. On the other hand; functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the construction of substituted heterocycles. Given the importance of sulfone and carboxamide groups containing isoxazoles, and our ongoing interest on the synthesis various bioactive heterocycles using novel ketene dithioacetals starting from acetoacetanilides, encouraged us to utilized these ketene dithioacetals for the construction of small molecule library of 3-methyl-5-(methylsulfonyl)-*N*-arylisoxazole-4-carboxamide derivatives in aqueous medium. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. The biological screening of the synthesized compounds is under process. The chemical purity of all the synthesized compounds was examined by UPLC.

3.5 RESULTS AND DISCUSSION

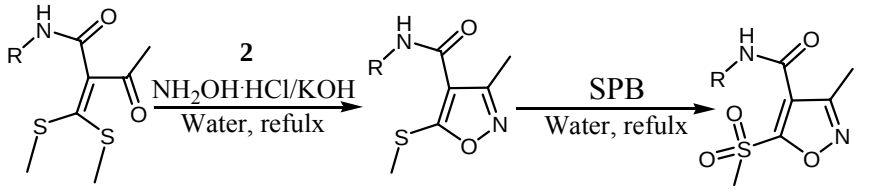
Various substituted 3-oxo-*N*-arylbutanamide were prepared by refluxing substituted amines and ethyl acetoacetate in toluene with a catalytic amount of NaOH or KOH (**Scheme 1**). The reaction mixtures were refluxed for 12-15 h. The synthesized acetoacetanilides were further converted to 2-(bis(methylthio)methylene)-3-oxo-*N*-arylbutanamide **1a-w** through the reaction with carbon disulfide, base and followed by methylation.

Scheme 1: Synthesis of various acetoacetanilides



AAA 1-23

Scheme 2: Water mediated synthesis of isoxazoles containing methyl, sulfone and carboxamide groups



1a-w **3a-w** **OPMS 1-23**

Under the optimized condition (described in chapter 3), the various ketene dithioacetals were reacted with hydroxyl amine hydrochloride **2** and potassium hydroxide to furnished isoxazoles in excellent yields using water as a solvent. (Scheme 2, Table 1). The synthesized compounds were characterized by spectral data. In the ^{1}H NMR of isoxazole **3b** a characteristic singlet for amide proton appeared at δ 9.19 and hydrogen of methylthio group displayed a singlet at δ 2.63.

Table 1: 3-mehtyl, 5-methylthio, 4-carboxamide substituted isoxazoles.

Entry	R	Time h	Yield %	mp ºC
3a	Ph	2.5	94	135-137
3b	4-CH_3Ph	2.8	92	141-142
3c	4-OCH_3Ph	3.0	92	128-130
3d	4-FPh	3.2	90	142-144
3e	2-OCH_3Ph	2.6	88	136-138
3f	2-CH_3	3.0	87	133-135
3g	4-ClPh	2.9	89	145-147
3h	4-EtPh	3.3	90	147-148
3i	4-NO_2Ph	2.5	87	149-151
3j	3-Cl,4-FPh	3.4	88	134-136
3k	5-Cl,2-OCH_3Ph	3.6	90	151-153
3l	2,5-diClPh	2.8	89	142-144
3m	2,5-diCH_3Ph	2.9	98	136-138
3n	4-Cl,2-CH_3Ph	3.0	87	137-139
3o	3,4-diFPh	3.2	91	146-148
3p	2-ClPh	3.1	94	142-144
3q	2-FPh	3.3	96	140-142
3r	4-BrPh	3.5	95	151-153
3s	3,4-diClPh	2.8	90	137-139
3t	3-NO_2Ph	3.2	88	125-127
3u	3-CH_3Ph	3.0	94	130-132
3v	2,3-diCH_3Ph	3.7	85	138-140
3w	2-OCH_3,4-NO_2Ph	2.8	96	124-126

As we described the significant utility of sulfone group in medicinal chemistry and to develop library of isoxazole functionalized with alkyl, carboxamide and sulfone groups, we next planned to oxidize the sulfides to sulfones. With the optimized condition in hand for

oxidation, all the isoxazoles were oxidized to make sulfone group containing isoxazole derivatives and the results are gather in table 2.

Table 2: 3-mehtyl, 5-sulfone, 4-carboxamide functionalized library of isoxazoles.

Entry	R	Time min	Yield %	mp ºC
OPMS-1	Ph	60	94	186-188
OPMS-2	4-CH_3Ph	50	95	192-194
OPMS-3	4-OCH_3Ph	60	93	188-190
OPMS-4	4-FPh	55	91	191-193
OPMS-5	2-OCH_3Ph	65	94	184-186
OPMS-6	2-CH_3	55	96	179-181
OPMS-7	4-ClPh	60	92	185-187
OPMS-8	4-EtPh	65	95	192-194
OPMS-9	4-NO_2Ph	60	93	196-198
OPMS-10	3-Cl,4-FPh	55	92	188-190
OPMS-11	5-Cl,2-OCH_3Ph	55	91	195-197
OPMS-12	2,5-diClPh	60	90	188-190
OPMS-13	2,5-diCH_3Ph	55	91	181-183
OPMS-14	4-Cl,2-CH_3Ph	50	88	187-189
OPMS-15	3,4-diFPh	65	89	189-191
OPMS-16	2-ClPh	60	93	191-192
OPMS-17	2-FPh	55	87	182-184
OPMS-18	4-BrPh	65	85	162-164
OPMS-19	3,4-diClPh	60	82	155-157
OPMS-20	3-NO_2Ph	60	90	164-166
OPMS-21	3-CH_3Ph	65	94	185-187
OPMS-22	2,3-diCH_3Ph	60	92	188-190
OPMS-23	2-OCH_3,4-NO_2Ph	55	97	172-174

The chemical purity of all the newly synthesized compounds was examined using UPLC. Among all the final compounds, compound **OPMS 12** has shown less than 95% chemical purity and other shown more than 95% chemical purity (**Figure 38**). The ^{1}H NMR of compound **OPMS-1** displayed a characteristic singlet for amide proton at δ 9.88 and two singlets for methyl and mehtylthio hydrogen, respectively, at δ 2.69 and 3.33. The overall study indicates that this is the simple and facile methodology to introduce sulfone and caboxamide group to isoxazole scaffold in excellent yield and chemical purity.

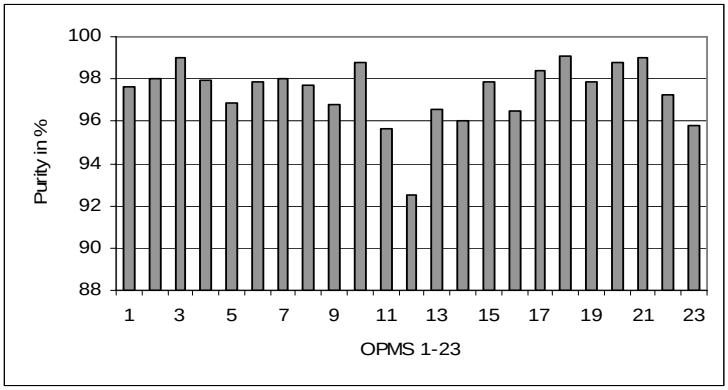


Figure 38: Chemical purity of trifunctionalized isoxazoles using UPLC

In ketene dithioacetal system the carbonyl carbon and β-carbon atoms regarded as hard and soft electrophilic centers, since the carbonyl carbon is adjacent to the hard-base oxygen while the β-carbon is flanked by the soft-base methylthio groups. Thus, the nucleophile of hydroxylamine hydrochloride attack on β-carbon of systems and formed heterocyclic product by removal of methylthio group as good leaving group (**Figure 39**).

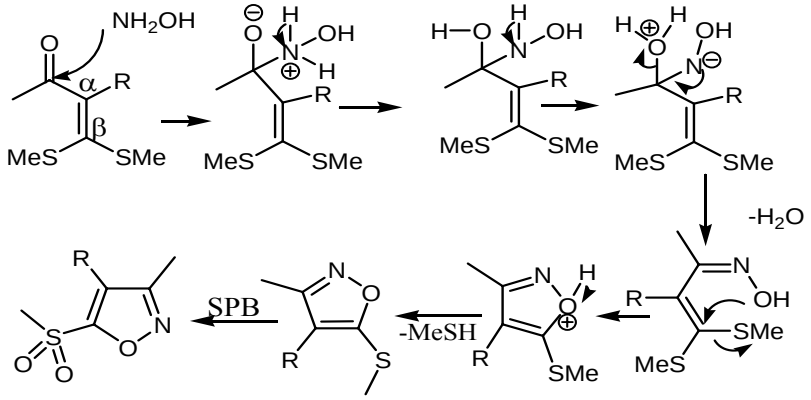


Figure 39: Mechanism for the formation of Isoxazole.

3.6 CONCLUSION

In Summary, we have synthesized solution-phase library of isoxazoles functionalized with methyl, sulfone and carboxamide moieties in two steps with excellent yield and chemical purity for medicinally interesting molecules. Water was emerged as an efficient and green solvent in the condensation reaction of various ketene dithioacetals with hydroxyl amine hydrochloride. Further, the facile synthesis of isoxazole containing sulfone group was achieved *via* oxidation of sulfide to sulfone. SPB was more efficient and effective oxidizing agent for oxidation of sulfide to sulfone in aqueous medium. This procedure offers a good scope for the synthesis of a wide variety of isoxazoles containing caboxamide and sulfone in two steps.

3.7 EXPERIMENTAL SECTION

Melting points were determined in open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F_{254} (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Chemical purity was determined on Waters Acquity UPLC with PDA Detector using Acquity BEH C18, 50 × 2.1, 1.7µm column at 210-400nm.

- **Gradient Program for UPLC:** (Same as chapter 2)
- **General synthesis of 3-oxo-*N*-arylbutanamide AAA 1-23.**

A mixture containing the primary amine (10 mmol), ethyl acetoacetate (10 mmol), and catalytic amount of sodium or potassium hydroxide lie (10 %) was reflux at 110 ^{o}C for the approximately 12-15 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under *vaccuo* and the solid or oil was crystallized from methanol which afforded pure products.

- **General procedure for the synthesis of trisubstituted isoxzoles 3a-w.**

To a well stirred solution of hydroxyl amine hydrochloride (15 mmol), potassium hydroxide (15 mmol) in water (25 mL) was added suspension of various ketene dithioacetals **1a-w** and refluxed the resulting mixture for appropriate time (Table 1) with constant stirring. After completion of the reaction, the reaction mixture was allowed to come to room temperature and add cold water (50 mL). The separated suspension was filtered, washed with water (2 × 50 mL), dried and crystallized from methanol to afford analytically pure products which were used for next step without further purification.

- **General procedure for the oxidation of sulfide to sulfones OPMS 1-23.**

The appropriate compound **3a-w** (6 mmol) in water (15 mL) was added sodium per borate (18 mmol) and the resulting mixture was then heated to reflux for appropriate time (Table 2). The mixture was cooled to room temperature and extracted with ethyl acetate (2 × 15 mL). The organic layer was washed with water (2× 20 mL) and dried over magnesium

sulphate. The solvent was evaporated at room temperature and product was isolated by crystallization technique in excellent yield.

3-methyl-5-(methylsulfonyl)-*N*-phenylisoxazole-4-carboxamide (OPMS-1): White solid; *R*f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3207, 2922, 1647, 1496, 1406, 1207 cm^{-1}; ^1H NMR: δ 2.69(s, 3H, CH_3), 3..33 (s, 3H, CH_3), 7.09-7.15 (q, 1H, ArH), 7.66-7.73 (t, 2H, ArH), 7.75-7.97 (t, 2H, ArH), 9.88 (s, 1H, NH); ^{13}C NMR: δ 12.47, 40.97, 111.18, 121.41, 122.76, 132.52, 132.24, 140.19, 159.75, 169.74; MS (*m/z*): 280 (M^+); Anal. Calcd for $C_{12}H_{12}N_2O_4S$: C, 51.42; H, 4.32; N, 9.99; Found: C, 51.29; H, 4.24; N, 9.89.

***N*-(4-methylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-2):** White solid; *R*f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3275, 2822, 1687, 1526, 1406, 1284 cm^{-1}; ^1H NMR: δ 2.32 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 3.32 (s, 3H, CH_3), 7.13-7.16 (q, 2H, ArH), 7.52-7.56 (t, 2H, ArH), 9.79 (s, 1H, NH); ^{13}C NMR: δ 12.43, 25.99, 40.59, 111.38, 124.74, 124.95, 132.59, 137.43, 138.16, 138.24, 142.32, 159.47, 159.98, 169.05, 169.96; MS (*m/z*): 294 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_4S$: C, 53.05; H, 4.79; N, 9.52; Found: C, 53.06; H, 4.65; N, 9.44.

***N*-(4-methoxyphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-3):** White solid; *R*f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3319, 3034, 1663, 1514, 1423, 1251 cm^{-1}; MS (*m/z*): 310 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_5S$: C, 50.31; H, 4.55; N, 9.03; Found: C, 50.27; H, 4.43; N, 9.09.

***N*-(4-fluorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-4):** White solid; *R*f 0.36 (6:4 hexane-EtOAc); IR (KBr): 3210, 2921, 1675, 1547, 1458, 1284, 1105 cm^{-1}; MS (*m/z*): 298 (M^+); Anal. Calcd for $C_{12}H_{11}FN_2O_4S$: C, 48.32; H, 3.72; N, 9.39; Found: C, 48.21; H, 3.60; N, 9.31.

***N*-(2-methoxyphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-5):** White solid; *R*f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3266, 2741, 1674, 1529, 1444, 1124 cm^{-1}; MS (*m/z*): 310 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_5S$: C, 50.31; H, 4.55; N, 9.03; Found: C, 50.29; H, 4.44; N, 9.07.

***N*-(2-methylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-6):** White solid; *R*f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3314, 2844, 1714, 1629, 1481, 1224 cm^{-1}

^{1}; MS (*m/z*): 294 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_4S$: C, 53.05; H, 4.79; N, 9.52; Found: C, 53.03; H, 4.67; N, 9.45.

***N*-(4-chlorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-7):** White solid; *R*f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3214, 2787, 1841, 1681, 1482, 1124 cm^{-1}; MS (*m/z*): 314 (M^+); Anal. Calcd for $C_{12}H_{11}ClN_2O_4S$: C, 45.79; H, 3.52; N, 8.90; Found: C, 45.71; H, 3.43; N, 8.82.

***N*-(4-ethylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-8):** White solid; *R*f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3334, 2781, 1681, 1481, 1242 cm^{-1}; MS (*m/z*): 308 (M^+); Anal. Calcd for $C_{14}H_{16}N_2O_4S$: C, 54.53; H, 5.23; N, 9.08; Found: C, 54.41; H, 5.11; N, 8.98.

***N*-(4-nitrophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-9):** White solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3215, 2741, 1658, 1511, 1411, 1174 cm^{-1}; ^{13}C NMR: δ 13.24, 40.41, 113.38, 125.51, 127.43, 132.39, 135.06, 136.40, 141.56, 144.50, 164.49, 171.75; MS (*m/z*): 325 (M^+); Anal. Calcd for $C_{12}H_{11}N_3O_6S$: C, 44.31; H, 3.41; N, 12.92; Found: C, 44.20; H, 3.29; N, 12.81.

***N*-(3-chloro-4-fluorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-10):** White solid; R_f 0.54 (7:3 hexane-EtOAc); IR (KBr): 3210, 2754, 1711, 1629, 1511, 1124 cm^{-1}; MS (*m/z*): 332 (M^+); Anal. Calcd for $C_{12}H_{10}ClFN_2O_4S$: C, 43.32; H, 3.03; N, 8.42; Found: C, 43.19; H, 2.90; N, 8.44.

***N*-(5-chloro-2-methoxyphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-11):** White solid; *R*f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3114, 2914, 1619, 1577, 1481, 1141 cm^{-1}; MS (*m/z*): 344 (M^+); Anal. Calcd for $C_{13}H_{13}ClN_2O_5S$: C, 45.29; H, 3.80; N, 8.13; Found: C, 45.16; H, 3.71; N, 8.08.

***N*-(2,5-dichlorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-12):** White solid; *R*f 0.51 (7:3 hexane-EtOAc); IR (KBr): 3210, 2754, 1681, 1229, 1581, 1142 cm^{-1}; MS (*m/z*): 349 (M^+); Anal. Calcd for $C_{12}H_{10}Cl_2N_2O_4S$: C, 41.28; H, 2.89; N, 8.02; Found: C, 41.19; H, 2.76; N, 7.92.

***N*-(2,5-dimethylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-13):** White solid; *R*f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3110, 2741, 1617, 1529, 1311, 1124

cm^{-1}; MS (*m/z*): 308 (M^+); Anal. Calcd for $C_{14}H_{16}N_2O_4S$: C, 54.53; H, 5.23; N, 9.08 Found: C, 54.44; H, 5.10; N, 8.97.

***N*-(4-chloro-2-methylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-14):** White solid; *Rf* 0.34 (6:4 hexane-EtOAc); IR (KBr): 3224, 2871, 1658, 1519, 1311, 1121 cm^{-1}; MS (*m/z*): 328 (M^+); Anal. Calcd for $C_{13}H_{13}ClN_2O_4S$: C, 47.49; H, 3.99; N, 8.52; Found: C, 47.38; H, 3.87; N, 8.39.

***N*-(3,4-difluorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-15):** White solid; *R*f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3147, 2871, 1611, 1531, 1321, 1124 cm^{-1}; MS (*m/z*): 316 (M^+); Anal. Calcd for $C_{12}H_{10}F_2N_2O_4S$: C, 45.57; H, 3.19; N, 8.86; Found: C, 45.44; H, 3.07; N, 8.72.

***N*-(2-chlorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-16):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3221, 2851, 1895, 1684, 1371, 1118 cm^{-1}; MS (*m/z*): 314 (M^+); Anal. Calcd for $C_{12}H_{11}ClN_2O_4S$: 45.79 H, 3.52; N, 8.90; Found: C, 45.84; H, 3.43; N, 8.85.

***N*-(2-fluorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-17):** White solid; R_f 0.35 (6:4 hexane-EtOAc); IR (KBr): 3247, 2851, 1678, 1499, 1281, 1245 cm^{-1}; MS (*m/z*): 298 (M^+); Anal. Calcd for $C_{12}H_{11}FN_2O_4S$: C, 48.32; H, 3.72; N, 9.39; Found: C, 48.21; H, 3.61; N, 9.27.

***N*-(4-bromophenyl)-3-methyl-5-(methylsulfonyl) isoxazole-4-carboxamide (OPMS-18):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3277, 2811, 1581, 1448, 1227 cm^{-1}; MS (*m/z*): 359 (M^+); Anal. Calcd for $C_{12}H_{11}BrN_2O_4S$: C, 40.13; H, 3.09; N, 7.80; Found: C, 40.11; H, 3.07; N, 7.68.

***N*-(3,4-dichlorophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-19):** White solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3182, 2841, 1675, 1458, 1368, 1147 cm^{-1}; MS (*m/z*): 349 (M^+); Anal. Calcd for $C_{12}H_{10}Cl_2N_2O_4S$: C, 41.28; H, 2.89; N, 8.02; Found: C, 41.14; H, 2.75; N, 8.11.

***N*-(3-nitrophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-20):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3265, 2835, 1679, 1428, 1221 cm^{-1}; MS

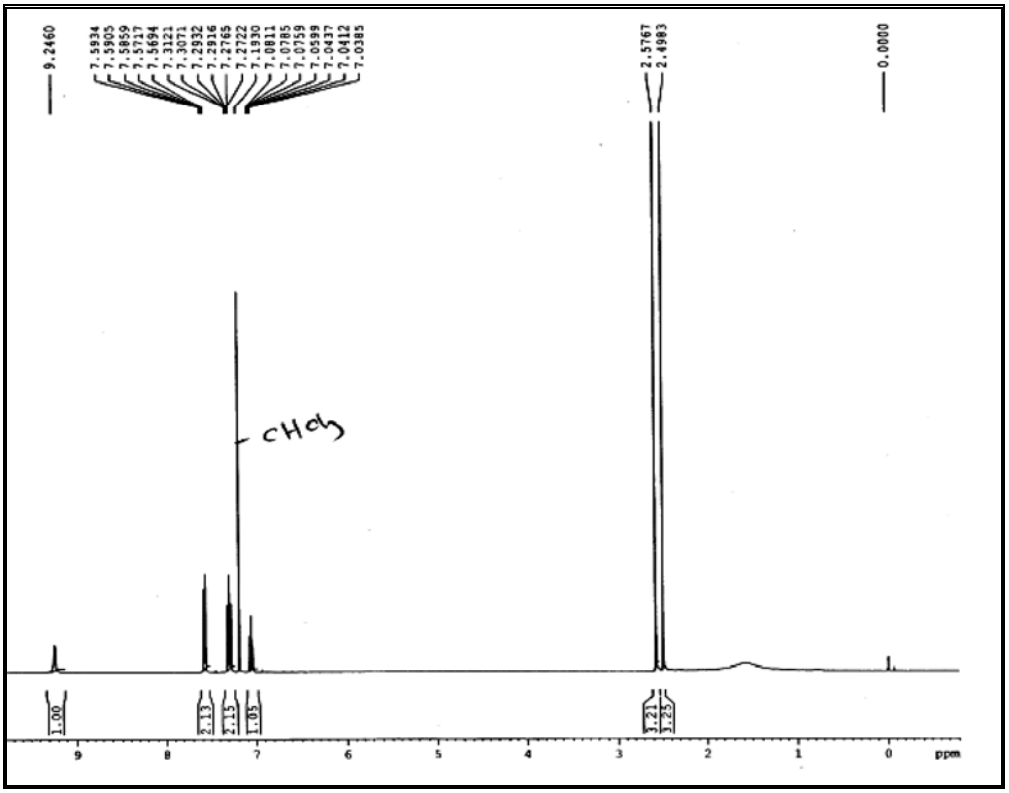
(*m/z*): 325 (M^+); Anal. Calcd for $C_{12}H_{11}N_3O_6S$: C, 44.31; H, 3.41; N, 12.92; Found: C, 44.22; H, 3.30; N, 12.82.

***N*-(3-methylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-21):** White solid; R_f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3243, 2813, 1685, 1477, 1118 cm^{-1}; MS (*m/z*): 294 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_4S$: C, 53.05; H, 4.79; N, 9.92; Found: C, 52.92; H, 4.66; N, 9.84.

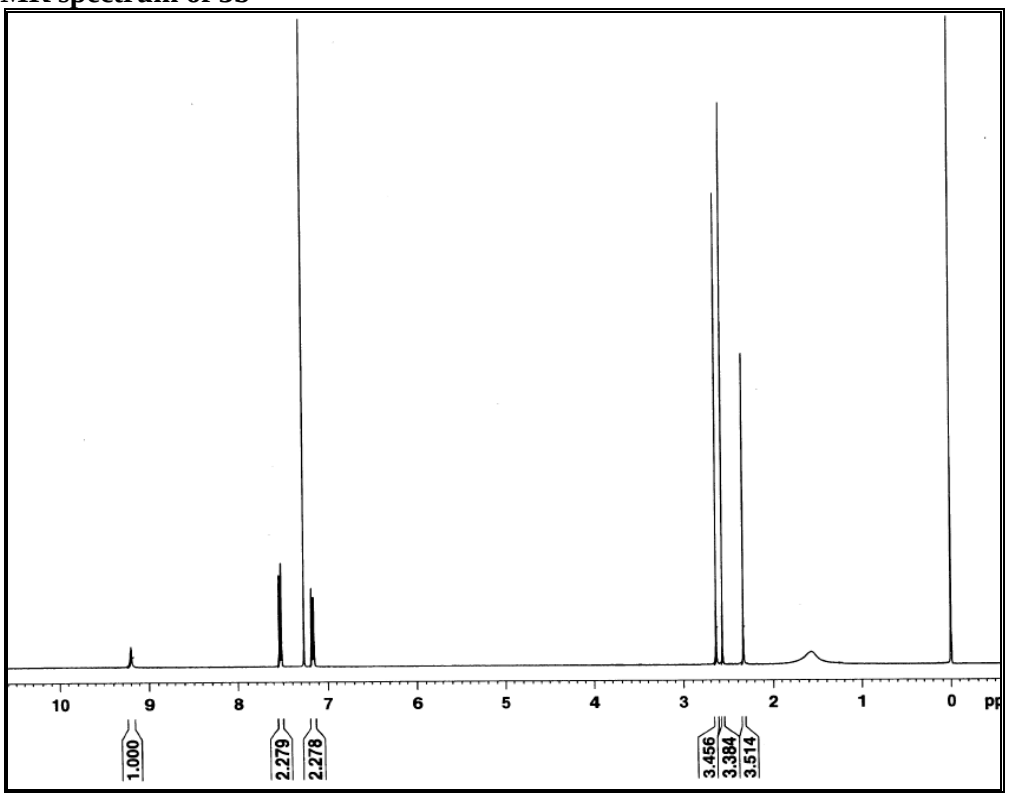
***N*-(2,3-dimethylphenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-22):** White solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3274, 2817, 1688, 1478, 1311, 1264 cm^{-1}; MS (*m/z*): 308 (M^+); Anal. Calcd for $C_{14}H_{16}N_2O_4S$: C, 54.53; H, 5.23; N, 9.08; Found: C, 54.45; H, 5.21; N, 8.98.

***N*-(2-methoxy,4-nitrophenyl)-3-methyl-5-(methylsulfonyl)isoxazole-4-carboxamide (OPMS-23):** White solid; R_f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3157, 2872, 1765, 1668, 1348, 1274 cm^{-1}; MS (*m/z*): 355 (M^+); Anal. Calcd for $C_{13}H_{13}N_3O_7S$: C, 43.94; H, 3.69; N, 11.83; Found: C, 43.81; H, 3.55; N, 11.71.

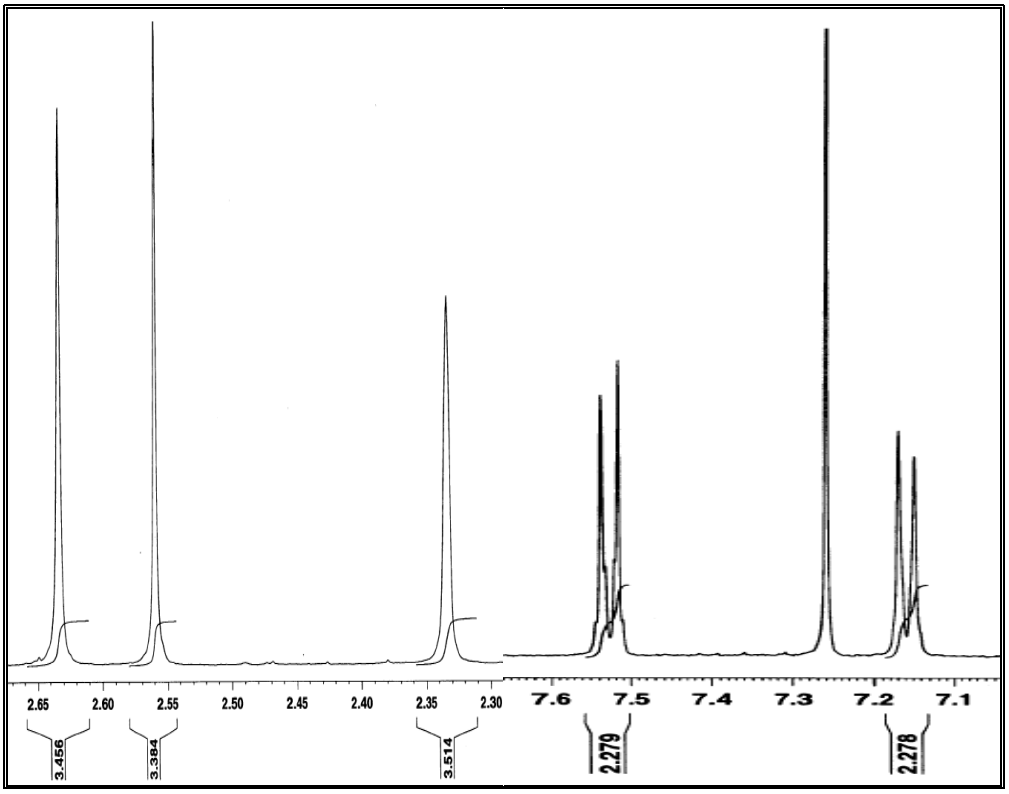
^{1}H NMR spectrum of 3a



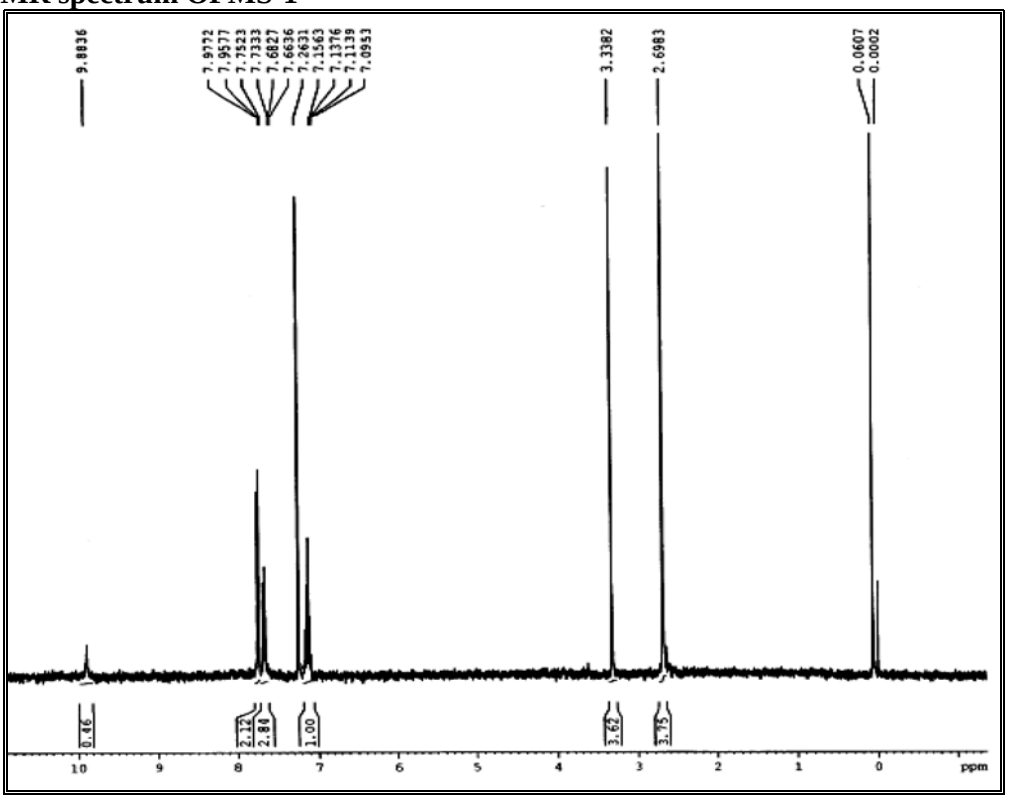
^{1}H NMR spectrum of 3b



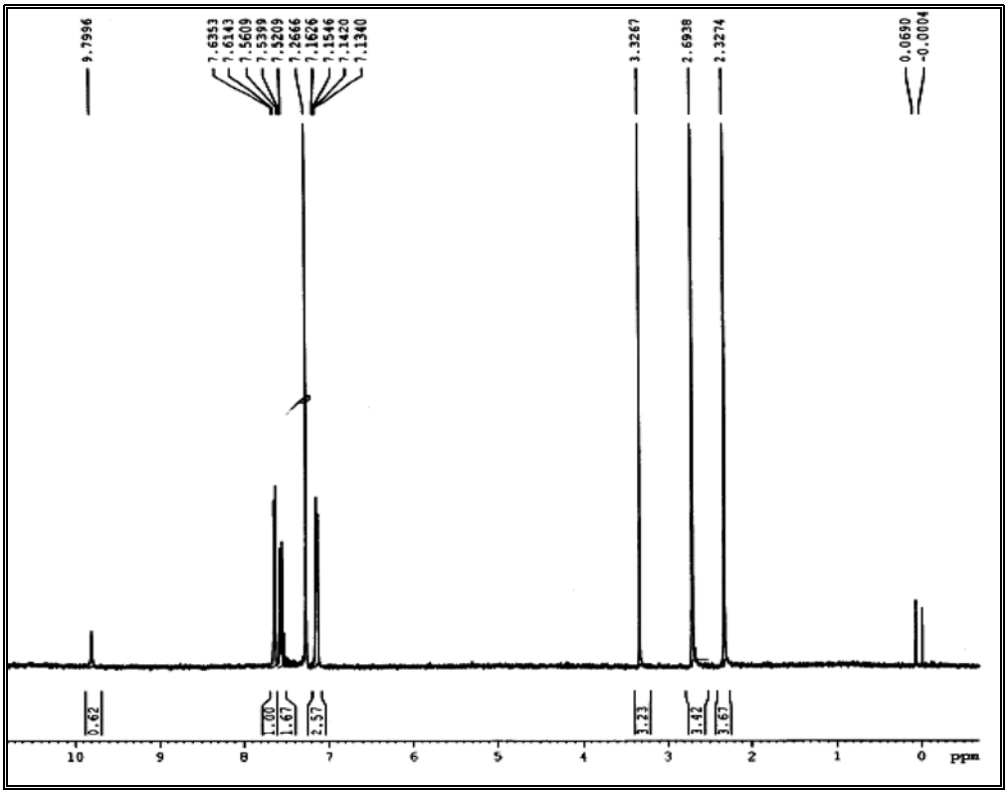
Expanded ^{1}H NMR spectrum of 3b



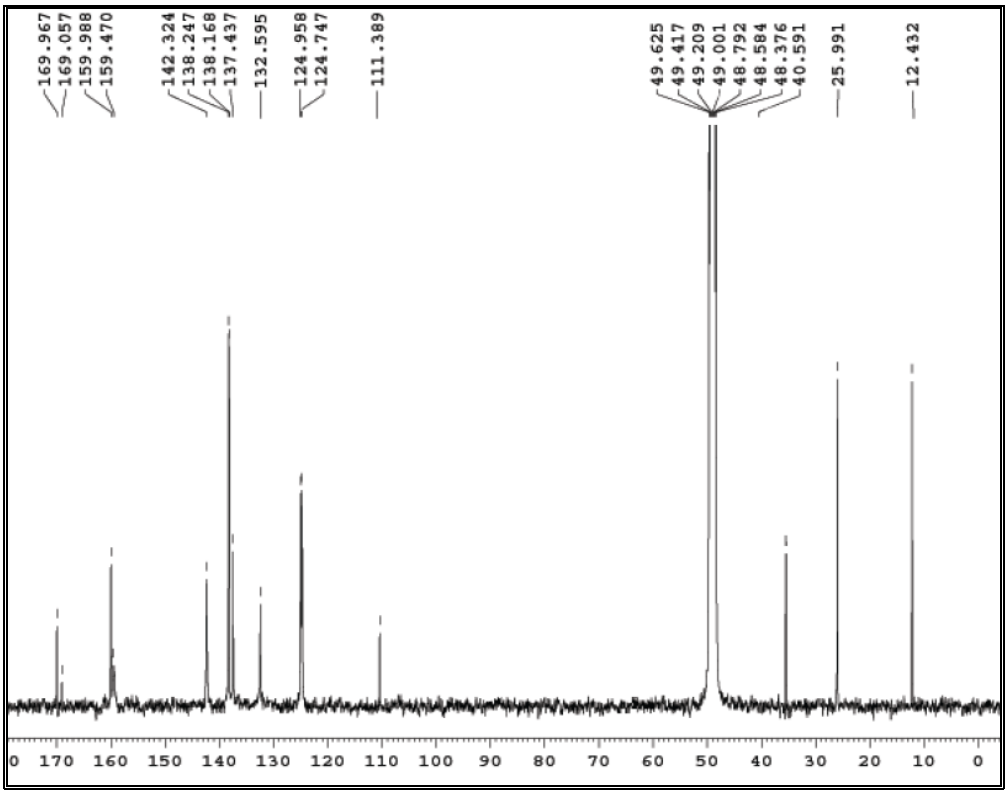
^{1}H NMR spectrum OPMS-1



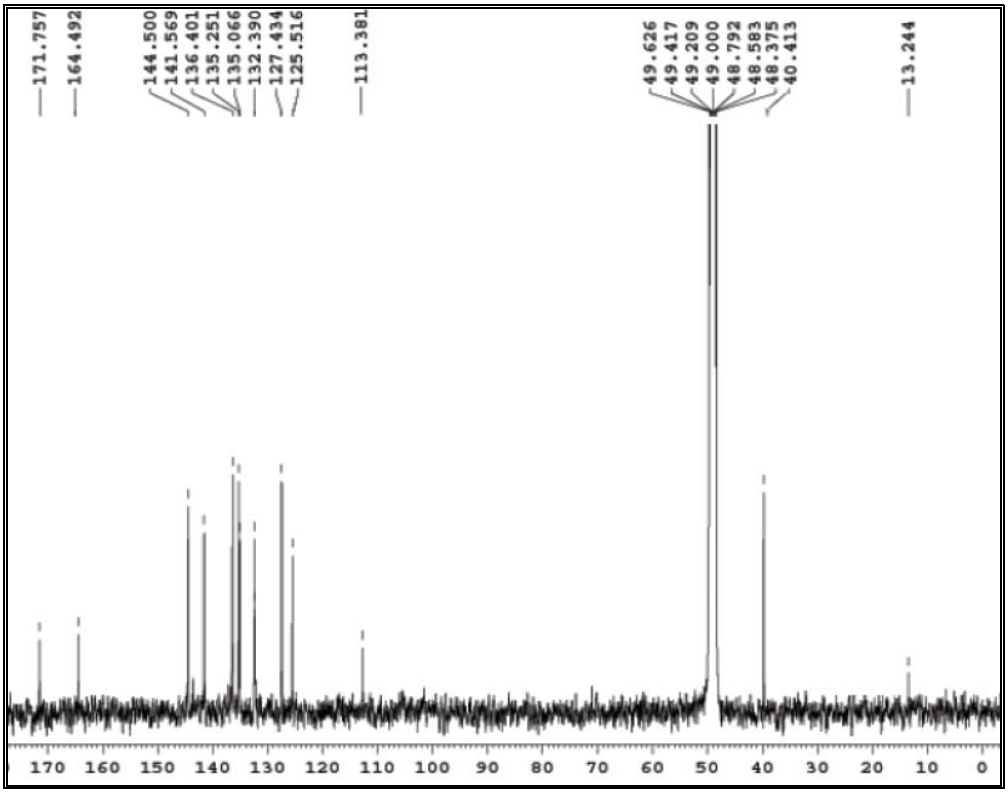
^{1}H NMR spectrum of OPMS-2



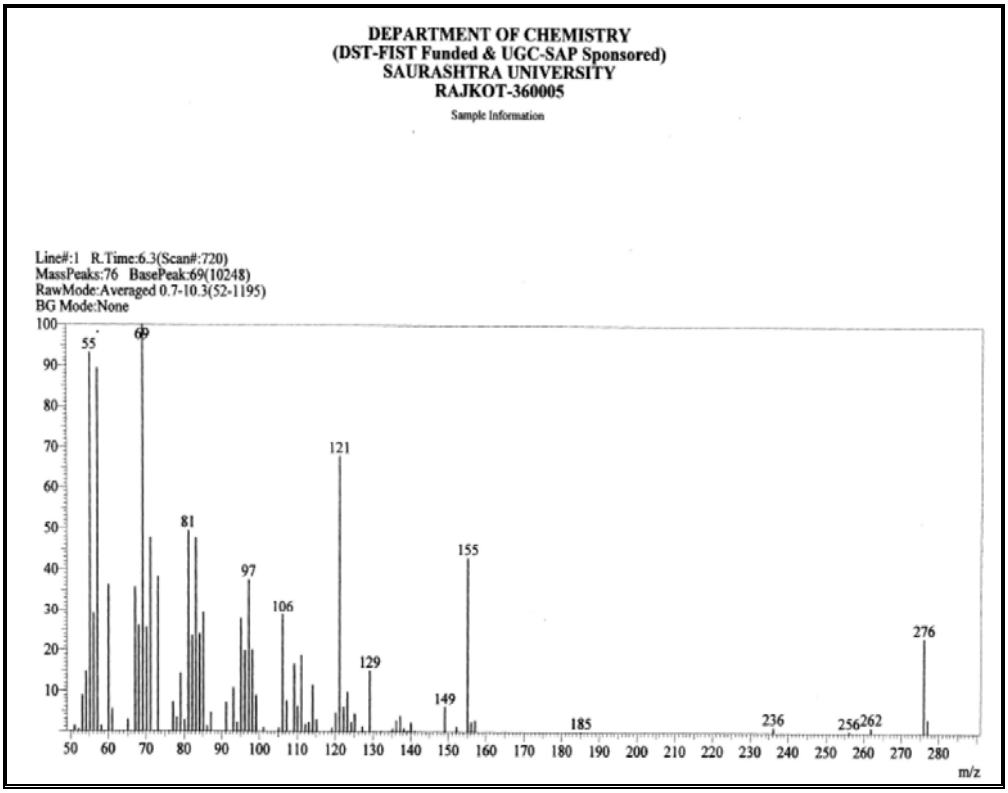
^{13}C NMR of compound OPMS-2



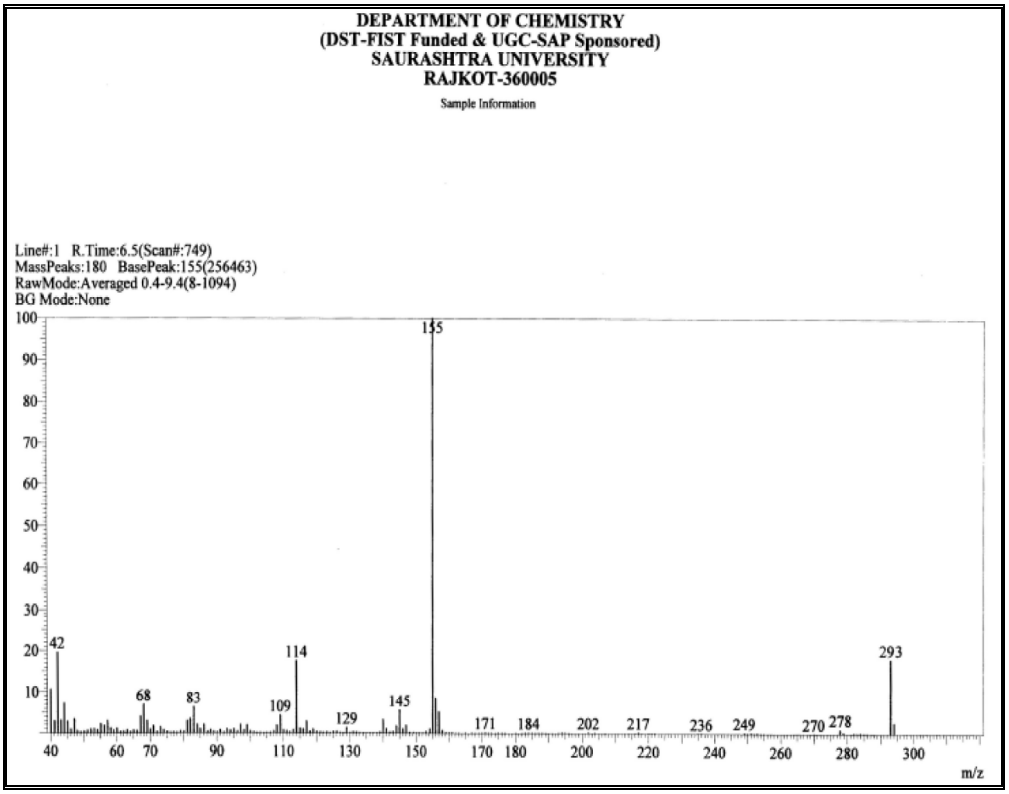
^{13}C NMR spectrum OPMS-9



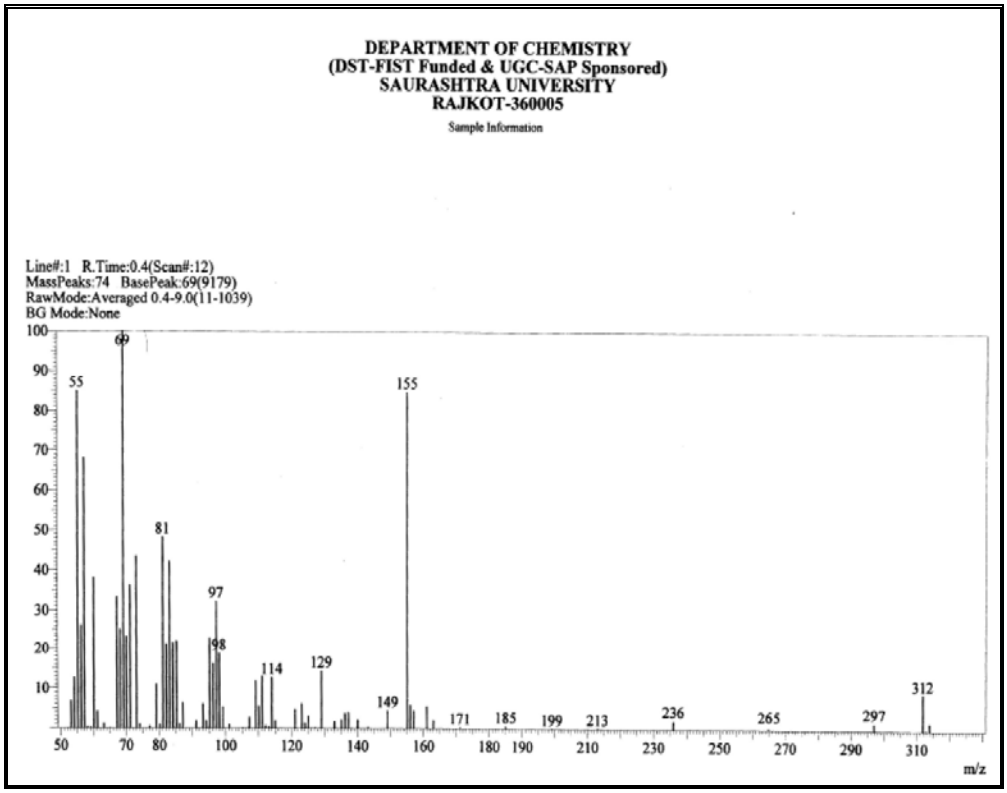
Mass spectrum of 3h



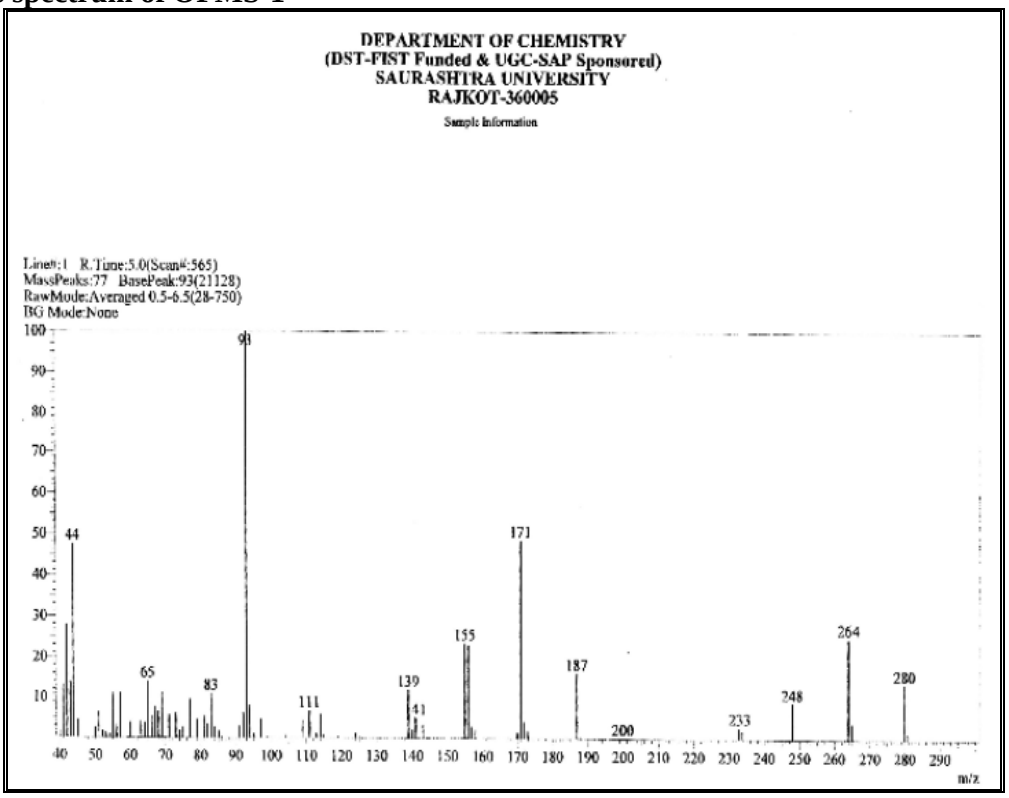
Mass spectrum of 3i



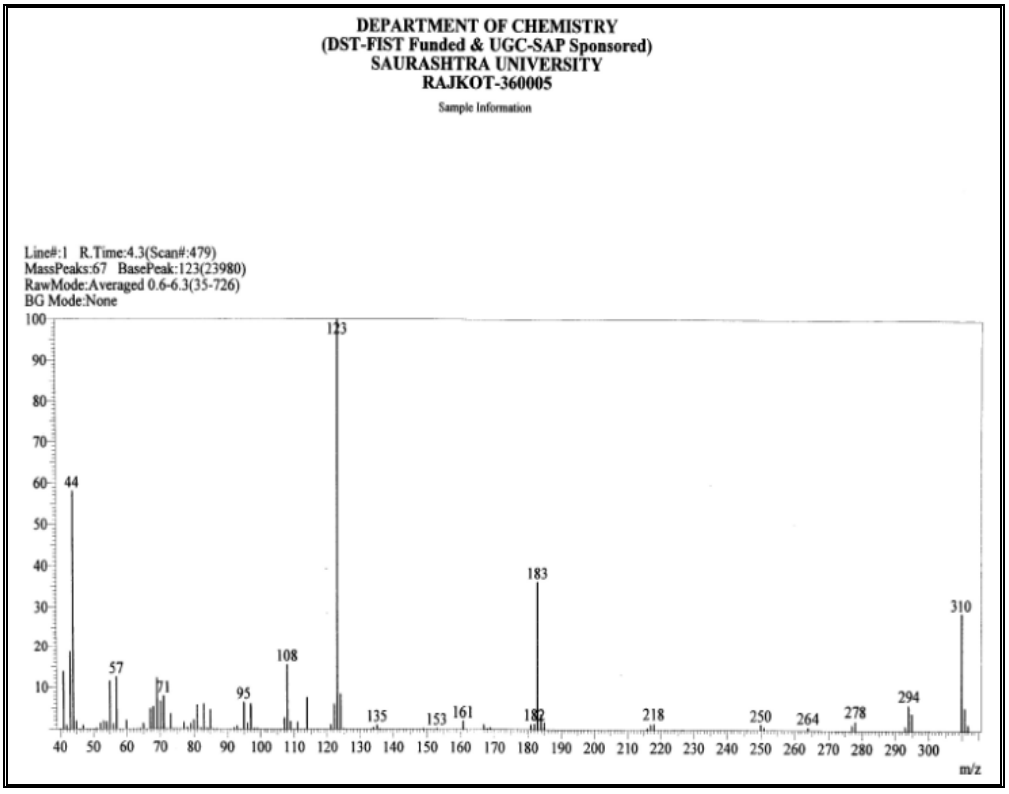
Mass spectrum of 3k



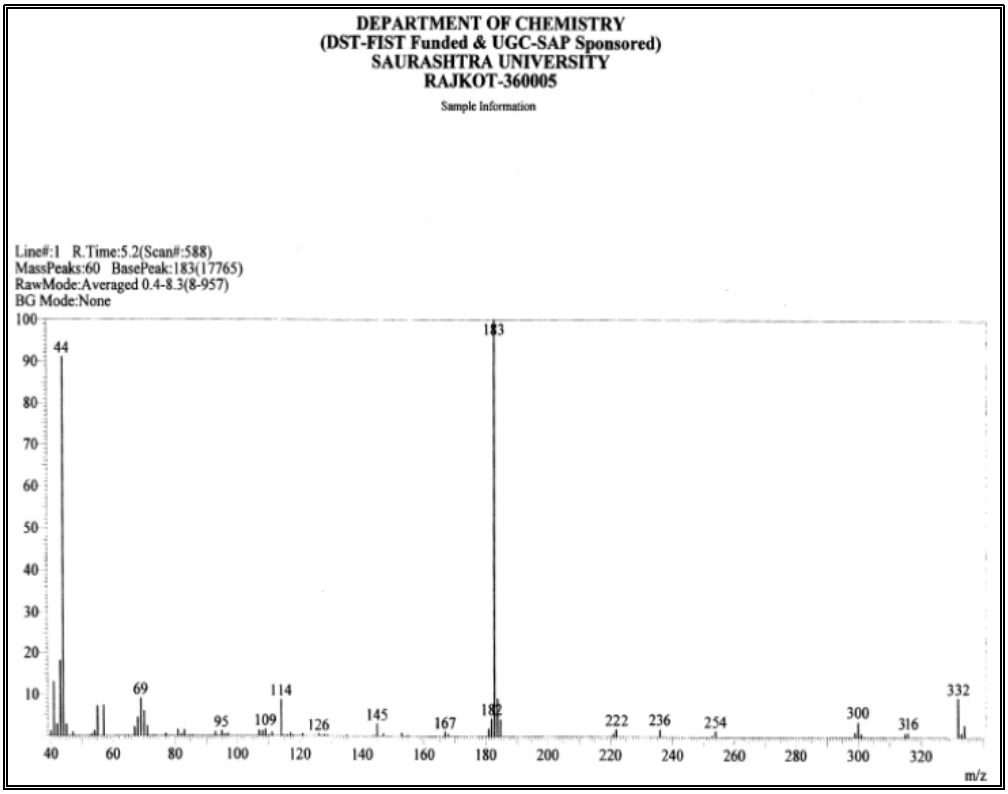
Mass spectrum of OPMS-1



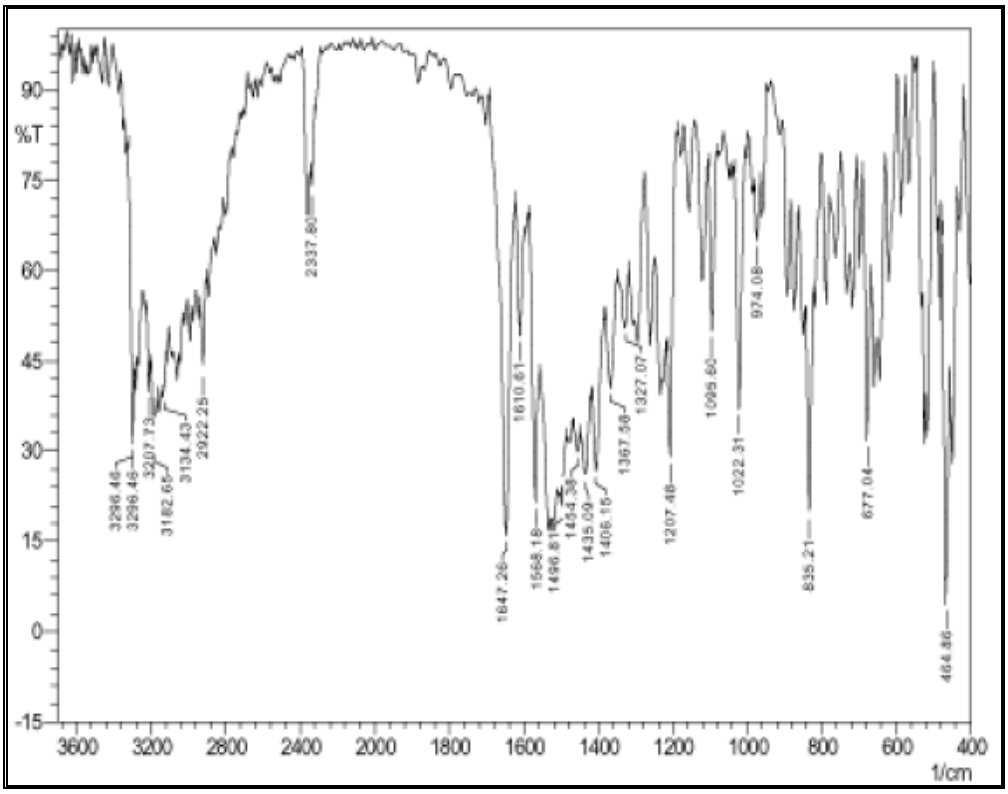
Mass spectrum OPMS-3



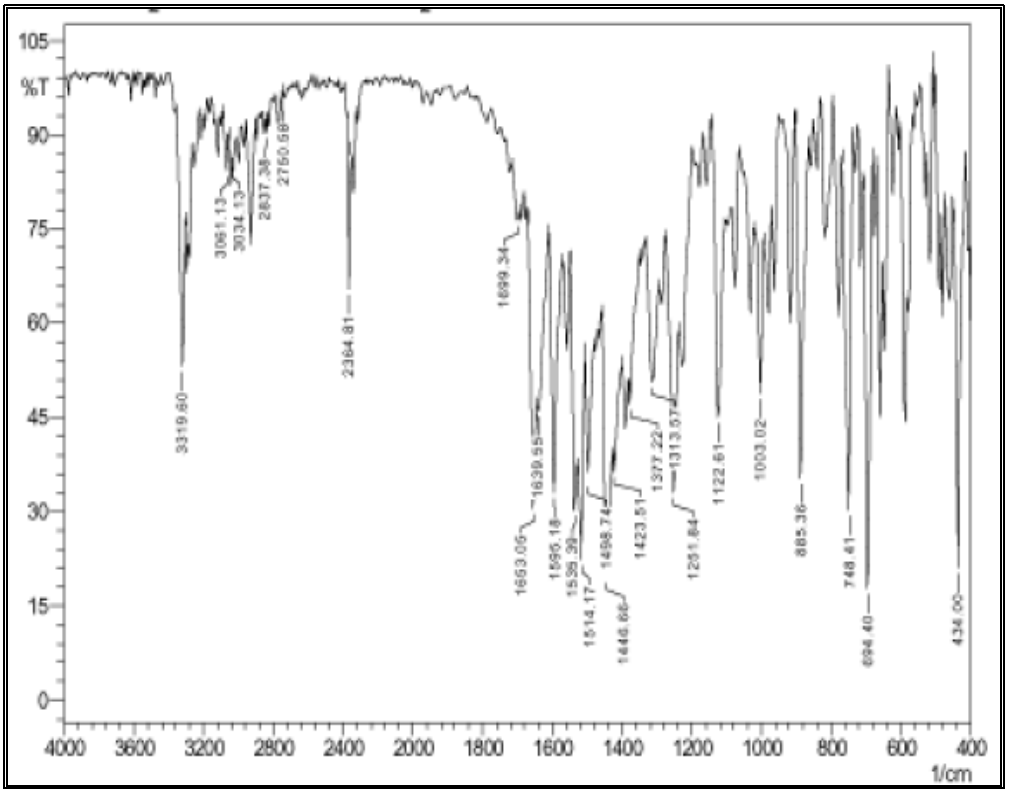
Mass spectrum of OPMS-10



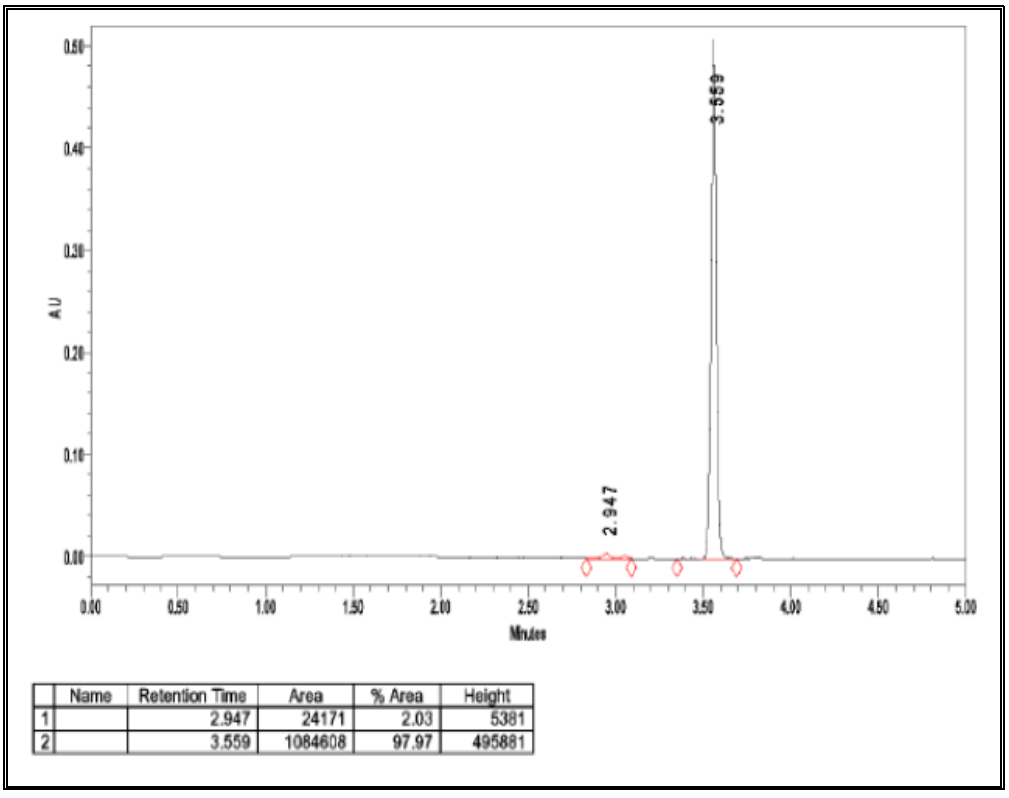
IR spectrum of OPMS-1



IR spectrum of OPMS-3

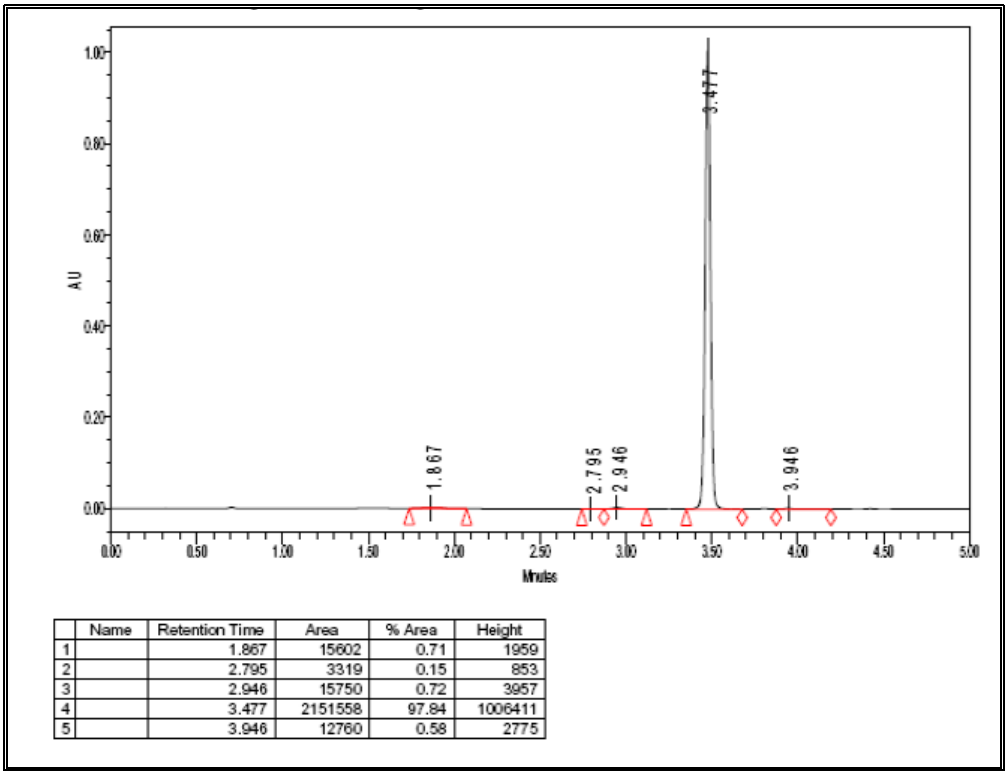


Chemical purity of OPMS-2



	Name	Retention Time	Area	% Area	Height
1		2.947	24171	2.03	5381
2		3.559	1084608	97.97	495881

Chemical purity of OPMS-6



	Name	Retention Time	Area	% Area	Height
1		1.867	15602	0.71	1959
2		2.795	3319	0.15	853
3		2.946	15750	0.72	3957
4		3.477	2151558	97.84	1006411
5		3.946	12760	0.58	2775

3.8 REFERENCES

1. Claisen, L.; Lowmann, O. *Chem. Ber.*, **1888**, *21*, 1149.

2. Quelico, A. *Chem. Heterocycl.Compd.,* **1962**, *17*, 1.

3. For a brief review see: Carlsen, L.; Dopp, D.; Dopp, H.; Duus, F.; Hartmann, H.; Lang-Fugmann, S.; Schulze, B.; Smalley, R. K.; Wakefield, B. J. In *Houben-Weyl, Methods in Organic Chemistry*; E. Schaumann, Ed.; Georg Thieme Verlag: Stuttgart, Germany, **1992**; Vol. E 8a, pp 45-204.

4. Rowley, M.; Broughton, H. B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Ragan, C. I. *J. Med. Chem.* **1996**, *39*, 1943.

5. Frolund, B.; Jorgensen, A. T.; Tagmose, L.; Stensbol, T. B.; Vestergaard, H. T.; Engblom, C.; Kristiansen, U.; Sanchez, C.; Larsen, P. K.; Liljefors, T. *J. Med. Chem.* **2002**, *45*, 2454.

6. Daidone, G.; Raffa, D.; Maggio, B.; Plescia, F.; Cutuli, V. M. C.; Mangano, N. G.; Caruso, A. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 50.

7. Tomita, K.; Takahi, Y.; Ishizuka, R.; Kamamura, S.; Nakagawa, M.; Ando, M.; Nakanishi, T.; Nakamura, T.; Udaira, H. *Ann. Sankyo Res. Lab.* **1973**, *1*, 25; *Chem. Abstr.* **1974**, *80*, 120808.

8. (a) Talley, J. J. *Prog. Med. Chem.* **1999**, *13*, 201. (b) Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.

9. Giovannoni, M. P.; Vergelli, C.; Ghelardini, C.; Galeotti, N.; Bartolini, A.; Kal Piaz, V. *J. Med. Chem.* **2003**, *46*, 1055.

10. Li, W. T.; Hwang, D. R.; Chen, C. P.; Shen, C. W.; Huang, C. L.; Chen, T. W.; Lin, C. H.; Chang, Y. L.; Chang, Y. Y.; Lo, Y. K.; Tseng, H. Y.; Lin, C. C.; Song, J. S.; Chen, H. C.; Chen, S. J.; Wu, S. H.; Chen, C. T. *J. Med. Chem.* **2003**, *46*, 1706.

11. Deng, B. L.; Zhao, Y.; Hartman, T. L.; Watson, K.; Buckheit, R. W.; Cushman, M. *Eur. J. Med. Chem.,* **2009**, *44*, 1210.

12. Clercq, E. D. *Chem*. *Biodivers*. **2004**, *1*, 44.

13. Pauwels, R. *Curr*. *Opin*. *Pharmacol*. **2004**, *4*, 437.

14. Clercq, E. D. *J. Clin*. *Virol*. **2004**, *30*, 115.

15. Frolund, B.; Jensen, L. S.; Storustovu, S. I.; Stensbol, T. B.; Ebert, B.; Kehler, J.; Larsen, P. K.; Liljefors, T. *J. Med. Chem.* **2007**, *50,* 1988.

16. Agirbas, H.; Guner, S.; Budak, F.; Keceli, S.; Kandemirli, F.; Shvets, N. *Bioorg. & Med. Chem.* **2007**, *15*, 2322.

17. Eddington, N. D.; Cox, D. S.; Roberts, R. R.; Butcher, R. J. *Eur*. *J. Med. Chem*. **2002**, *37*, 635.

18. Cesari, N.; Biancalani, C.; Vergelli, C.; Piaz, V. D.; Graziano, A.; Biagini, P.; Ghelardini, C.; Galeotti, N.; Giovannoni, M. P. *J. Med. Chem.* **2006,** *49,* 7826.

19. Peifer, C.; Abadleh, M.; Bischof, J.; Hauser, D.; Schattel, V.; Hirner, H.; Knippschild, U.; Laufer, S. *J. Med*. *Chem*. doi: 10.1021/jm9005127.

20. Andresa, J. I.; Alcazar, J.; Alonso, J. M.; De Lucas, A. I.; Iturrino, L.; Biesmans, I.; Megens, A. A. *Bioorg. & Med. Chem.* **2006**, *14*, 4361.

21. Carr, J. B.; Durham, H. G.; Kendall Hass D. *J. of Med. Chem.* **1977***, 20,* 935.

22. Talley, J. A.; Brown, D. L.; Carter, J. S.; Masferrer, M. J.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.

23. (a) Lawrence, S. A.; Roth, V.; Slinger, R.; Toye, B.; Gaboury, I.; Lemyre, B. *BMC Pediatr* **2005**, *5*, 49. (b) Miranda, N. G.; Leanos, M. B. E.; Vilchis, P. M.; Solorzano, S. F. *Ann. Clin. Microbiol. Antimicrob.* **2006**, *5*, 25. (c) Sutherland, R.; Croydon, E. A.; Rolinson, G. N. *Br. Med. J.* **1970**, *4*, 455.

24. Balsamo, A.; Coletta, I.; Guglielmotti, A.; Martinelli, A.; Milanese, C.; Rapposelli, S.; Rossello, A.; *Eur*. *J. Med. Chem*., **2003**, *38*, 157.

25. Habeeb, A. G.; Praveen Rao, P. N.; Knaus, E. E. *J. Med. Chem.* **2001,** *44,* 2921.

26. Talley, J. J.; S. R. Bertenshaw, D. L. Brown, J. S. Carter, M. J. Graneto, M. S. Kellogg, C. M. Koboldt, J. Yuan, Y. Y. Zhang, K. Seibert, *J. Med. Chem.* **2000,** *43,* 1661.

27. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.

28. Batra, S.; Srinivasan, T.; Rastogi, S. K.; Kundu, B.; Patra, A.; Bhaduria, A. P.; Dixit, M. *Bioorg. & Med. Chem. Let.* **2002**, *12*, 1905.

29. Pruitt, J. R.; Pinto, D. J.; Estrella, M. J.; Bostrom, L. L.; Knabb, R. M.; Wong, P. C.; Wright, M. R.; Wexler, R. R. *Bioorg. & Med. Chem. Let.* **2000,** *10*, 685.

30. (a) Nantermet, P. G.; Barow, J. C.; Lundell, G. F.; Pellicore, J. M.; Rittle, K. E.; Young, M. B.; Friedanger, R. M.; Connoly, T. M.; Condra, C.; Karczewski, J.; Bednar, R. M.; Gaul, S. L.; Gould, R. J.; Prendergast, K.; Selnick, H. G. *Bioorg*. *Med*. *Chem*. *Lett*. **2002**, *12*, 319. (b) Xue, C. B.; Roderick, J. J.; Mousa, S. A.; Olson, R. E.; Degrado, W. F. *Bioorg*. *Med*. *Chem*. *Let*. **1998**, *8*, 3499.

31. (a) Baraldi, P. G.; Basco, A.; Benetti, S.; Pollini, G. P.; Simoni, D. *Synthesis* **1987**, 857. (b) Kozikowski, A. P. *Acc*. *Chem*. *Res*. **1984**, *17*, 410.

32. Tweedie, S. R.; Schulte, J. P. *Synlett* **2007**, *15*, 2331.

33. Yavari, I.; Moradi, L. *Tetra. Lett.* **2006**, *47*, 1627.

34. Burkhart, D. J.; Zhou, P.; Blumenfeld, A.; Twamley, B.; Natale, N. R. *Tet.* **2001**, *57*, 8039.

35. Bode, J. W. Hachisu, Y. Suzuki, K. *Org. Lett.*, **2003**, *5*, 391.

36. Lautens, M.; Roy, A. *Org. Lett.* **2000**, *2*, 555.

37. Kislyi, V. P.; Danilova, E. B.; Semenov, V. V. *Russ. Chem. Bull.0*, **2005**, *54* (5), 1189.

38. Holzer, W.; Hahn, K. *J. Hetero. Chem.*, **2003**, *40*, 303.

39. Bourbeau, M. P.; Rider, J. T. *Org. Lett.,* **2006**, *8*, 3679.

40. Wang, K.; Xiang, D.; Liu, J.; Pan, W.; Dong, D. *Org. Lett.*, **2008**, *10,* 1691.

41. Duan, H.; Sun, X.; Liao, W.; Petersen, J. L.; Shi, X. *Org. Lett.,* **2008**, *10*, 4113.

42. Daidonea, G.; Raffaa, D.; Maggioa, B.; Plesciaa, F. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332,* 50.

43. Gupta, A. K.; Yadav, K. M.; Patro, B.; Ila, H.; Junjappa, H. Synthesis, **1995**, *7*, 841.

44. Kumar, S.; Ila, H.; Junjappa, H. *Tet.*, **2007**, *63*, 10067.

45. Kuettel, S.; Zambon, A.; Kaiser, M.; Brun, R.; Scapozza, L.; Perozzo, R. *J. Med. Chem.* **2007**, *50,* 5833.

46. Mahata, P. K.; Syam Kumar, U. K.; Sriram, V.; Ila, H.; Junjappa, H. *Tet.* **2003**, *59*, 2631.

47. Dieter, R. K.; Chang, H. J. *J. Org. Chem.* **1989**, *54*, 1088.

48. Tu, S. J.; Zhang, X. H.; Han, Z. G.; Cao, X. D.; Wu, S. S.; Yan, S.; Hao, W. J.; Zhang, G.; Ma, N. *J. Comb. Chem.* **2009,** *11,* 428.

49. Wenli, M.; Peterson, B.; Kelson, A.; Laborde, E. *J. Comb. Chem.* **2009**, *11*, 697.

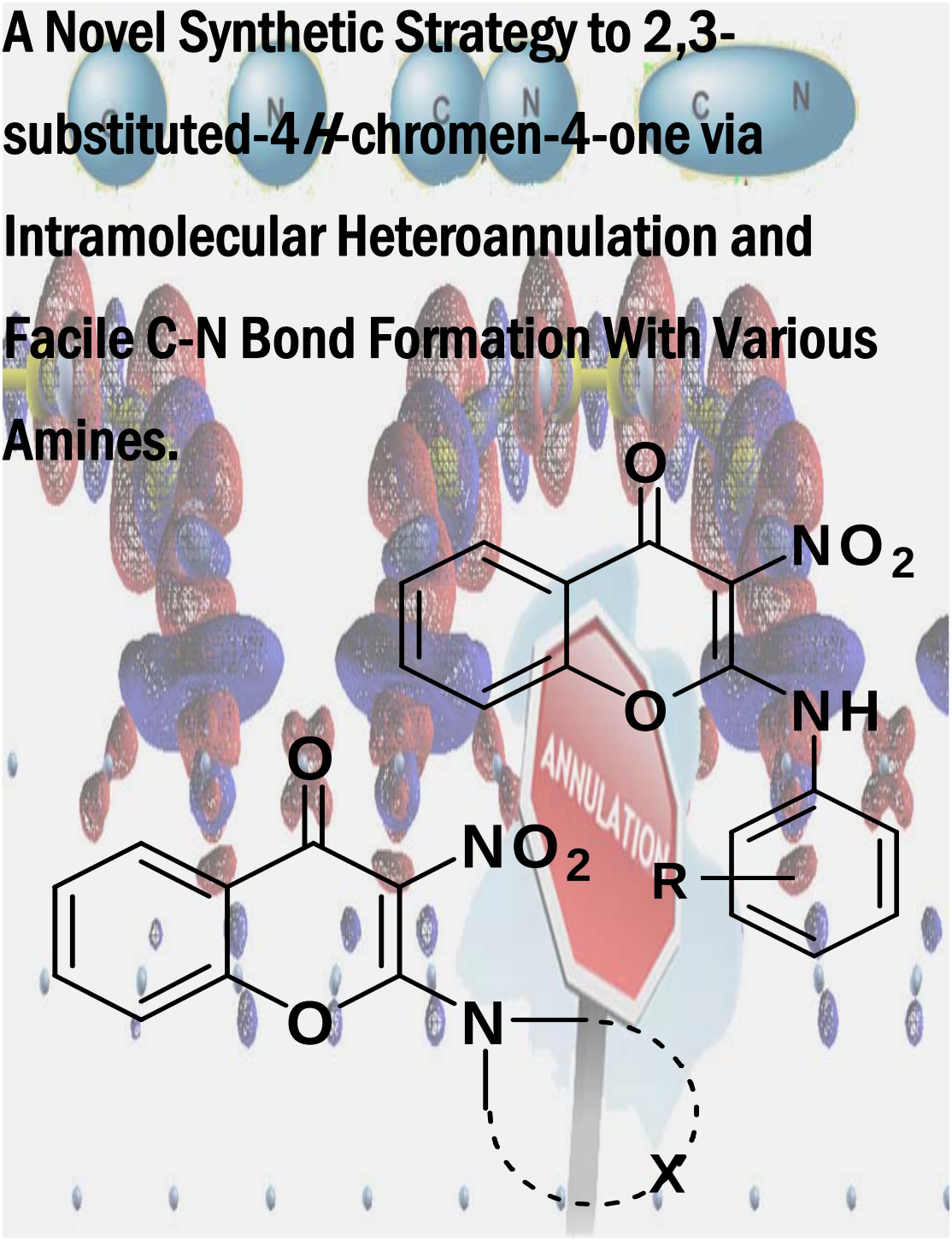
50. Waldo, J. P.; Mehta, S.; Neuenswander, B.; Lushington, G, H,; Larock, R. C. *J. Comb. Chem.* **2008,** *10,* 658.

51. Jeddeloh, M. R.; Holden, J. B; Nouri, D. H.; Kurth, M. J. *J. Comb. Chem.* **2007,** *9,* 1041.

52. (a) Talley, J. J. *Prog. Med. Chem.* **1999**, *13*, 201.

CHAPTER 4

A Novel Synthetic Strategy to 2,3-substituted-4*H*-chromen-4-one via Intramolecular Heteroannulation and Facile C-N Bond Formation With Various Amines.



4.1 INTRODUCTION

Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. To date about 3000 varieties of flovonoids are known.[1] Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring (A) condensed with a sixmembered ring (C), which in the 2-position carries a phenyl ring (B) as a substituent (**Figure 1**). Six-member ring condensed with the benzene ring is either a γ-pyrone (chromenols **3** and chromenones **4**) or its dihydroderivative (**1, 2**). The position of the benzenoid substituent divides the flavonoid class into flavonoids (**1-4**) and isoflavonoids (**5, 6**). Chromenols (**1**) differ from chromenones (**4**) by hydroxyl group the 3-position and C2-C3 double bonds.[2]

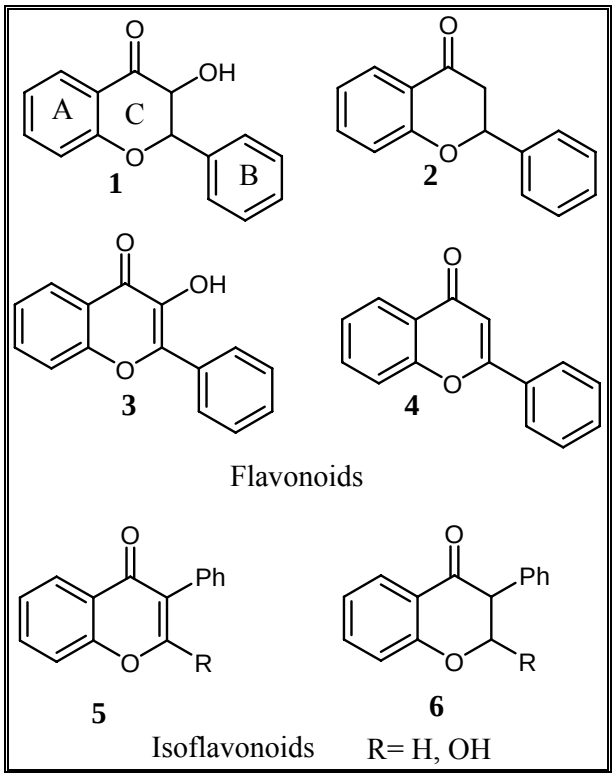


Figure 1

Many flavonoids possess low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity.[3] Concerning the chromenone moiety, besides forming the basic nucleus of an entire class of natural products, i.e. flavones,[4] it also forms the important component of pharmacophores for a large number of molecules of medicinal significance.[5] Consequently, considerable attention is being devoted to isolation from natural resources, chemistry and synthesis of chromenone derivatives, and evaluation of their biological activity with emphasis on their potential medicinal applications.[5–8]

4.2 Biological activity of various chromenone derivatives.

In recent decades, chromenone and its derivatives have attracted considerable attention from medicinal and synthetic organic chemists because of a wide range of biological activities displayed by this class of compounds which is described below. The corresponding 2-substituted-3-nitro chromenones are molecules of current interest as they have potent biological activity. It is well recognized that incorporation of nitro group into the chromenone skeleton have significant biological activity.[9,10]

Vasselin A. D. et al[11] have synthesized a new series of fluoro, methoxy and amino substituted isoflavones (**Figure 2**) and demonstrated as potent antitumor agents. The substituted isoflavones were synthesized using palladium catalyzed coupling methodologies to construct the central aryl carbon-carbon single bond. The new isoflavone derivatives were tested for *in vitro* activity in human breast (MDA-MB-468 and MCF-7) and colon (HT29 and HCT-116) cancer cell lines. Low micromolar GI_{50} values were obtained in a number of cases, with the MDA-MB-468 cell line being the most sensitive overall. This study is suggesting that isoflavone derivatives can act as substrates for CYP1A1 bioactivation.

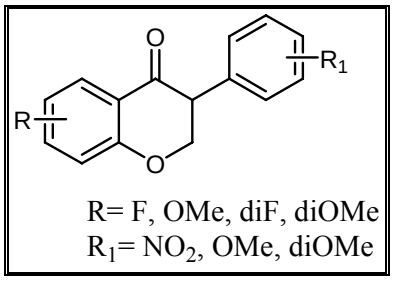


Figure 2

Chen S. F. et al[9] have developed a series of nitrocoumarin and nitrochromene derivatives (**Figure 3**) and shown to inhibit the phosphatidylinositol-specific phospholipase C(PLC) (ICW *C* 10 pg/mL) isolated from human melanoma. The inhibition of PLC by nitrocoumarin was time-dependent and irreversible. The inhibition of PLC was shown to interfere with inositide metabolism in whole cells in a manner consistent with their proposed mode of activity.

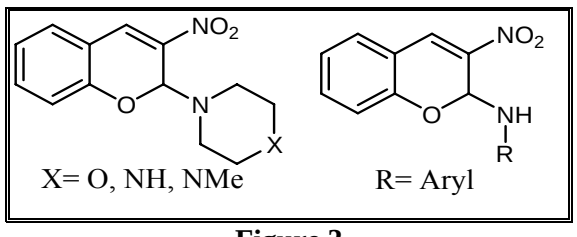


Figure 3

Dauzonne D. et al[10] have synthesized some novel flavone-8-acetic acid derivatives and evaluated for reversible inhibitors of aminopeptidase N(APN/CD13) activity. The cell surface APN/CD13, overexpressed in tumor cells, plays a critical role in angiogenesis. In this context, they have tested a series of novel flavone-8-acetic acid derivatives and found that the 2', 3-dinitroflavone-8-acetic acid proved to be the most efficient and exhibited an IC_{50} of 25 μM which is 2.5 times higher than that of bestatin, the natural known inhibitor of APN/CD13. The presence of other substituents such as OMe groups at the 3 or 4 position of the A phenyl group, or the existence of steric constraints, did not improve selectivity and potency. The results were indicated that derivatives, which bear a CH_2COOH group in the 8-position and two NO_2 substituents in both 2' and 3 positions (**Figure 4**), inhibited efficiently APN activity and this to the same extent as bestatin. Deletion or replacement of the NO_2 group in the 2'-position gave compounds with a lesser degree of potency against APN activity whereas the presence of an electron-donating methoxy group in the ortho or para position of the nitro substituent led to slightly lowered inhibitory effects.

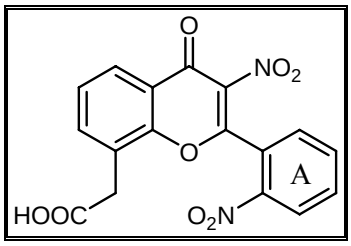


Figure 4

Balbi A. et al,[13] have synthesized some chromenone derivatives (**Figure 5**) having nitro group and amine linkage at 3 and 2 positions, respectively, and demonstrated that they were potent stabilization agents for oligonucleotides. The newly synthesized chromenones having tri or pentamethylenamine linkers were tested and the T_m data and thermodynamic parameters for complex formation confirmed the ability of chromone (c-pyrone) derivatives to stabilize strongly the 7-mer/8-mer complementary complex. Moreover, benzochromone derivatives showed the capacity of stabilizing this 7-mer/8-mer complementary complex. The effect of all these chromenones on the stability of the oligonucleotide complexes ($\Delta\Delta G$ at 37 ºC ranged from -1.2 to -2.0 kcal/mol) was shown to be comparable to the effect of one nucleotide base pair and similar to the effect ($\Delta\Delta G$ at 37 ºC ranged from -1.5 to -2.0 kcal/mol) found for acridine oligonucleotide conjugates, which served as a reference in this study.

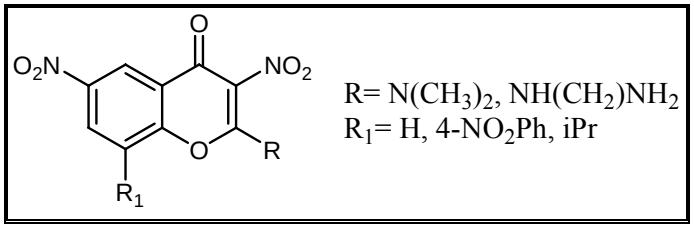


Figure 5

Cantello C. C. et al[14] have synthesized some substituted 4-hydroxy-3-nitrocoumarins and found these compounds possess potent antiallergic activity (**Figure 6**). The antiallergic activity was measured by the homocytotropic antibody-antigen induced passive cutaneous anaphylaxis reaction in the rat.

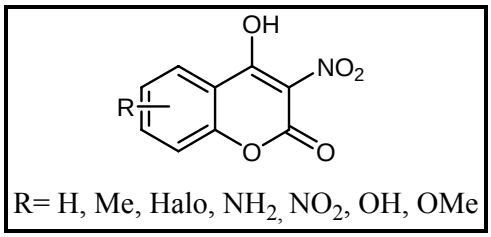


Figure 6

The design and synthesis of a small library of 8-amidoflavone, 8-sulfonamidoflavone, 8-amido-7-hydroxyflavone (**Figure 7**) and heterocyclic analogs of flavopiridol was reported by Georg G. I. and coworker.[15] The potential activity of these compounds as kinase inhibitors was evaluated by cytotoxicity studies in MCF-7 and ID-8 cancer cell lines and inhibition of CDK2-Cyclin A enzyme activity *in vitro*. The antiproliferative and CDK2- Cyclin A inhibitory activity of these analogs was significantly lower than the activity of flavopiridol. They were carried out molecular docking simulations for those molecules and these studies suggested a different binding orientation inside the CDK2 binding pocket for these analogues compared to flavopiridol.

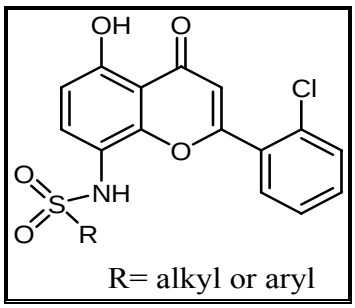


Figure 7

Griffin R. J. et al[16] have synthesized a diverse range of chromen-2-one, chromen-4-one and pyrimidoisoquinolin-4-one derivatives and evaluated for inhibitory activity against the DNA

repair enzyme DNAdependent protein kinase (DNA-PK), with a view to elucidating structure-activity relationships for potency and kinase selectivity. DNA-PK inhibitory activity varied widely over the series of compounds evaluated (IC_{50} values ranged from 0.19 to >10 μM), with excellent activity being observed for the 7,8-benzochromenone and pyrimido[2,1-*a*]isoquinolinone templates. They have revealed a very constrained structure-activity relationship at the 2-position of the benzopyranone and pyrimido[2,1-*a*]-isoquinolin-4-one pharmacophore, with only a 2-morpholino or 2-(2'-methylmorpholino) group being tolerated at this position. More detailed biological studies conducted with the most potent inhibitor NU7163 demonstrated ATP-competitive DNA-PK inhibition, with a *K*i value of 24 nM, and compound (**Figure 8**) exhibited selectivity for DNA-PK compared with the related enzymes ATM, ATR, mTOR, and PI 3-K (p110alpha). This study was identify these structural classes as novel DNA-PK inhibitors and delineated initial structure activity relationships against DNA-PK.

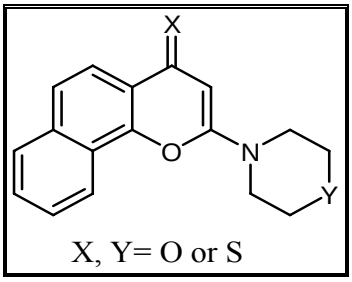


Figure 8

Dauzonnel D. et al[17] have synthesized some new 3-aminoflavones (**Figure 9**) using various aromatic aldehydes. They have also demonstrated these chromenones possess potent cytotoxic activity *in vitro*. Those results in the 3-aminoflavones series indicated that the 4'-methoxy group was important for cytotoxic activity. Moreover, they were observed that the flavone analogs bearing 3-amino of 3-nitro group have potent cytotoxic activity. Methoxy groups on the 6 and 7 positions of flavonoids also appear to be important for antiproliferative activity.

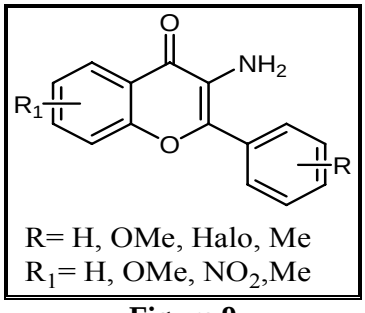
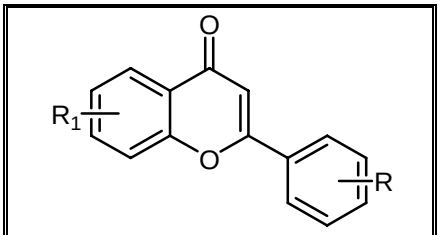


Figure 9

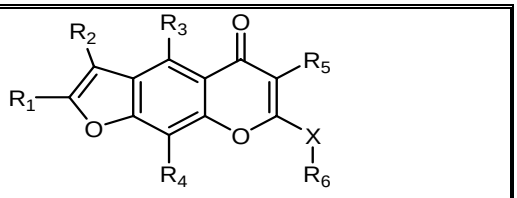
Chen K. et al[18] have studied three-dimensional quantitative structure-activity relationship (3D-QSAR) for flavone (**Figure 10**) template in GABAA receptors. A reasonable pharmacophore model was built through CoMFA, CoMSIA, and HQSAR analyses and electrostatic potential calculations. A plausible binding mode for flavonoids with GABAA receptors was rationalized on the basis of commonly recognized binding site and the specific S1 and S2 subsites relating to substituent positions.



R, R_1= H, OMe, Halo, Me, NO_2

Figure 10

In 2008,[19] several fused chromenones (**Figure 11**) have been synthesized and found useful in the modulation of potassium channel activity in cells, in particular the activity of Kv1.3 channels found in T cells. These chromenone derivatives were also useful in the prevention of autoimmune and inflammatory diseases, including multiple sclerosis.



R_1= H, Me, alkyl, aryl
R_2= H, OMe, Halo, Me,alkyl
R_3= H, Me, Halo, alkyl
R_4= H, Me, R_5= H, Me, alkyl, NO_2, aryl, halo,
R_6= H, Me, alkyl, aryl, X= N, S, O

Figure 11

Lewin C. et al[20] have synthesized several aminoflavones and examined its antiproliferative activity, activation of apoptosis and inhibition of tubulin assembly. These flavones mostly contained 5,6,7,8-tetra- or 5,7-dioxygenated groups on A ring. Among them, flavones having 5-hydroxy-6,7,8-trimethoxy substitution pattern on the A-ring exhibited promising antiproliferative activity (**Figure 12**).

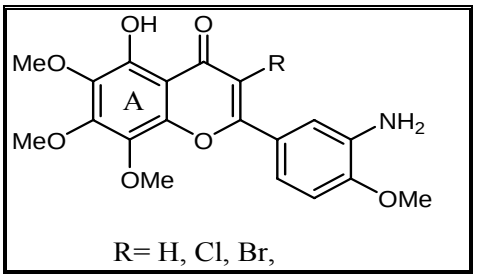


Figure 12

A new series of flavonoid derivatives have been designed, synthesized and evaluated as potent AChE inhibitors by Hua Y. and coworkers.[21] Most of them showed more potent inhibitory activities to AChE than rivastigmine. The most potent inhibitor isoflavone derivative (**Figure 13**) inhibit AChE with an IC_{50} of 4 nM and showed high BChE/AChE inhibition ratio (4575-fold), superior to donepezil (IC_{50} = 12 nM, 389-fold). They were also performed molecular docking studies to explore the detailed interaction with AChE.

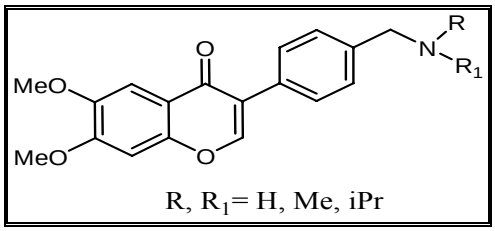


Figure 13

In search for a new antibacterial agent with improved antimicrobial spectrum and potency, Shingare M. S. et al[22] have designed and synthesized a series of novel 3-((Z)-2-(4-nitrophenyl)-2-(1*H*-tetrazol-5-yl) vinyl)-4*H*-chromen-4-ones (**Figure 14**) by convergent synthetic approach. All the synthesized compounds were assayed for their *in vitro* antibacterial activities against gram-negative and gram-positive bacteria. They were performed preliminary structure activity relationship to elucidate the essential structure requirements for the antimicrobial activity. Amongst the synthesized chromenones, few compounds were found to possess activity against methicillin resistant *S. aureus* in addition to the activity against other bacterial strains such as *E. faecalis, S. pneumoniae,* and *E. coli*.

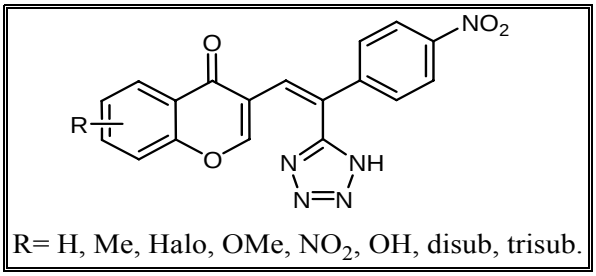


Figure 14

Moreover, a new series of quinoxaline fused chromenones have been synthesized and evaluated for antibacterial and antifungal activities.[23] The results of the antimicrobial screening showed the compound (**Figure 15**) being the most effective among the various treatments in antimicrobial screening. However, other molecules showed moderate activity against the microorganisms tested.

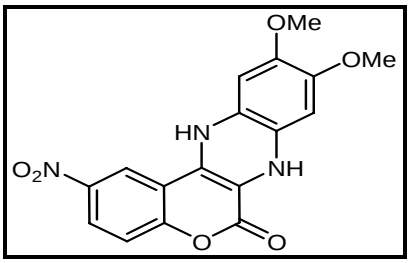


Figure 15

4.3 Synthesis of various chromenone derivatives.

Numerous methods have been developed for the synthesis of substituted chromenone molecules. The two most important methods for the synthesis of chromenone derivatives are due to Perkin W. H.[24] and Pechmann V[25]. The naturally occurring chromenones[26] have been obtained either (i) by the closure of the lactonic ring with the necessary substitutents in the benzene nucleus, or (ii) by the introduction of the aliphatic acid and its anhydride on an *o*-hydroxy aldehyde with the intermediate formation of the *o*-hydroxy cinnamic acid (Perkin's method) and the action of malic acid on phenols in the presence of sulphuric acid (Pechmann's method) have been very convenient methods for the synthesis of naturally occurring chromenones. The *o*-hydroxy cinnamic acids have also been prepared by other methods and them easily lactonize to chromenones.

The phenols have been used by Ruhemann S.[27] and Simonis H.[28] for the synthesis of chromone derivatives. Ruhemann condensed sodium phenolates with ethyl chloro fumarate, ethyl phenyl propiolate and ethyl *β*-chloro crotonate and treated the intermediate products, thus obtained, with con. H_2SO_4 or better with PCl_5 and $AlCl_3$ whereby the desired chromones were obtained (**Figure 16**).

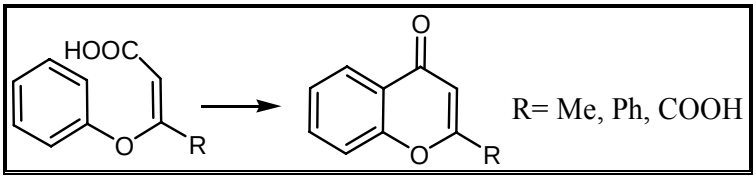


Figure 16

The only attempt at synthesizing chromones from *o*-hydroxy acid was made by Kostanecki S. V.,[29a] who condensed ethyl *o*-methoxy benzoate with acetone and acetophenone and obtained the chromones on heating the intermediate *β*-diketones with HCl (**Figure 17**).

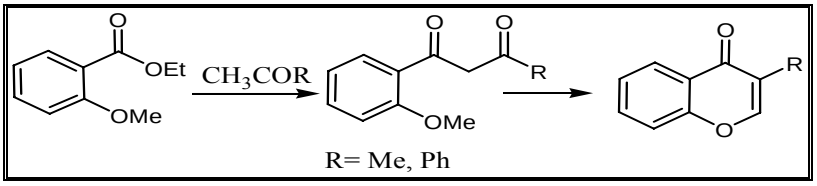


Figure 17

The *o*-hydroxy acetophenones have been largely used for the synthesis of chromone derivatives. Kostanecki[29b] et al have condensed *o*-hydroxy acetophenones with aldehydes giving rise to chalkones, the dibromides of which on treatment with alkali form chromones (**Figure 18**).

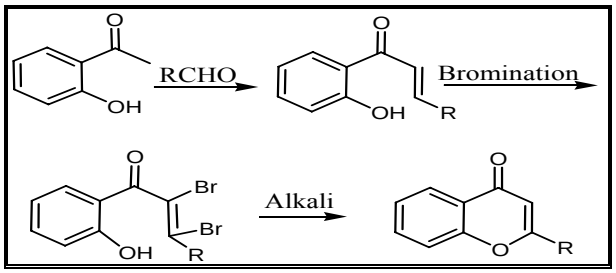


Figure 18

Moreover, Kostanecki[29c] et al have synthesized chromenones by the reaction of esters with *o*-methoxy acetophenones and the resulted intermediate *β*-diketones then heated with hydroiodic acid (**Figure 19**).

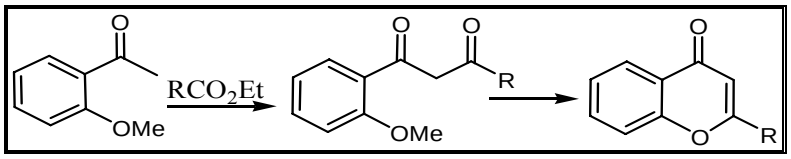


Figure 19

Spath E.[30] et al have condensed *o*-hydroxy phenyl benzyl ketones with ethyl formate and the intermediate oxymethylene ketones gave isoflavones on ring closure with HCl (**Figure 20**).

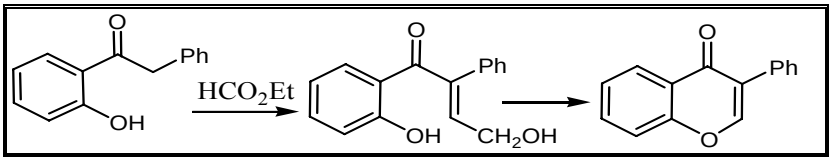


Figure 20

Nagai W. N.[31a] and Tahara Y.[31b] et al have utilized resacetophenone and phenol for the synthesis of chromenones in the presence of sodium acetate and acetic anhydride (**Figure 21**). However, this method was further developed by Robinson[32] et al and used by them in synthesizing a large number of chromones and chromonols occurring in nature by heating various *o*-hydroxy aryl ketones with the anhydrides and the corresponding sodium salts of aliphatic and aromatic acids.

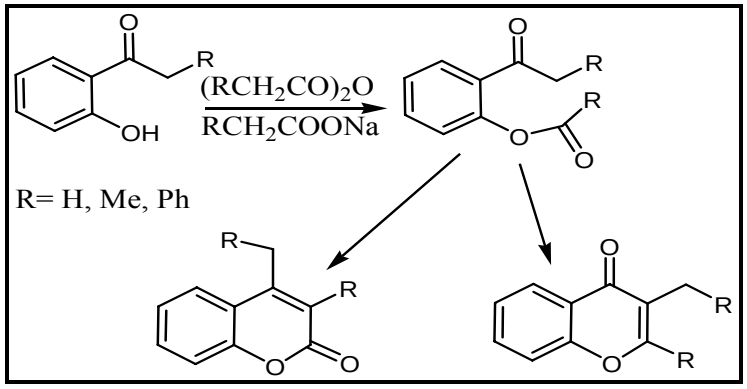


Figure 21

Recently, Alfredo C. et al[33] have synthesized flavones *via* a microwave assisted, One-Pot Sonogashira-carbonylation-annulation reaction starting from 2-iodo phenol and acetylene (**Figure 22**). They were observed that palladium complexes of 1,3,5,7-tetramethyl-2,4,8-trioxa-6-phenyl-6-phosphaadamantane are shown to be effective catalytic systems facilitating the sequential application of a microwave-assisted Sonogashira and carbonylative annulation reaction.

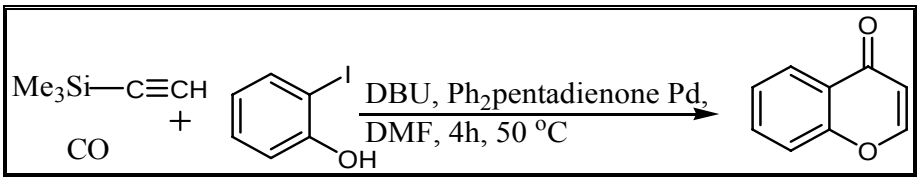


Figure 22

In addition, Zhen Y. et al[34] have developed a Pd-catalyzed copper-free carbonylative Sonogashira coupling reaction to synthesize alkynyl ketones and flavones from terminal alkynes and aryl iodides using water as a solvent (**Figure 23**). The reaction was carried out at room temp under balloon pressure of CO with Et_3N as a base. The developed method was successfully applied to the synthesis of flavones.

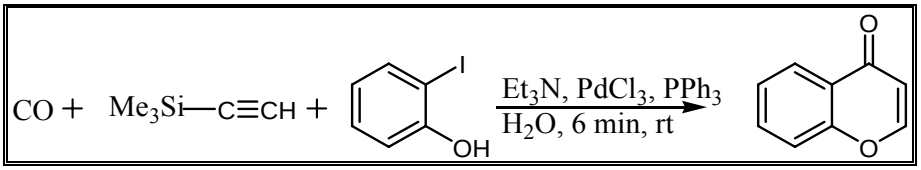


Figure 23

In 2002, Rao V. K. et al[35] have demonstrated the utility of *o*-hydroxy benzoylacetone for the synthesis of some substituted chromenones, chelating agents and related materials. The synthesis of 3-nitro chromenone was achieved by the reaction of *o*-hydroxy nitroacetophenone and ethyl orthoformate using pyridine (**Figure 24**).

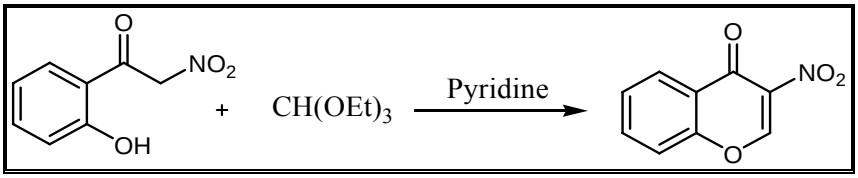


Figure 24

Chen S. F. et al[9] have synthesized 4-(3-nitro-2*H*-chromen-2-yl)morpholine (**Figure 25**) with the reaction of salisaldehyde and 4-((*Z*)-2-nitrovinyl)morpholine refluxing in morpholine. The synthesized chromenes further substituted at 2-position with various primary and secondary amines and evaluated as potent phospholipase C inhibitors.

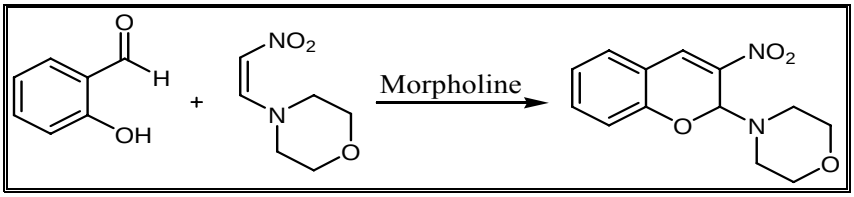


Figure 25

In 1976, Ellis G. P. et al[36] have first synthesized some substituted 3-nitro chromones by the reaction of nitroacetophenone and acetic formic anhydride (**Figure 26**)**.**

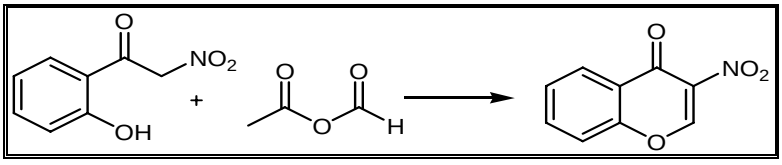


Figure 26

In addition, et al[37] have utilized substituted 2-hydroxy-*ω*-nitroacetophenone and chromenone-3-carbaldehyde for the synthesis of various 2-substituted-3-nitro chromenones in good yields (**Figure 27**).

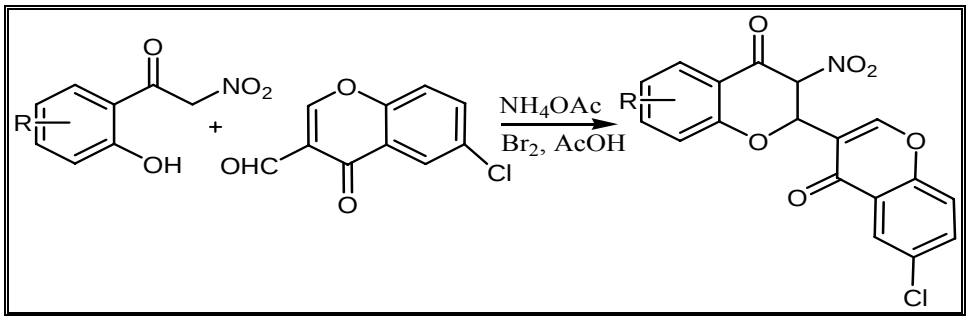


Figure 27

Balbi A. et al[13] have synthesized substituted 2-amino-3-nitro chromenone derivatives starting from various phenols and ethyl 2-(dimethylcarbamoyl) acetate followed by $POCl_3$ mediated cycliczation and nitration (**Figure 28**). The synthesized chromenones were evaluated as potent stabilizing agents for oligonucleotides.

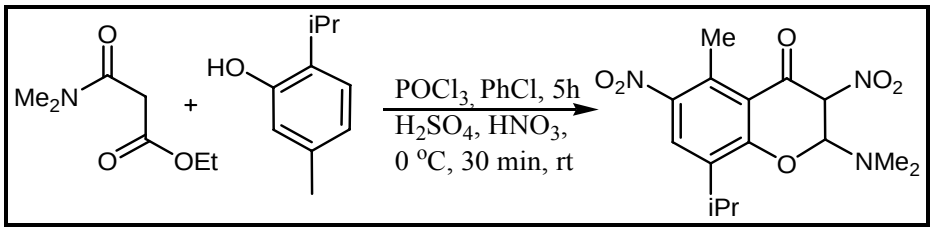


Figure 28

In 1995, Dauzonne D. et al[38] have described an efficient synthesis of 3-nitrochromones. The reaction of 2-hydroxy-2-nitroacetophenone with acetic formic anhydride and sodium formate without external heating or cooling gave an almost quantitative yield of 3-nitrochromone (**Figure 29**).

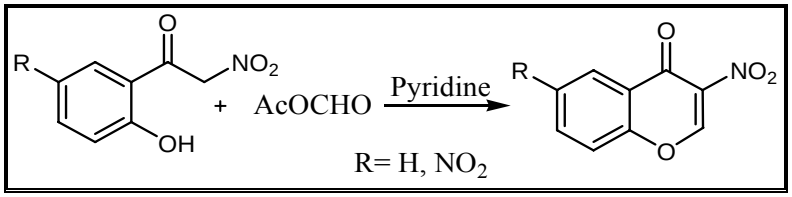


Figure 29

Moreover, Dauzonne D. et al[10] have synthesized flavone-8-acetic acid using 3-allyl-2-hydroxybenzaldehyde **1** and 1-((*Z*)-2-chloro-2-nitrovinyl) benzene **2**. Further, the product **3** was treated with PCC in DCM for 19 h then reaction of **4** with DBU afforded 2-aryl-3-nitro chromenone **5.** Compound **5** on reaction with ruthenium chloride, sodium hypoiodate in acetonitrile and CTC afforded product **6** which on reduction gave the target molecule **7** (**Figure 30**).

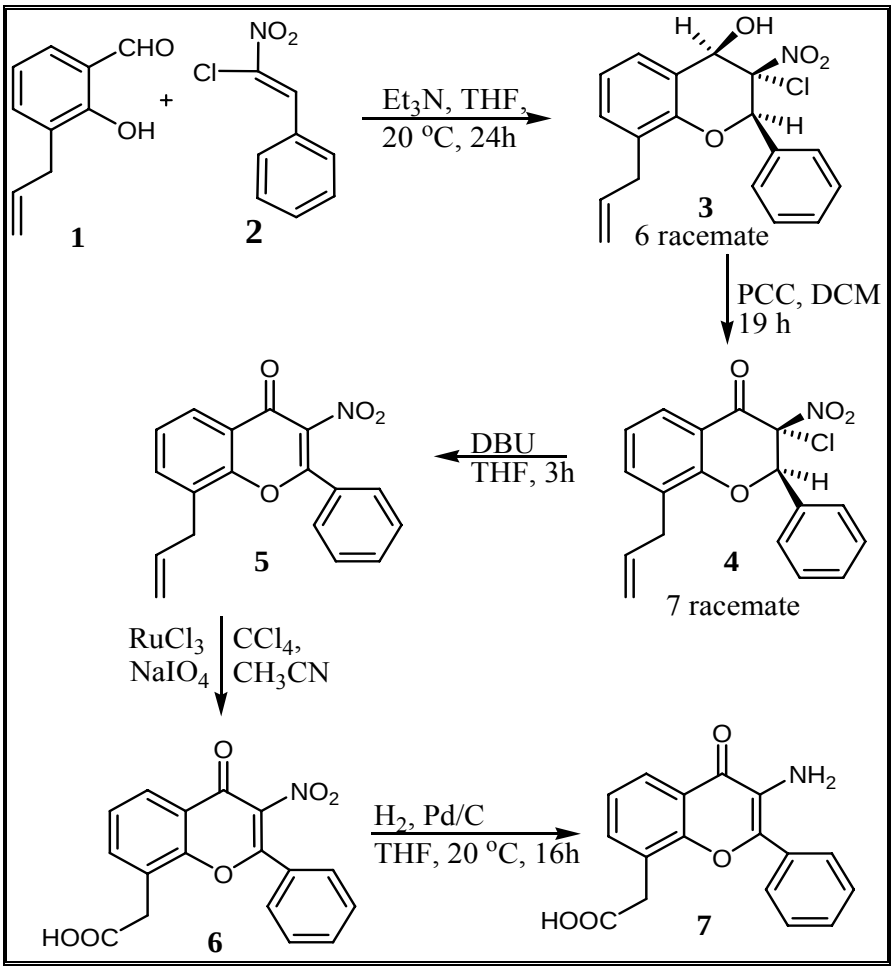


Figure 30

Recently, an efficient route to a new class of tetrahydrochromeno[2,3-*b*]carbazoles and tetrahydrochromeno[3,2-*f*]indazoles has been developed by Tsoleridis A. et al.[39] The cycloaddition reactions of chromones **1** and pyrazole-*o*-quinodimethane **2** were more regioselective giving only cycloadducts **3** (**Figure 31**).

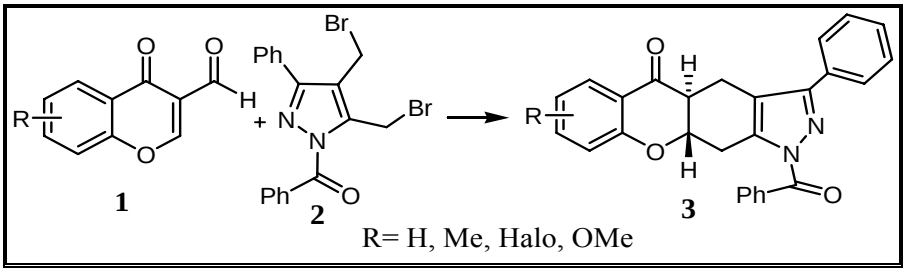


Figure 31

Hu W. et al[40] have synthesized, and examined crystal and molecular structure of 3-chloro-3,6-dinitro-2,2- dimethyl-4-chromanone **3**, the first 3-chloro-3-nitro-4-chromanone by nitrating its corresponding 4-chromanone **1** at the 3 position and then chlorinating the nitration product **2**. They were observed that the nitro group at the 6 position conjugates with

the benzene ring. The pyranone ring has a half-chair conformation; the chloro group occupies the pseudoequatorial position, and the nitro group occupies the pseudoaxial position. The 2 and 3 positions are essentially antiperiplanar, minimizing steric interaction (**Figure 32**).

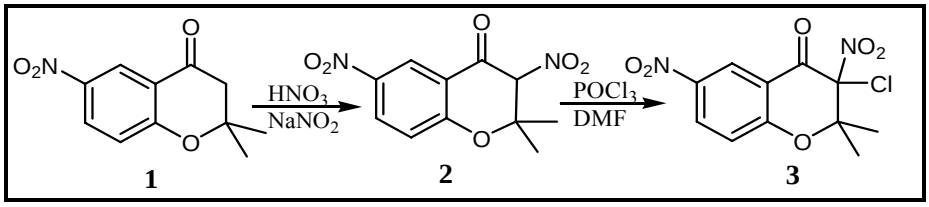


Figure 32

Recently, Rao H. S. et al[51a] have developed a combinatorial library of the 2-alkylamino-3-nitro 4-alkylsufanyl-4*H*-chromenes (**Figure 33**). They were demonstrates the reaction of nitroketene *N,S*-acetals and substituted salisaldehydes afforded the nitrochromenes in the presence of base with excellent yields. Further, they have replaced the C4 methylthio group with various thiols to obtained substituted chromenones in good yields.

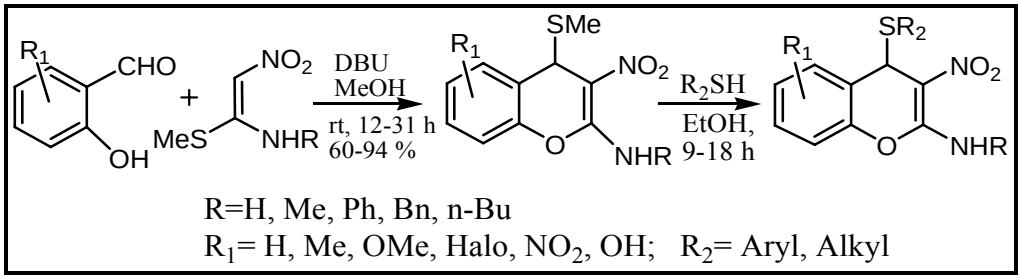


Figure 33

❖ **Nitro group in various chemical transformation reactions.**

Nitro group can be converted to amine functionality using various reducing agents. As we described above,[10] the 3-nitroflavone-8-acetic acid on reaction with palladium and carbon at 20 ^{o}C for 16 h gave 3-aminoflavone-8-acetic acid by reduction of nitro group (**Figure 34**).

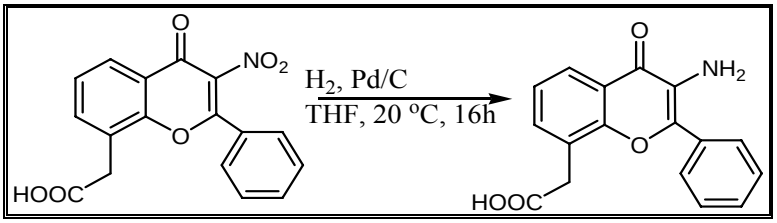


Figure 34

Fringuelli F. et al[41] have utilized 3-nitrocoumarins as dienophiles in the Diels-Alder reaction in water. They were described new approach to the Synthesis of nitrotetrahydrobenzo[*c*]-chromenones and dihydrodibenzo[*b*,*d*]furans (**Figure 35**). The reactions of 8-hydroxy-3-

nitrocoumarins with various diene were investigated in aqueous medium, in organic solvent and under solventless conditions. The reactions performed in water occurred in heterogeneous phase but were faster than those executed organic solvents.

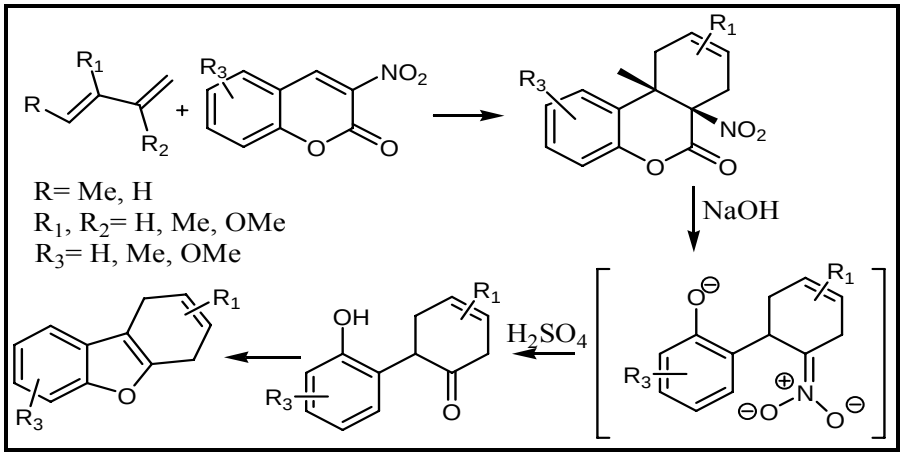


Figure 35

Vinokurov V. G. et al[42] have synthesized 6,7,8,13-tetrahydro[l]benzopyrano-[4,3-*b*][l,4]-benzodiazepine-6,8-dione by the condensation of 3-nitro-4-chlorocoumarin and anthranilic acid. Thus, the obtained amides were reduced to *N*-(3-amino-4-coumarinyl)anthranilic acid amides and further cyclized in the presence of catalytic amount of hydrochloric acid to diazepines (**Figure 36**).

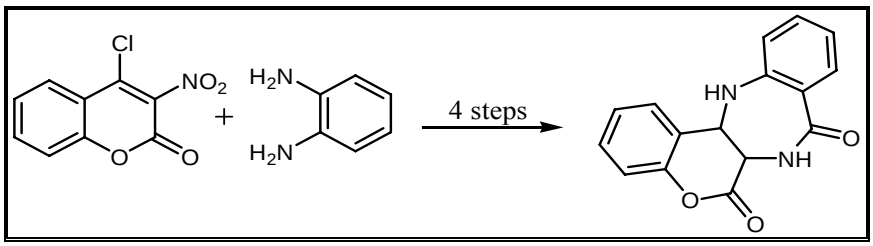


Figure 36

Junjappa H. et al[43] have developed a novel highly regioselective synthesis of substituted quinoxalines (**Figure 37**) through $POCl_3$ mediated heteroannulation of nitroketene *N,S*-arylaminoacetals. The 3-chloro-2-(methylthio)quinoxalines further substituted with various nucleophiles.

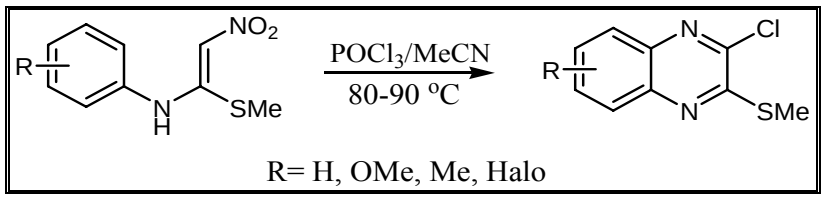


Figure 37

A new synthesis of 2-substituted[1]benzopyrano[3,4-*d*]imidazol-4(3*H*)-ones, starting from 3-nitrocoumarin *N*-functionalized amidines **3**, has been developed by Trimarco P. et al.[44] When the 3-nitro-amidines **3** were treated with $NaBH_4$ in the presence of 10% palladium on charcoal, 2-substituted [1]benzopyrano[3,4-*d*]imidazol-4(3*H*)-ones **4** were produced. Structure elucidation of compounds **4** revealed that they exist as one of the three possible tautomeric structures (**Figure 38**).

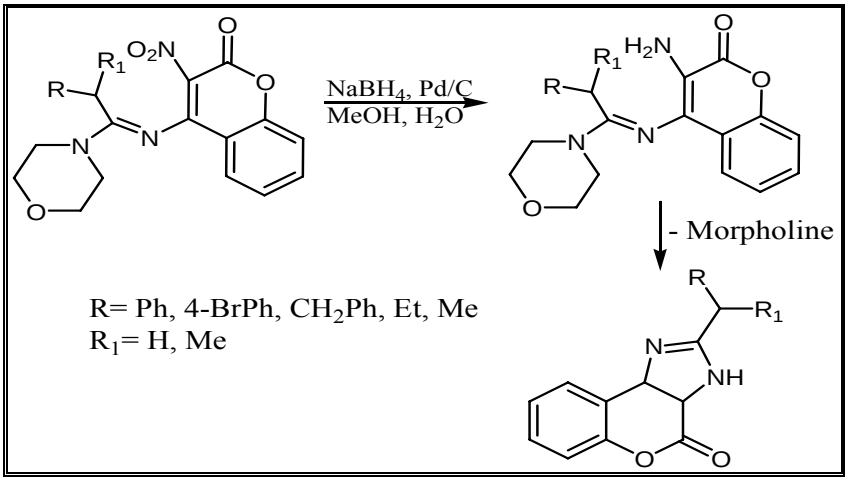


Figure 38

4.4 CURRENT RESEARCH WORK

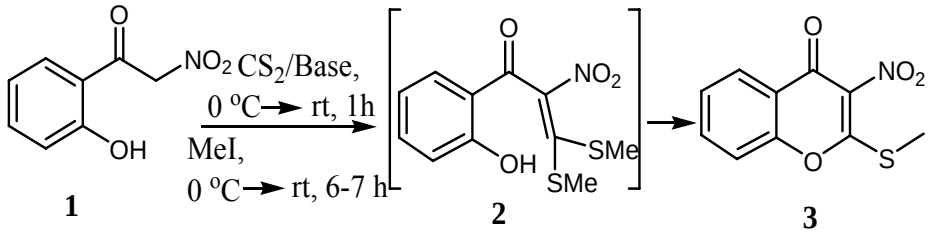
The chromenone and its derivatives have attracted considerable interest to medicinal and synthetic organic chemists because of a wide range of biological activities. As we mentioned, the corresponding 2-substituted-3-nitro chromenones are molecules of current interest as they have potential biological activity. Moreover, Chromenones bearing nitro group at C3, afford the possibility of further modifications through which, one can generate, a large number of pharmacologically important compounds. Given the biological significance of 2,3-substituted chromenones, several routes are reported in the literature for the synthesis of substituted chromenone *via* [4+2] annulation,[45] [5+1] addition,[36,46] [4+1+1] addition reaction,[47] or electrophilic substitution reactions over the preconstructed chromone motifs. However, these potentially useful methods have not been thoroughly explored. Further more, the existing few examples are rather limited in scope and suffer from several practical disadvantages such as extremely vigorous conditions or low yields.[48] Therefore, developing a simple and effective method to synthesize 2-substituted-3-nitro chromenones *via* readily available starting material is essential.

In chapter 1, we have demonstrates the utility of ω-nitro acetophenone for the synthesis of nitro functionalized pyrimidine derivatives. Nowadays, functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the synthesis of substituted heterocycles (Chapter 2). These studies motivated us to utilize the 2HNA as a versatile synthon to make new nitro ketene dithioacetals and to study its utility for the synthesis of novel NO_2 group functionalized chromenones. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. In addition the newly synthesized 2-methylthio-3-nitro-4*H*-chromen-4-one was confirmed by X-Ray diffraction technique. The detail study of X-Ray crystallography is described in chapter 6. The biological screening of the newly synthesized compounds is under process.

4.5 RESULTS AND DISCUSSION

The reaction of 2HNA **1** with carbon disulfide in the presence of base and followed by methylation with methyl iodide, to our surprise, the product isolated was not the expected **2** but was characterized as 2-methylthio-3-nitro-4*H*-chromen-4-one **3**. In addition the structure of **3** was confirmed unequivocally by the analysis of single crystal X-Ray diffraction technique (Chapter 6).

Scheme 1: Synthesis of novel 2-methylthio-3-nitro-4*H*-chromen-4-one (3) through intramoleculer heteroannulation of 2.



Having discovered a facile synthesis of chromenone **3**, we next planned to optimize the reaction condition using various bases and solvents (Table 1). The yield of desired product **3** was poor when strong bases such as, NaO*i*-Pr and NaOMe were used in THF and DMF (Table 1, entry 1-4). However, the reaction of **1** with KF in THF and DMF did not improve the yield and the reaction needed longer time (Table 1, entry 7,8). It was found that the reaction proceeded smoothly in the presence of K_2CO_3 and THF to furnish the product **3** in excellent yield (Table 1, entry 5).

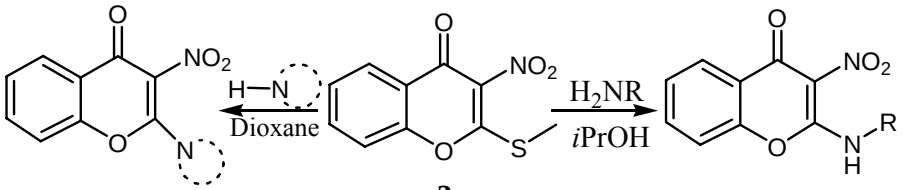
Further, to develop facile route for 2-substituted-3-nitro chromenones through nucleophilic substitution of 2-methylthio group, we carried out the reaction of compound **3** with various amines. Extensive literature survey revealed, the elimination of methylthio group from aromatic or heterocyclic moieties required more drastic conditions.[49-52] It is important to note that nucleophilic displacement at C2 possessing methylthio group for C-N bond formation required either oxidation of sulfide to sulfone then reaction with nucleophiles or transition metal catalysts.[43,49a] However, our studies revealed that elimination of methylthio group by various amines in the chromenone **3** was simple and required neither oxidation nor transition

metal catalysts. This can be explained by the fact that the adjacent electron-withdrawing effect of nitro and carbonyl groups makes the labile methylthio moiety a good leaving group.

Table 1: Optimization of the reaction condition for the synthesis of 3.

Entry	Base(2 equiv)	Solvent	Time h	Yield (%)
1	NaO*i*-Pr	THF	1.5	52
2	NaO*i*-Pr	DMF	1.7	48
3	NaOMe	THF	2.0	64
4	NaOMe	DMF	2.3	60
5	K_2CO_3	THF	1.5	88
6	K_2CO_3	DMF	1.5	80
7	KF	THF	3.0	68
8	KF	DMF	3.2	66

Scheme 2: Reaction of chromenone 3 with primary alkyl/aryl and secondary amines.



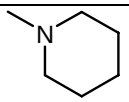
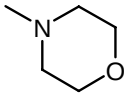
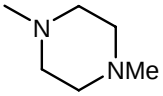
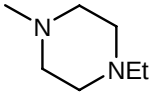
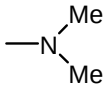
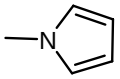
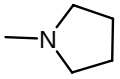
FPMS 24-30 **3** **FPMS 1-23**

Thus, under the optimized condition, the reaction of primary alkyl/aryl amines with chromenone **3** in isopropyl alcohol afforded substituted 2-aryl/alkylamine-3-nitro-4*H*-chromenones **FPMS 1-23** in excellent yields (Table 2, Scheme 2). However, the reaction of various secondary amines with chromenone was conducted using dioxane as solvent which afforded chromenones **FPMS 24-29** with good to excellent yields (Table 3, Scheme 2).

Table 2: Synthesis of 2-aryl/alkylamine-3-nitro-4*H*-chromen-4-ones FPMS 1-23.

Entry	R	Yield %	mp ^{o}C	Purity %
FPMS-1	Ph	97	220-222	97.89
FPMS-2	3-CH_3Ph	95	165-167	95.49
FPMS-3	4-OCH_3Ph	94	174-176	97.75
FPMS-4	2-FPh	94	186-188	95.56
FPMS-5	3,4-diClPh	95	185-187	98.91
FPMS-6	4-CH_3Ph	92	155-157	95.56
FPMS-7	4-FPh	97	160-162	99.73
FPMS-8	3,4-diFPh	92	191-193	94.89
FPMS-9	4-EtPh	95	210-312	92.78
FPMS-10	2-OCH_3,4-NO_2Ph	88	188-190	94.88
FPMS-11	3-Cl,4-FPh	92	154-156	91.37
FPMS-12	3-BrPh	93	182-184	96.78
FPMS-13	4-NO_2Ph	96	177-179	96.24
FPMS-14	2,3-diCH_3Ph	95	181-183	94.99
FPMS-15	2-OCH_3Ph	91	170-172	96.65
FPMS-16	2,4-diCH_3Ph	91	185-187	98.80
FPMS-17	4-ClPh	90	161-163	93.38
FPMS-18	3-OCH_3Ph	94	167-168	93.56
FPMS-19	3-NO_2Ph	93	172-174	95.82
FPMS-20	2-ClPh	92	151-153	96.71
FPMS-21	CH_3	96	186-188	94.70
FPMS-22	Cyclo propyl	97	196-198	95.52
FPMS-23	Furfuryl	96	178-180	94.07

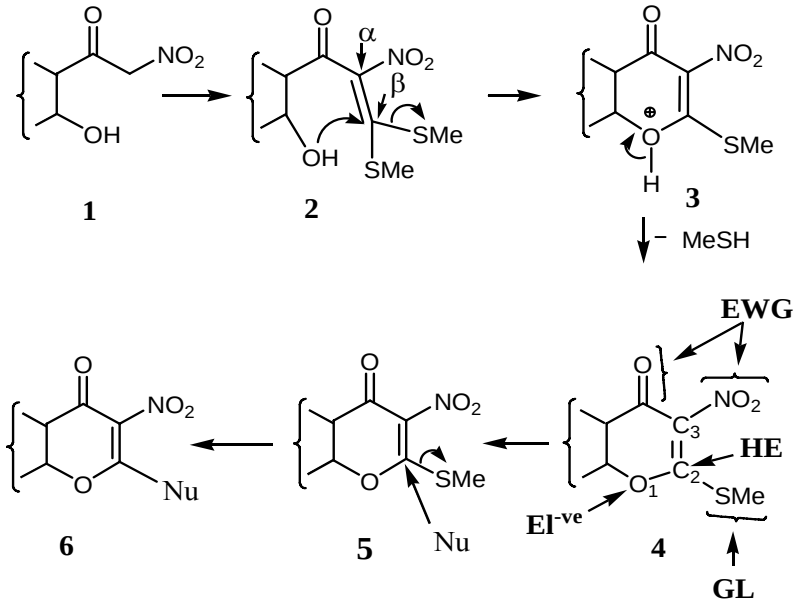
Table 3: Synthesis of novel chromenones FPMS 24-30.

Entry	—N	Yield %	mp °C	Purity %
FPMS-24		93	133-135	99.49
FPMS-25		94	136-138	97.54
FPMS-26		97	126-128	96.77
FPMS-27		93	132-134	96.17
FPMS-28		95	141-143	95.68
FPMS-29		96	124-126	96.10
FPMS-30		91	142-144	97.03

The plausible mechanism for the formation of chromenone **4** from **1** may involve initial formation of nitroketene dithioacetals **2** and followed by intramoleculer heteroannulation (Scheme 3). The *o*-hydroxy group appears to undergo nucleophilic attack on suitably located *β*-C atom, which mainly activated by the presence of nitro group at the *α*-position of ketene dithioacetals and followed by elimination of methylthio group **3** to furnish the corresponding chromenone in good yields (scheme 1). The mechanism for the direct nucleophilic addition with chromenone involves activation of C2 which is triggered by NO_2 and carbonyl groups present at C3. The excellent electron-withdrawing effect of nitro and carbonyl groups create the C2═C3 bond as polarized push-pull alkene with electron delocalizing from methylthio to nitro group. Due to this polarization effect, the C2 exhibits electrophilic characteristics. In addition, the C2 is adjacent to oxygen and methylthio groups which are electronegative (El^{-ve}) atom and good leaving group (GL), respectively **4**. As a result of these entire affects the C2 act as hard electrophile, which resulted in facile C-N bond formation with various

nucleophiles through elimination of the methylthio group **5** and furnished novel 2-substituted chromenones **6.**

Scheme 3: Proposed mechanism for the formation of novel chromenone.



4.6 CONCLUSION

In summary, we have developed a novel synthetic strategy for the synthesis of 2-substituted 3-nitro-4*H*-chromen-4ones through [5+1] heteroannulation of readily accessible 2-hydroxy-ω-nitro acetophenone with carbon disulfide and followed by straightforward nucleophilic addition through elimination of methylthio with various amines. The direct C-N bond formation reaction at C2 was achieved by the presence of nitro functionality at C3 position of chromenone. The newly developed methodology allows direct access to 2-substituted-3-nitro chromenone in excellent yield and high chemical purity. The presence of nitro functionality further makes them useful substrates for various transformations for biological interest.

4.7 EXPERIMENTAL SECTION

The solvents and chemicals were analytical grade. THF was distilled over sodium/benzophenone prior to use and anhydrous K_2CO_3 was used. Analytical thin layer chromatography (TLC) was performed on 0.2-mm precoated plates of Silica Gel 60 F_{254} precoated plates. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO, and TMS was used as an internal reference on a Bruker AVANCE II spectrometer. Mass spectra were determined using direct inlet probe on a GCMSQP2010 mass spectrometer. Chemical purity was determined on Waters Acquity UPLC with PDA Detector using Acquity BEH C18, 50 × 2.1, 1.7µm column at 210-400nm. Melting points were measured in open capillaries and are uncorrected.

❖ **Gradient Program for UPLC**

Mobile Phase: A - 10 mM Ammonium dihydrogenphosphate,

B – Acetonitrile

Entry	Time	Flow	%A	%B
1	Initial	0.2 mL	90	10
2	5.0	0.2 mL	10	90
3	5.1	0.2 mL	90	10
4	6.0	0.2 mL	90	10

❖ **General method for the synthesis of substituted *ω*-nitro acetophenone**:

To a suspension of substituted 4-hydroxycoumarin (26 mmol) in glacial acetic acid 10 ml was added slowly a solution of con. HNO_3 (2.20 mL) in glacial acetic acid 2 ml. the reaction mixture was then heated to 80 ºC at which a vigorous reaction starts. The reaction mix was cooled in ice-cold water to keep the reaction mix under control. After completion of the reaction, crushed ice was added to the reaction mixture. The separated substituted 3-nitro-4-hydrxycoumarin was filtered and washed with water and dry it in oven. The product of substituted 3-nitro-4-hydroxycouamarin (12 mmol) was dissolved in sodium hydroxide solution (5%, 75 mL) and the reaction mixture was kept at rt for 24 h. insoluble material was filtered and the clear filtrate acidified with con. HCl. The separated product was filtered and washed with water. It is crystallized from methanol.

❖ **A typical procedure for the synthesis of 2-(methylthio)-3-nitro-4*H*-chromen-4-one**

To a well-stirred suspension of K_2CO_3 (55 mmol) in dry THF (15 mL) at 0-5 ºC was added CS_2 (27.5 mmol) diluted with 10 mL THF along with substituted 2-hydroxy-*ω*-nitroacetophenone (27.5 mmol) over a period of 30 min. After completion of the addition, the reaction mixture was stirred at 0-5 ºC for 30 min and rt for 30 min. Appearance of orange yellow solid in the reaction medium indicated the formation of disodium salt. To this reaction, a solution of methyl iodide (55 mmol) in THF (7 mL) was added dropwise within 15 min at 0-5 ºC. The mixture was allowed to warm to room temperature and stirred for 5-6 h, and then poured onto crushed ice under stirring. The separated solid was collected by filtration, washed with water (2 × 100 mL) then hexane, dried in *vacuo* and crystallized from chloroform to furnish the analytically pure products in excellent yield which were used for next step without further purification.

2-(methylthio)-3-nitro-4*H*-chromen-4-one (3): Yellow solid; *Rf* 0.41 (6:4 hexane-EtOAc); Yield 98 %; purity 98.91%; mp 185-187 °C; IR (KBr): 3088, 1656, 1512, 1448, 1366, 1321, 1222, 989; MS *m/z*: 237(M^+); ^{1}H NMR (400 MHz): *δ* 3.13(s, 3H, CH_3), 7.56-7.61(t, 1H, ArH), 7.79(d, *J* = 8.8Hz, 1H, ArH), 7.85-7.91(t, 1H, ArH), 8.11(d, *J* = 8.6 Hz, 1H, ArH); Anal. Calcd. for $C_{10}H_7NO_4S$: C, 50.63; H, 2.97; N, 5.90%. Found: C, 50.57; H, 2.90; N, 5.84%.

❖ **General procedure for the reaction of various alkyl/aryl amines with chromenone 3.**

To a solution of various amines (1.15 mmol) in isopropyl alcohol (5 mL) was added suspension of **3** (1.05 mmol) and reflux the resulting mixture for 45-60 min. After completion of the reaction, the reaction mixture was allowed to come to room temperature then cooled it at 0-5 ºC in ice bath. The separated suspension was filtered, washed with water (20 mL), dried in *vacuo* and crystallized from methanol to afford analytically pure products.

3-nitro-2-(phenylamino)-4*H*-chromen-4-one (**FPMS-1):** Creamish solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3439, 3377, 1645, 1510, 1213, 1093; MS *m/z*: 282(M^+); ^{1}H NMR (400 MHz): *δ* 7.24(d, *J* = 0.76Hz, 1H, ArH), 7.40-7.55(m, 6H, ArH), 7.61-7.66 (t, 1H, ArH), 8.29(dd, *J* = 7.92Hz, 1H, ArH), 11.65(s, 1H, NH); ^{13}C NMR (100 MHz): 88.17, 116.84, 118.21, 122.19, 125.00, 125.76, 125.87, 126.93, 128.96, 134.18, 134.88, 151.01, 158.83, 167.32, Anal. Calcd. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.93%. Found: C, 63.77; H, 3.50; N, 9.86%.

2-(m-tolylamino)-3-nitro-4*H*-chromen-4-one (FPMS-2): White solid; *Rf* 0.52 (6:4 hexane-EtOAc); IR (KBr): 3389, 3105, 1685, 1478, 1233, 1124, 985; MS *m/z*: 296(M^+); Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46%. Found: C, 64.79; H, 4.01; N, 9.40%.

2-(4-methoxyphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-3): Creamish solid; *Rf* 0.49 (6:4 hexane-EtOAc); IR (KBr): 3274, 3160, 1698, 1491, 1171, 1024; MS *m/z*: 312(M^+); ^{13}C NMR (100 MHz): 55.33, 87.64, 117.58, 118.51, 122.18, 125.79, 126.66, 134.88, 149.37, 154.56, 167.54, Anal. Calcd. for $C_{16}H_{12}N_2O_5$: C, 61.54; H, 3.87; N, 8.97%. Found: C, 61.49; H, 3.82; N, 8.93%.

2-(2-fluorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-4): White solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3591, 3300, 1658, 1512, 1435, 1402, 1288; MS *m/z*: 300(M^+); Anal. Calcd. for $C_{15}H_9FN_2O_4$: C, 60.01; H, 3.02; N, 9.33%. Found: C, 59.89; H, 3.04; N, 9.21%.

2-(3,4-dichlorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-5): Yellowish solid; *Rf* 0.50 (6:4 hexane-EtOAc); IR (KBr): 3287, 3087, 1696, 1436, 1296, 1114; MS *m/z*: 351(M^+); Anal. Calcd. for $C_{15}H_8Cl_2N_2O_4$: C, 51.31; H, 2.30; N, 7.98%. Found: C, 51.19; H, 2.18; N, 7.86%.

2-(p-tolylamino)-3-nitro-4*H*-chromen-4-one (FPMS-6): Pale yellow solid; *Rf* 0.50 (6:4 hexane-EtOAc); IR (KBr): 3419, 3115, 1665, 1489, 1265, 1214, 1174; MS *m/z*: 296(M^+); Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46%. Found: C, 64.79; H, 4.01; N, 9.40%.

2-(4-fluorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-7): Creamish solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3468, 3160, 1666, 1590, 1375, 1232, 1188; MS *m/z*: 300(M^+); Anal. Calcd. for $C_{15}H_9FN_2O_4$: C, 60.01; H, 3.02; N, 9.33%. Found: C, 59.89; H, 3.04; N, 9.21%.

2-(3,4-difluorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-8): White solid; *Rf* 0.48 (6:4 hexane-EtOAc); IR (KBr): 3337, 3027, 1696, 1590, 1417, 1392, 1258, 1172; MS *m/z*: 318(M^+); Anal. Calcd. for $C_{15}H_8F_2N_2O_4$: C, 56.61; H, 2.53; N, 8.80%. Found: C, 56.49; H, 2.41; N, 8.68%.

2-(4-ethylphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-9): Yellowish solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3328, 3120, 1676, 1500, 1385, 1242, 1088; MS *m/z*:

310(M^+); Anal. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03%. Found: C, 65.69; H, 4.41; N, 8.91%.

2-(2-methoxy,4-nitrophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-10): Yellow solid; *Rf* 0.48 (6:4 hexane-EtOAc); IR (KBr): 3389, 3360, 1693, 1507, 1473, 1312, 1288; MS *m/z*: 357(M^+); Anal. Calcd. for $C_{16}H_{11}N_3O_7$: C, 53.79; H, 3.10; N, 11.76%. Found: C, 53.67; H, 2.94; N, 11.62%.

2-(3-chloro,4-fluorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-11): White solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3467, 3068, 1714, 1530, 1477, 1392, 1198; MS *m/z*: 334(M^+); Anal. Calcd. for $C_{15}H_8ClFN_2O_4$: C, 53.83; H, 2.41; N, 8.37%. Found: C, 53.71; H, 2.28; N, 8.25%.

2-(3-bromophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-12): Creamish solid; *Rf* 0.53 (6:4 hexane-EtOAc); IR (KBr): 3472, 3116, 1694, 1526, 1485, 1337, 1174; MS *m/z*: 361(M^+); Anal. Calcd. for $C_{15}H_9BrN_2O_4$: C, 49.89; H, 2.51; N, 7.76%. Found: C, 49.77; H, 2.37; N, 7.62%.

2-(4-nitrophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-13): Yellowish solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3357, 3071, 1695, 1541, 1351, 1237, 1108; MS *m/z*: 327(M^+); Anal. Calcd. for $C_{15}H_9N_3O_6$: C, 55.05; H, 2.77; N, 12.84%. Found: C, 54.91; H, 2.64; N, 12.72%.

2-(2,3-dimethylphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-14): White solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3392, 3070, 1681, 1470, 1335, 1221, 1108; MS *m/z*: 310(M^+); Anal. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03%. Found: C, 65.67; H, 4.42; N, 8.89%.

2-(2-methoxyphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-15): Creamish solid; *Rf* 0.50 (6:4 hexane-EtOAc); IR (KBr): 3368, 3170, 1657, 1413, 1271, 1224; MS *m/z*: 312(M^+); Anal. Calcd. for $C_{16}H_{12}N_2O_5$: C, 61.54; H, 3.87; N, 8.97%. Found: C, 61.48; H, 3.83; N, 8.91%.

2-(2,4-dimethylphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-16): White solid; *Rf* 0.52 (6:4 hexane-EtOAc); IR (KBr): 3424, 3187, 1697, 1584, 1335, 1231, 1192; MS *m/z*: 310(M^+); Anal. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03%. Found: C, 65.68; H, 4.42; N, 8.88%.

2-(4-chlorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-17): Yellowish solid; *Rf* 0.53 (6:4 hexane-EtOAc); 3487, 3018, 1734, 1680, 1577, 1439, 1108; MS *m/z*: 316(M^+); Anal. Calcd. for $C_{15}H_9ClN_2O_4$: C, 56.89; H, 2.86; N, 8.85%. Found: C, 56.84; H, 2.81; N, 8.78%.

2-(3-methoxyphenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-18): White solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3416, 3117, 1671, 1541, 1427, 1224; MS *m/z*: 312(M^+); Anal. Calcd. for $C_{16}H_{12}N_2O_5$: C, 61.54; H, 3.87; N, 8.97%. Found: C, 61.48; H, 3.83; N, 8.91%.

2-(3-nitrophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-19): Yellow solid; *Rf* 0.50 (6:4 hexane-EtOAc); IR (KBr): 3471, 3301, 1699, 1513, 1322, 1230, 1047; MS *m/z*: 327(M^+); Anal. Calcd. for $C_{15}H_9N_3O_6$: C, 55.05; H, 2.77; N, 12.84%. Found: C, 54.88; H, 2.69; N, 12.75%.

2-(2-chlorophenylamino)-3-nitro-4*H*-chromen-4-one (FPMS-20): Yellow solid; *Rf* 0.53 (6:4 hexane-EtOAc); IR (KBr): 3420, 3310, 1788, 1687, 1514, 1490, 1170; MS *m/z*: 316(M^+); Anal. Calcd. for $C_{15}H_9ClN_2O_4$: C, 56.89; H, 2.86; N, 8.85%. Found: C, 56.81; H, 2.72; N, 8.78%.

2-(methylamino)-3-nitro-4*H*-chromen-4-one (FPMS-21): Yellow solid; *Rf* 0.49 (6:4 hexane-EtOAc); IR (KBr): 3357, 3147, 1689, 1523, 1272, 1130, 1017; MS *m/z*: 220(M^+); ^{1}H NMR (400 MHz): δ 3.33(d, J = 5.16Hz, 3H, CH_3), 7.35(d, J = 8Hz, 1H, ArH), 7.41-7.45(t, 1H, ArH), 7.63-7.67(t, 1H, ArH), 8.28(dd, J = 7.88Hz, 1H, ArH), 10.06(s, 1H, NH); Anal. Calcd. for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72%. Found: C, 54.49; H, 3.61; N, 12.75%.

2-(cyclopropylamino)-3-nitro-4*H*-chromen-4-one (FPMS-22): Yellow solid; *Rf* 0.52 (6:4 hexane-EtOAc); IR (KBr): 3315, 3170, 1698, 1517, 1401, 1373, 1270; MS *m/z*: 232(M^+); Anal. Calcd. for $C_{11}H_8N_2O_4$: C, 56.90; H, 3.47; N, 12.06%. Found: C, 56.78; H, 3.35; N, 11.98%.

2-(furan-2-ylamino)-3-nitro-4H-chromen-4-one (FPMS-23): Yellow solid; *Rf* 0.51 (6:4 hexane-EtOAc); IR (KBr): 3320, 3240, 1788, 1587, 1314, 1280, 1170; MS *m/z*: 272(M^+); Anal. Calcd. for $C_{13}H_8N_2O_5$: C, 57.36; H, 2.96; N, 10.29%. Found: C, 57.24; H, 2.84; N, 10.17%.

❖ **General procedure for the reaction of various secondary amines with chromenone 3:**

To a solution of various secondary amines (1.15 mmol) in dioxane (5 mL) was added suspension of **3** (1.05 mmol) and reflux the resulting mixture for 75-80 min. After completion of the reaction, the reaction mixture was allowed to come to room temperature then add water 20 mL and extracted with chloroform (2 × 10 mL). The separated organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to afforded analytically pure products.

3-nitro-2-(piperidin-1-yl)-4*H*-chromen-4-one (FPMS-24): Reddish brown solid; *Rf* 0.27 (4:6 hexane-EtOAc); IR (KBr): 3061, 1662, 1591, 1508, 1492, 1421, 1178; MS *m/z*: 274(M^+); ^{13}C NMR (100 MHz): 22.82, 24.83, 48.09, 87.32, 117.16, 121.56, 125.65, 134.07, 151.46, 158.20, 168.89, Anal. Calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21%. Found: C, 61.27; H, 5.09; N, 10.17%.

2-morpholino-3-nitro-4*H*-chromen-4-one (**FPMS-25):** Reddish brown solid; *Rf* 0.28 (4:6 hexane-EtOAc); MS *m/z*: 276(M^+); IR (KBr): 3074, 1681, 1574, 1443, 1403, 1167; Anal. Calcd. for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14%. Found: C, 56.48; H, 4.39; N, 10.17%.

2-(4-methylpiperazin-1-yl)-3-nitro-4*H*-chromen-4-one (**FPMS-26):** Brown solid; *Rf* 0.26 (4:6 hexane-EtOAc); IR (KBr): 3049, 1679, 1587, 1463, 1280, 1140; MS *m/z*: 289(M^+); Anal. Calcd. for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53%. Found: C, 58.10; H, 5.18; N, 14.58%.

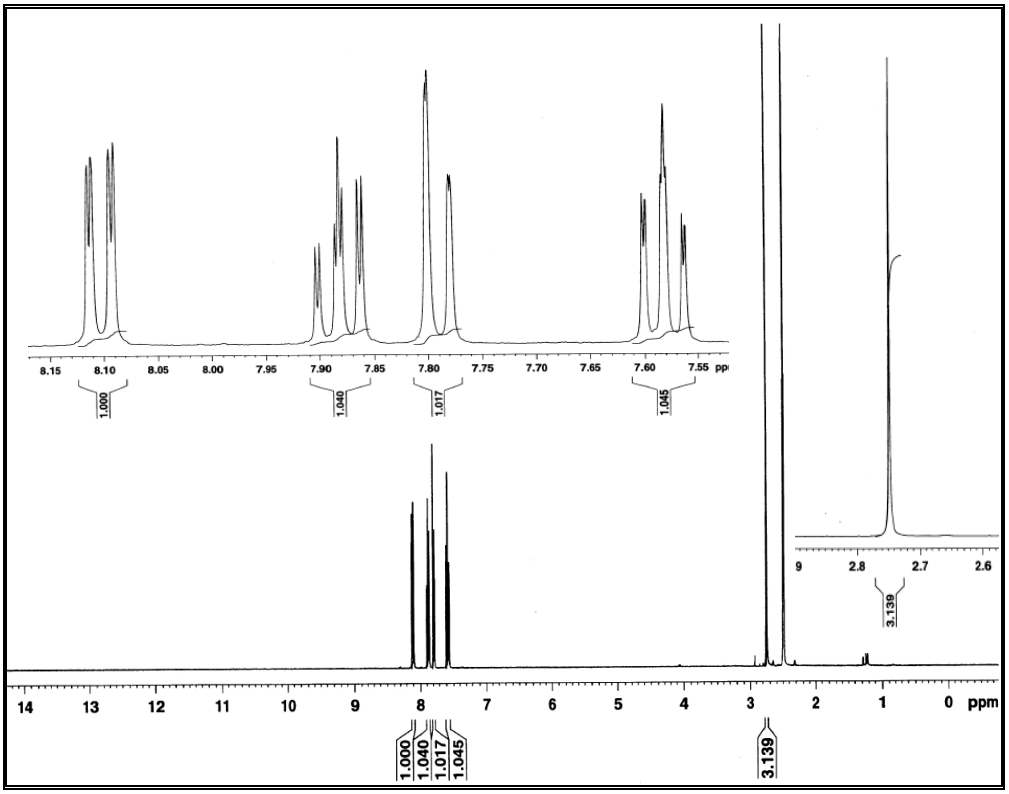
2-(4-ethylpiperazin-1-yl)-3-nitro-4*H*-chromen-4-one (**FPMS-27):** Reddish solid; *Rf* 0.25 (4:6 hexane-EtOAc); IR (KBr): 2932, 1682, 1574, 1325, 1248, 1109; MS *m/z*: 303(M^+); ^1H NMR (400 MHz): δ 1.11-1.14(t, 3H, CH_3), 2.48-2.52(q, 2H, CH_2), 2.62-2.64(t, 4H, CH_2), 3.60-3.63(t, 4H, CH_2), 7.31(d, J = 8.28Hz, 1H, ArH), 7.38-7.42(t, 1H, ArH), 7.61-7.66(t, 1H, ArH), 8.23(dd, J = 7.84Hz, 1H, ArH); Anal. Calcd. for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85%. Found: C, 59.37; H, 5.59; N, 13.81%.

2-(dimethylamino)-3-nitro-4*H*-chromen-4-one (FPMS-28): Reddish brown solid; *Rf* 0.24 (4:6 hexane-EtOAc); IR (KBr): 3071, 1688, 1495, 1326, 1105, 1030; MS *m/z*: 234(M^+); Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N, 11.96%. Found: C, 56.29; H, 4.18; N, 11.83%.

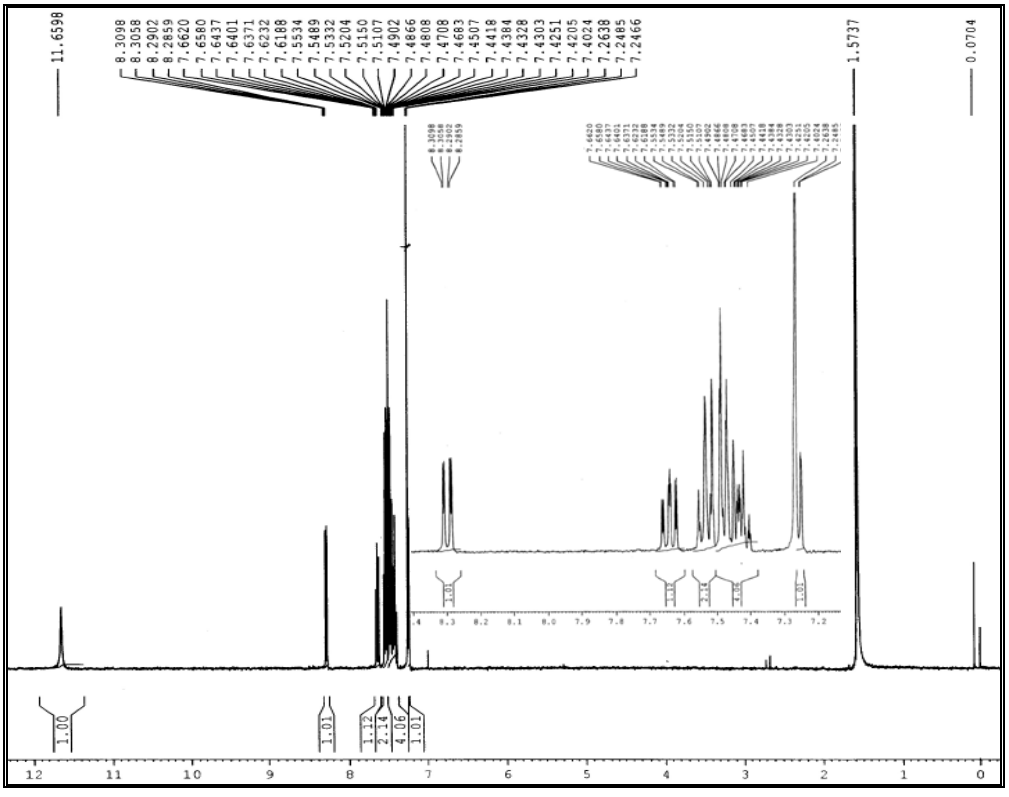
3-nitro-2-(1*H*-pyrrol-1-yl)-4*H*-chromen-4-one (FPMS-29): Brown solid; *Rf* 0.25 (4:6 hexane-EtOAc); IR (KBr): 3022, 1679, 1571, 1430, 1221, 1109; MS *m/z*: 256(M^+); Anal. Calcd. for $C_{13}H_8N_2O_4$: C, 60.94; H, 3.15; N, 10.93%. Found: C, 60.81; H, 3.02; N, 10.81%.

3-nitro-2-(pyrrolidin-1-yl)-4*H*-chromen-4-one (FPMS-30): Reddish solid; *Rf* 0.25 (4:6 hexane-EtOAc); IR (KBr): 3019, 1697, 1572, 1483, 1237, 1103; MS *m/z*: 260(M^+); Anal. Calcd. for $C_{13}H_{12}N_2O_4$: C, 60.00; H, 4.65; N, 10.76%. Found: C, 59.91; H, 4.58; N, 10.68%.

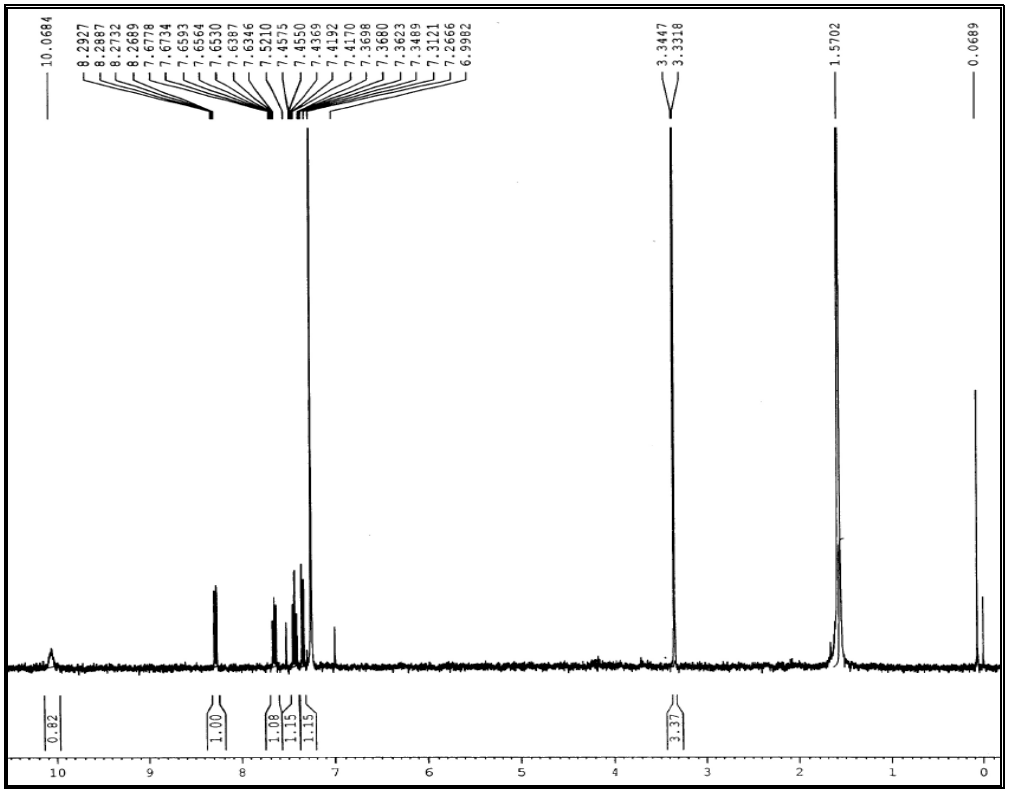
^{1}H NMR spectrum of compound 3



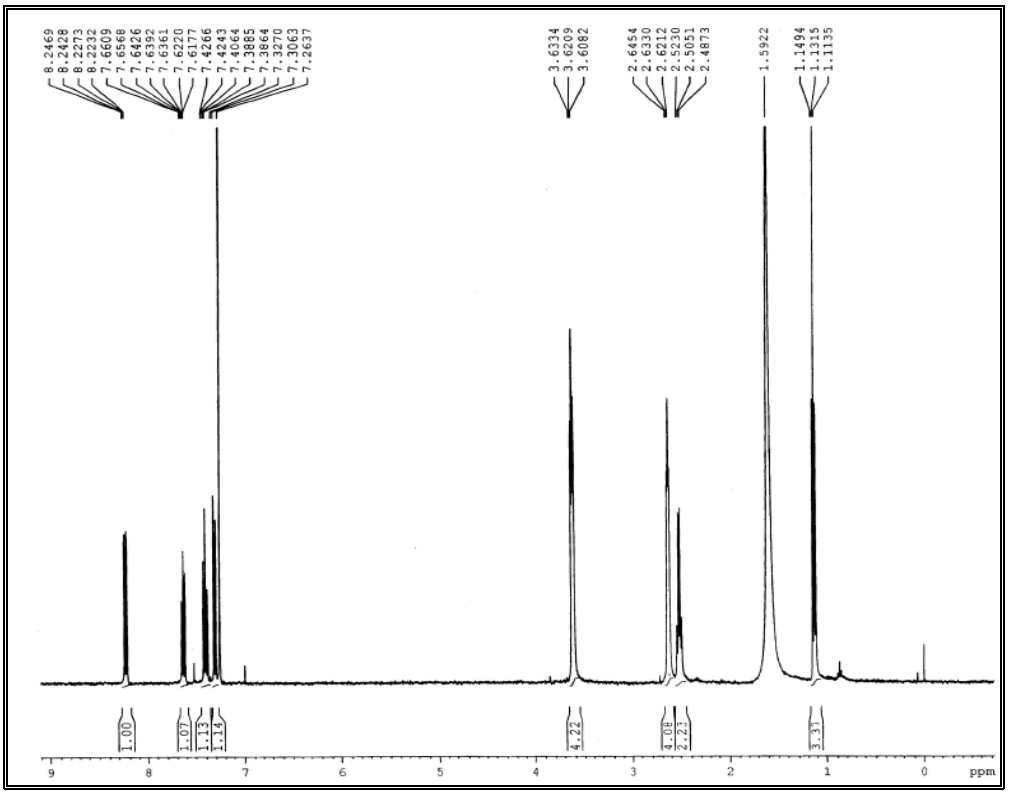
^{1}H NMR spectrum of compound FPMS 01



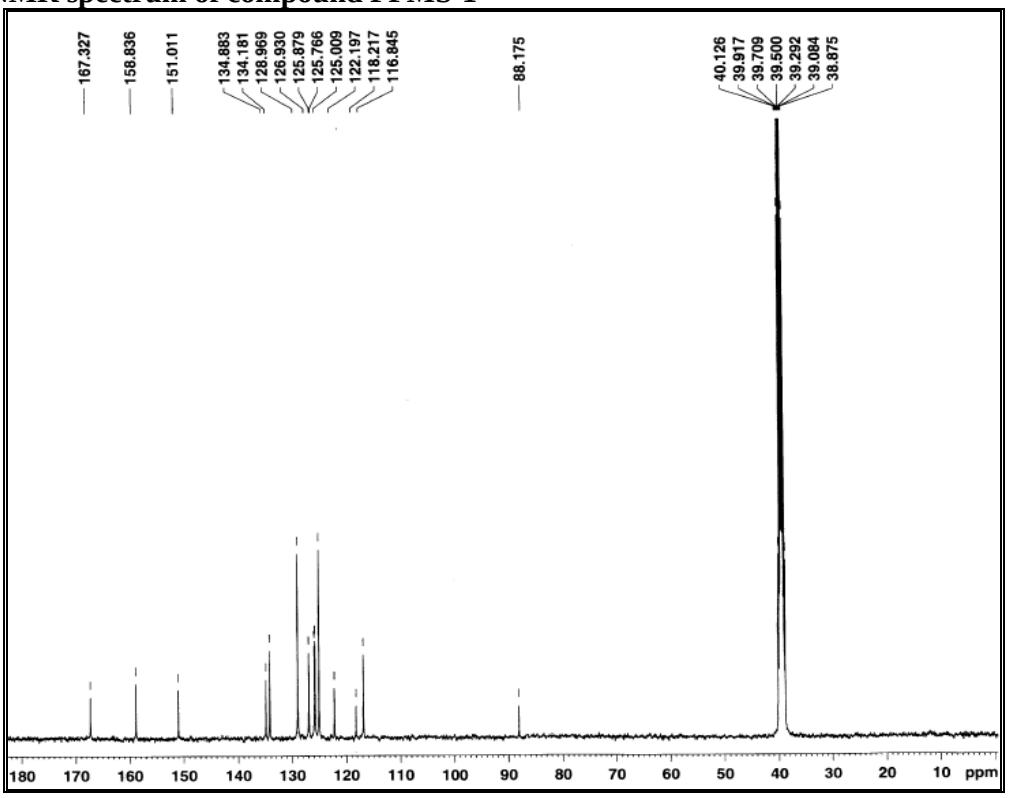
^{1}H NMR spectrum of compound FPMS 21



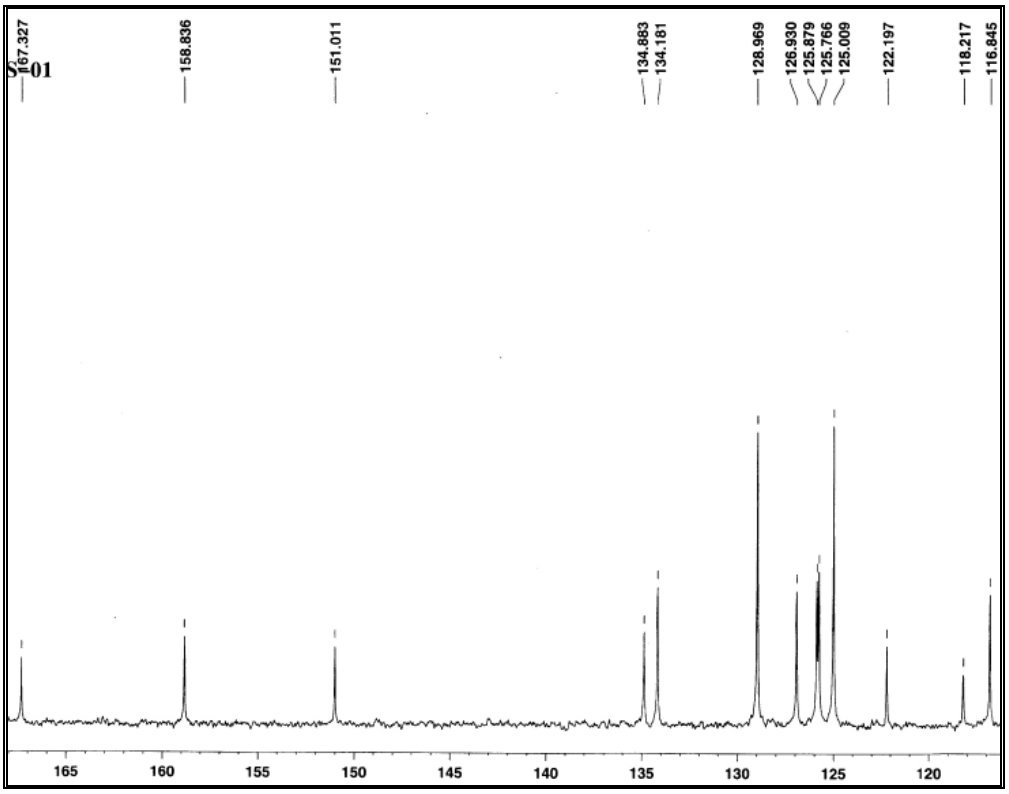
^{1}H NMR spectrum of FPMS 27



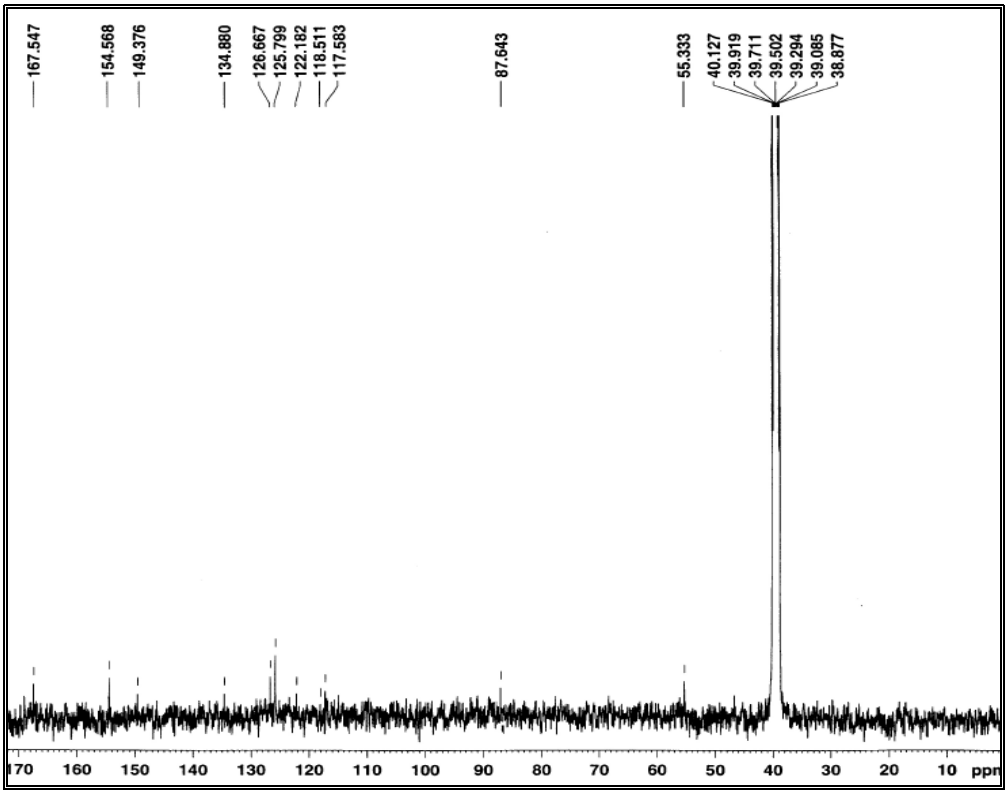
^{13}C NMR spectrum of compound FPMS-1



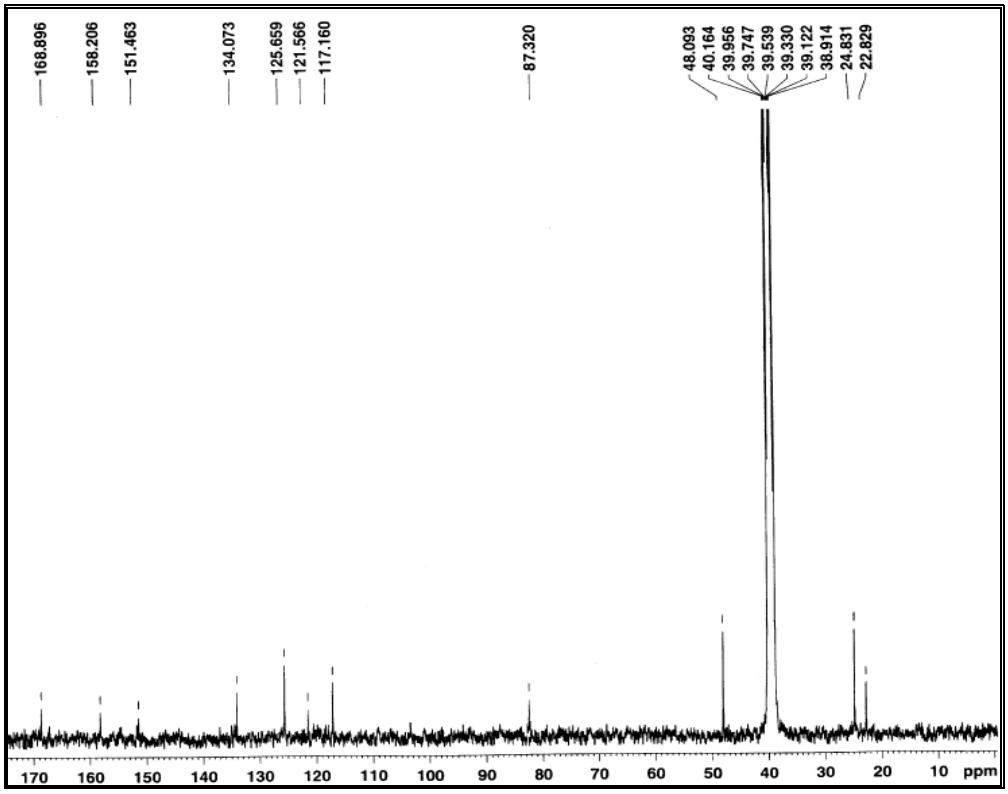
Expanded ^{13}C NMR spectrum of compound FPMS-1



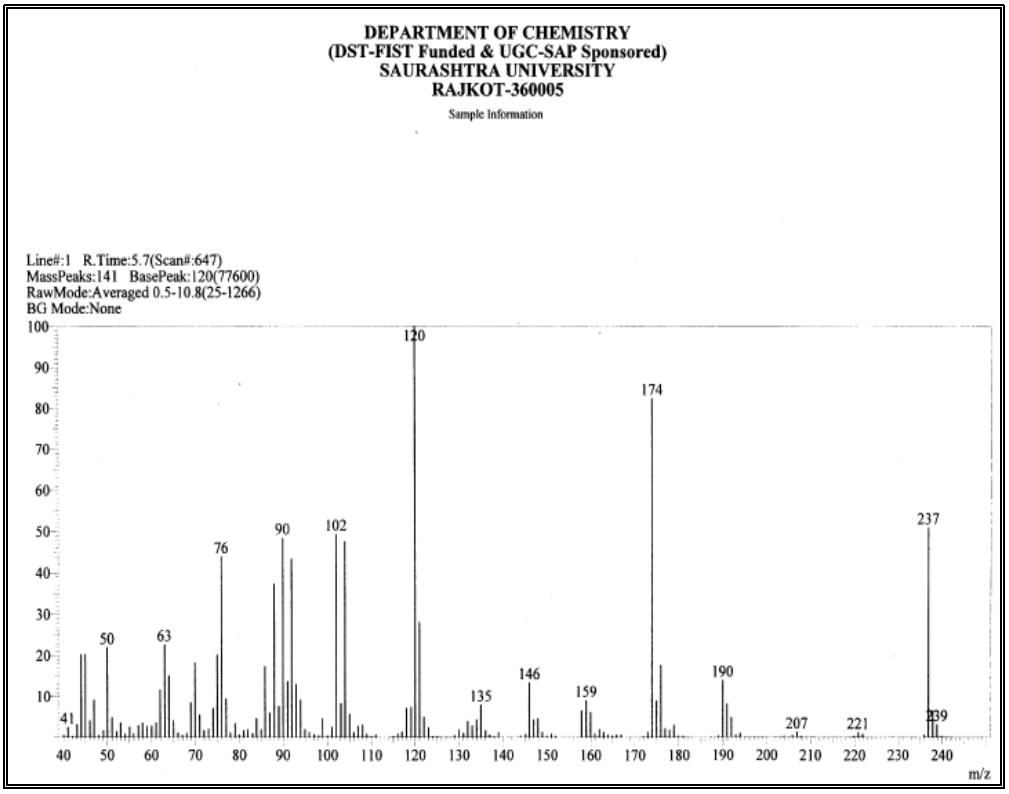
^{13}C NMR spectrum of compound FPMS-3



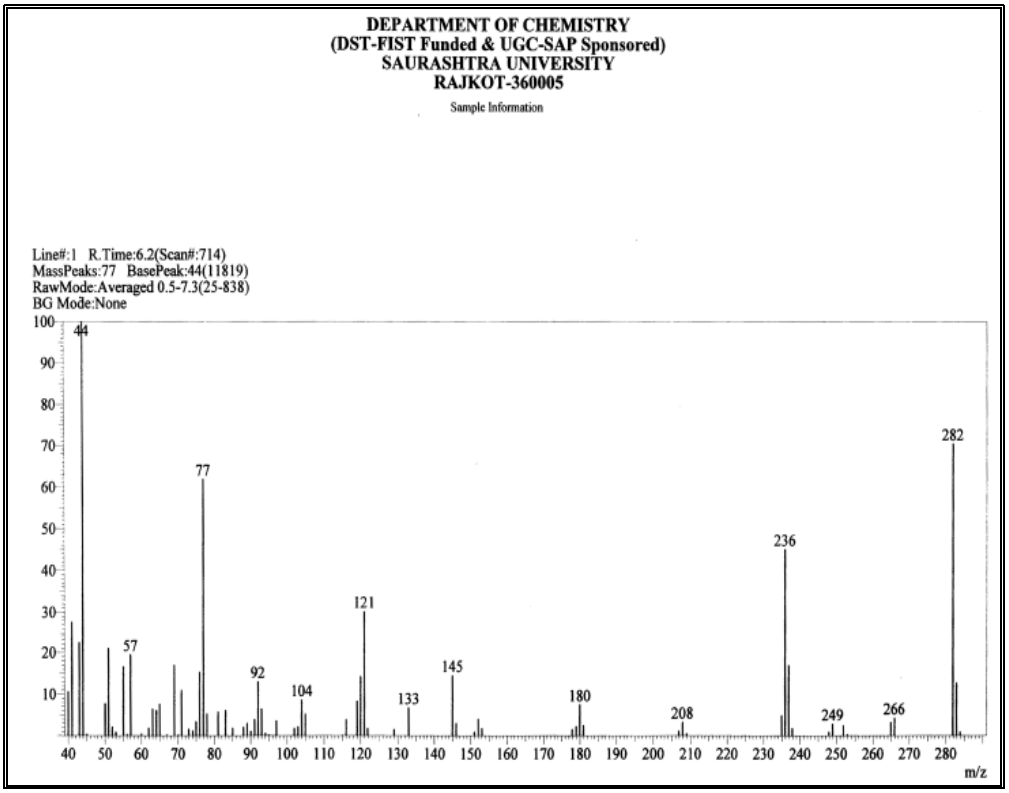
^{13}C NMR spectrum of compound FPMS-24



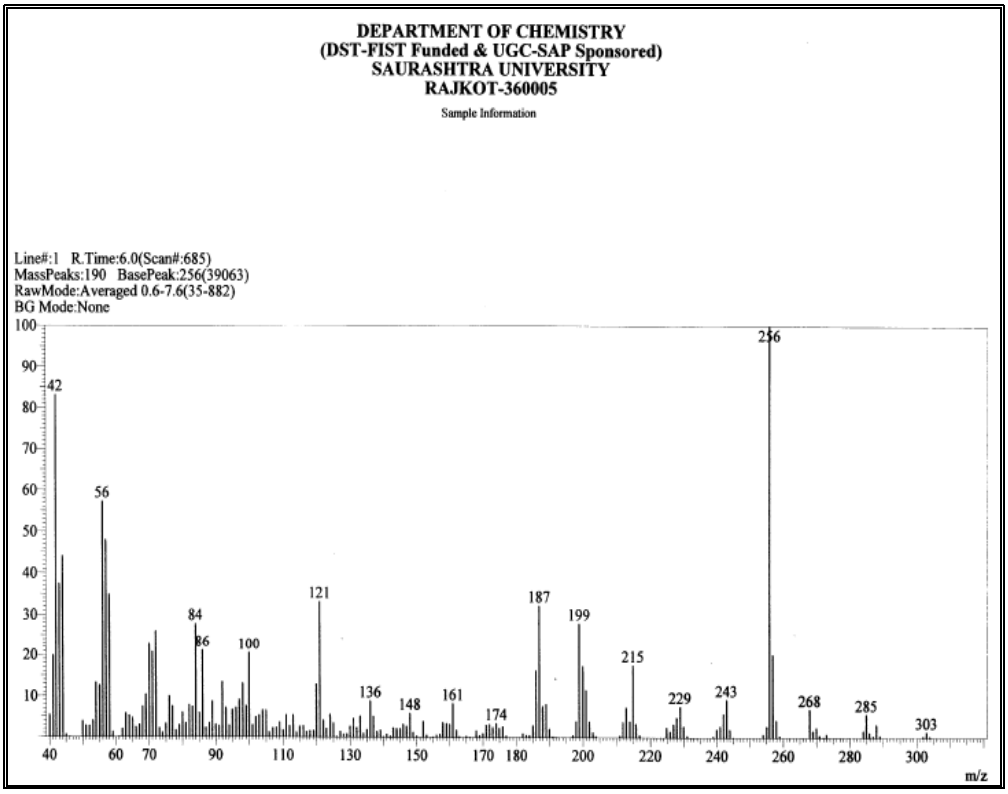
Mass spectrum of compound 3



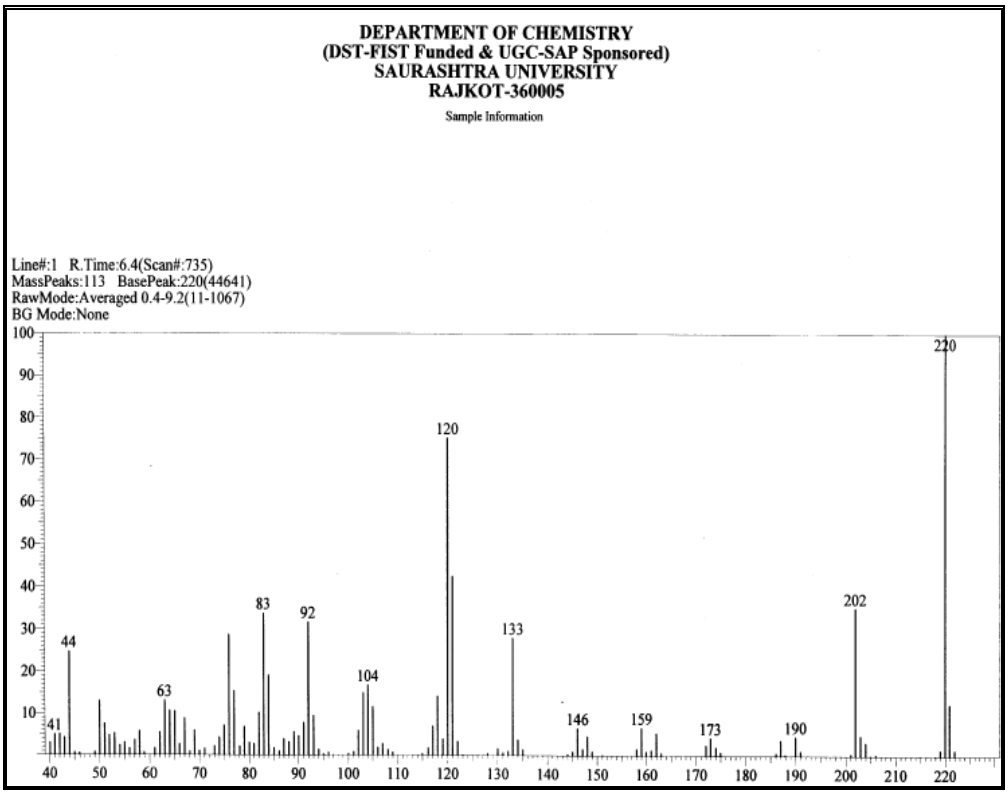
Mass spectrum of compound FPMS-1



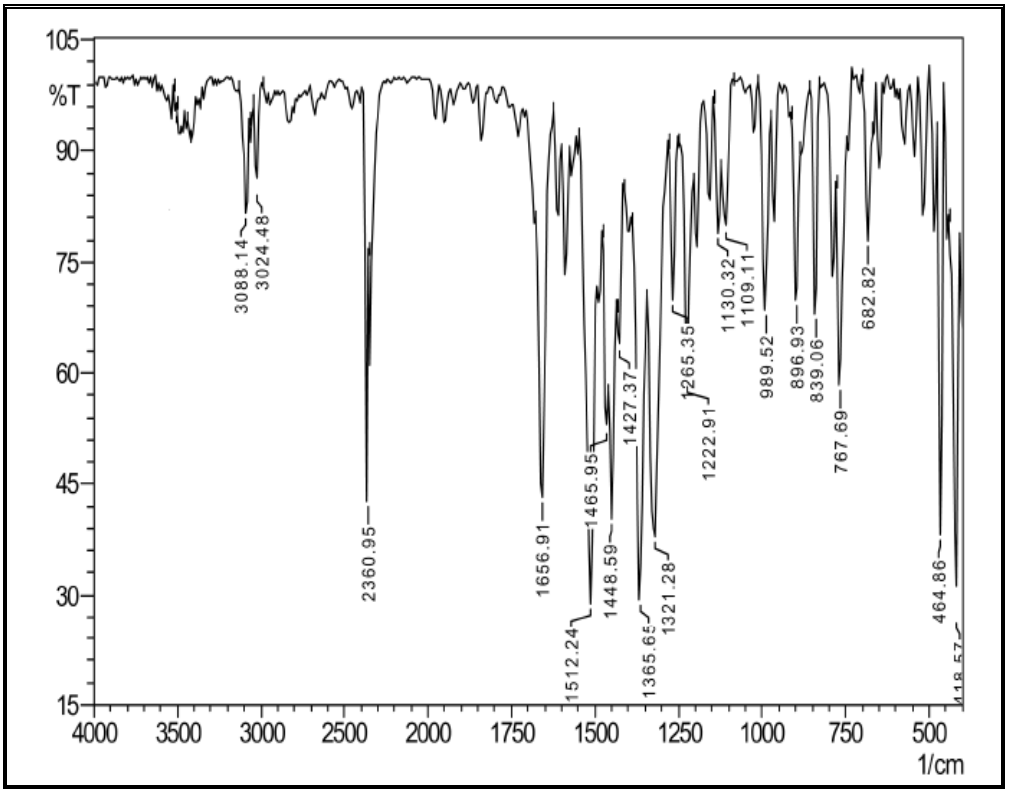
Mass spectrum of compound FPMS-27



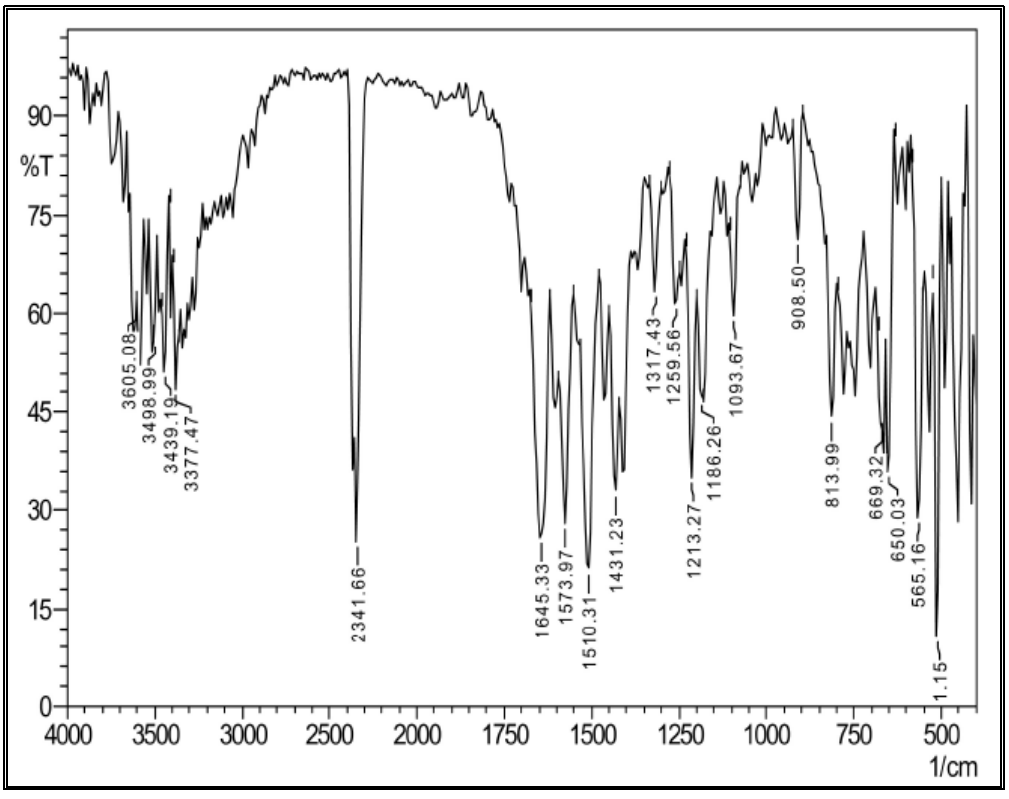
Mass spectrum of compound FPMS-21



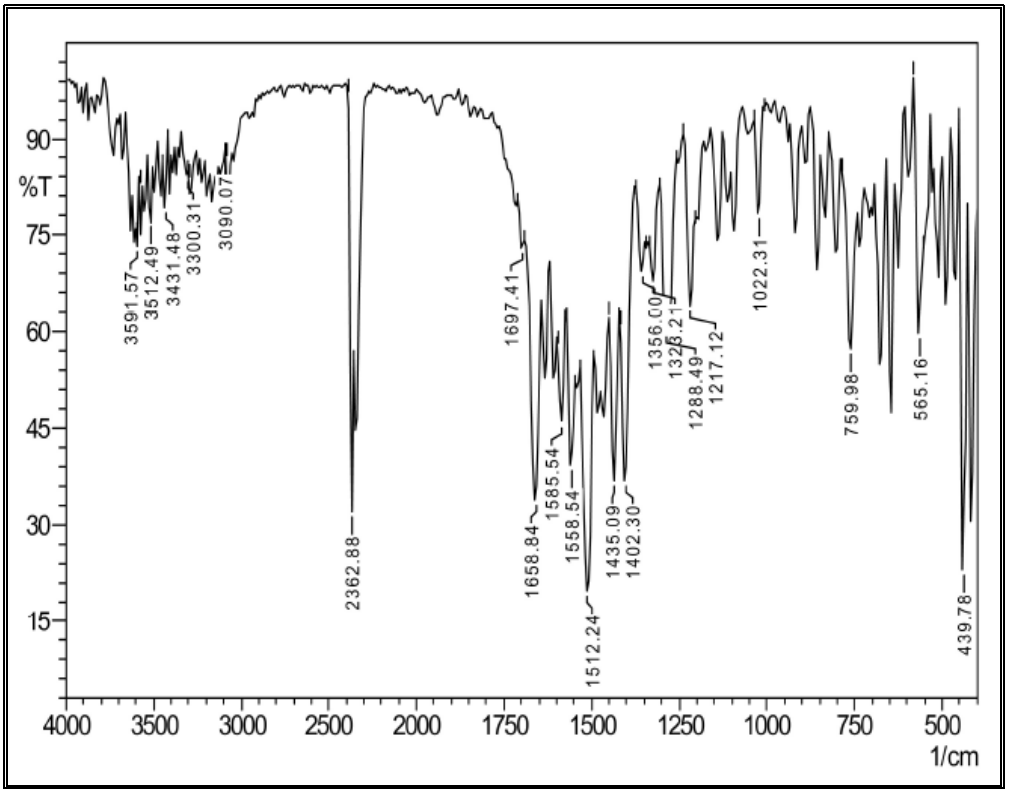
IR spectrum of compound 3



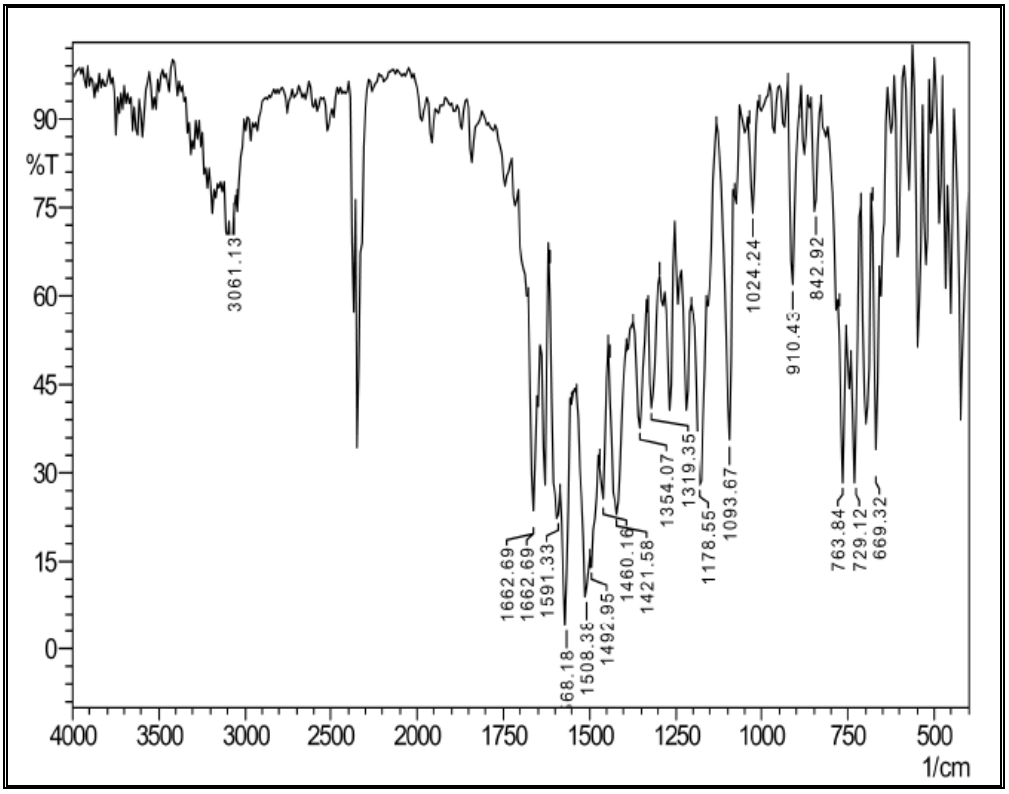
IR spectrum of compound FPMS 01



IR spectrum of compound FPMS 04



IR spectrum of compound FPMS-24



4.8 REFERENCES

1 Kuhnau, J. *World Res. Nut. Diet* **1976**, *24*,117.

2 Harsteen, B. *Biachem. Pharmacol.* **1983,** *32*, 1141.

3 Cesarone, M. R.; Laurora, G.; Ricci, A.; Belcaco, G.; Pomante, P. *J. Vas Disease* **1992**, *21*, 76.

4 (a) Dewick, P. M. The Flavonoids: Advances in Research Since1986; Harborne, J. B., Ed.; Chapmann & Hall: New York, **1994**; p 23. (b) Gill, M. In The Chemistry of Natural Products; Thomson, R. H. Ed.; 2nd Ed., Blackie: Surrey, **1993**; p 60. (c) Manthy, J. A.; Buslig, B. S.; Eds.; In Flavonoids in the Living Systems: Advances in Experimental Medicine and Biology; Plenum: New York, **1998**; 439. (c) Levai, A. *Arkivoc* **2004**, *7*, 15. (d) Silva, A. M. S.; Pinto, D. C. G. A.; Cavaleiro, J. A. S.; Levai, A.; Patonay, T. *Arkivoc* **2004**, *7*, 106.

5 (a) Korkina, G. L.; Alafanev, I. B. Advances in Pharmacology; Sies, H., Ed.; Academic: San Diego, **1997,** *38*. 151. (b) Hansch, C.; Sammes, P. G.; Taylor, J. B., Edt.; Comprehensive Medicinal Chemistry; Pergamon: London **1990**, Vol. 6.

6 (a) Ghosh, C. K.; Ghosh, C. *Indian J. Chem.* **1997**, *36*, 968. (b) Hsung, R. P. *J. Org. Chem.* **1997**, *62,* 7904. (c) Valenti, P.; Bisi, A.; Rampa, A.; Belluti, F.; Gobbi, S.; Zampiron, A.; Carrara, M. *Bioorg. Med. Chem.* **2000**, *8*, 239. (d) Malhotra, S.; Shakya, G.;Kumar, A.; Vanhoecke, B. W.; Cholli, A. L.; Raj, H.G.; Saso, L.; Ghosh, B.; Bracke, M. E.; Prasad, A. K.; Biswal, S.; Parmar, V. S. *Arkivoc* **2008**, *6*, 119. (e) Singh, G.; Singh, R.; Girdhar, N. K.; Ishar, M. P. S. *Tetrahedron* **2002**, *58*, 2471.

7 (a) Kumar, S. K. *J. Med. Chem.* **2003**, *46*, 2813. (b) Wang, Y. Q. *Bioorg. Med. Chem.* **2003**, *11*, 1569. (c) Gao, Y.; Li, D. J.; Keung, W. M. *Bioorg. Med. Chem.* **2003**, *11*, 4069. (d) Ishar, M. P. S.; Singh, G.; Singh, S.; Sreenivasan K. K.; Singh G. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1366.

8 For a review on the biological activities and synthesis of chromone derivatives see: Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Chem. Rev*. **2003**, *103*, 893.

9 Perrella, F. W.; Chen, S. F.; Behrens, D. L.; Kaltenbach, R. F.; Seitz, S. P. *J. Med. Chem.* **1994**, *37,* 2232.

10 Bauvois, B.; Puiffe, M. L.; Bongui, J. B.; Paillat, S.; Monneret, C.; Dauzonne, D. *J. Med. Chem.* **2003**, *46,* 3900.

11 Vasselin, A. D.; Westwell, A. D. ; Matthews, C. S.; Bradshaw, T. D.; Stevens, M. F. G. *J. Med. Chem.* **2006**, *49,* 3973.

12 Wu, E. S. C.; Cole, T. E.; Davidson, T. A.; Daiely, M. A.; Doring, K. G.; Fedorchuk, M.; Loch, J. T.; Thomas, T. L.; Blosser, J. C.; Borrelli, A. R.; Kinsolving, C. R.; Parker, R. B.; Strand, J. C.; Watkins, B. E. *J. Med. Chem.* **1989**, *32,* 182.

13 Sottofatoori, E.; Anzaldi, M.; Mazzei, M.; Meile, M.; Balbi, A.; Pyshnyi, D. S.; Zakhrovab, O. D.; Abramovab, T. V. *Bioorg. Med. Chem.* **2005**, *13,* 1515.

14 Buckle, D. R.; Barrie, C. C.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1975**, *18,* 391

15 Ahna, Y. M.; Vogetia, L.; Liu, C. J.; Santhapurama, H. K. R.; White, J. M.; Vasandani, V.; Mitscher, L. A.; Lushington, G. H.; Hanson, P. R.; Powell, D. R.; Himes, R. H.; Robye, K. F.; Yeb, Q.; Georg, G. I. *Bioorg Med Chem*. **2007**, *15*, 702.

16 Griffin, R. J.; Fontana, G.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Leahy, J. J.; Martin, N.; Richardson, C.; Rigoreau, L.; Stockley, M.; Smith, G. C. M. *J. Med. Chem.* **2005**, *48,* 569.

17 Dauzonnel, D.; Foleasl, B.; Martinez, L.; Chabot, G. G. *Eur. J. Med. Chem.* **1997**, *32*,7 l.

18 Huang, X.; Liu, T.; Gu, J.; Luo, X.; Ji, R.; Cao, Y.; Xue, H. J.; Wong, T. F.; Wong, B. L.; Pei, G.; Jiang, H.; Chen, K. *J. Med. Chem.*, **2001**, *44*, 1883.

19 Flynn, B. L.; Baell, J. B.; Harvey, A. J.; Chaplin, J. H.; Paul, D.; Mould, J. A. WO 040058 A1, **2008**.

20 Quintin, J.; Buisson, D.; Thoret, S.; Cresteil, T.; Lewin, G.; *Bioorg. & Med. Chem. Lett*., **2009**, *19*, 3502.

21 Sheng, R.; Lin, X.; Zhang, J.; Chol, K. S.; Huang, W.; Yang, B.; He, Q.; Hua, Y. *Bioorg. & Med. Chem*., **2009**, *17*, 6692.

22 Diwakar, S. D.; Bhagwat, S. S.; Shingare, M. S.; Gill, C. H. *Bioorg. & Med. Chem. Lett.* **2008**, *18*, 4678.

23 Kotharkar, S. A.; Shinde, D. B.; *Bioorg. & Med. Chem. Lett.* **2006**, *16*, 6181.

24 Perkin, W. H. *J. Chem. Soc.* **1868**, *21*, 53.

25 Pechmann, V. *Ber. Dtsch. Chem. Ges.* **1884**, *17*, 929.

26 Spath, E. *ibid*, **1937**, *70*, 83.

27 Ruhemann, S.; Stapleton, H. E. *J. Chem. Soc*. **1900**, *77*, 1179; *Ber. Dtsch. Chem. Ges*. **1913**, *46,* 2188.

28 Simonis, H.; Petschek, E. *Ber. Dtsch. Chem. Ges.* **1913**, *46*, 2014.

29 (a) Kostanecki, S. V. *Ber.Dtsch. Chem. Ges.***1903**, *33*, 32 (b) Kostanecki, S. V.; Emilewics, J. *ibid*. **1898**, *31*, 696.(c) Kostanecki, *ibid*. **1900**, *33*, 471

30 Spath, E.; Lederer, E. *ibid*. **1930**, *63*, 743.

31 (a) Nagai, W. N. *Beri*. **1892**, *25*, 1284. (b) Tahara, Y. *ibid*, **1892**, *25*, 1292.

32 Allan, J.; Robinson, R. *J. Chem. Soc*. **1924**, *125*, 2192.

33 Emelia, A.; Alfredo, C. *Org. Lett.*, **2009**, 11, 3210.

34 Bo, L.; Mengwei, H.; Zejin, Y.; Zhengchang, X.; Kui, L.; Reza, F.; Jiahua, C.; Zhen, Y. *J. of Org. Chem*. **2005**, *70*, 6097.

35 Rao, K. V. *Indus. & Eng. Chem. Res.*, **2002**, *41*, 3333.

36 Becket, G. J. P.; Ellis, G. P. *Tetra. Lett.* **1976**, *9*, 719.

37 Chebrolu, Paparao.; Sundaramurthy, V. *Ind. J. of Chem., Section B: Org. Chem. Include. Med. Chem.* **1986**, *25B*, 212.

38 Dauzonne, D.; Martinez, L. *Tetra. Lett.* **1995**, *36*, 1845.

39 Terzidis, M. A.; Tsoleridis, C. A.; Stephanatou, J. *Arkivoc* **2008,** *14*, 132.

40 Ji, M.; Hu, J.; Fu, T.; Hua, W.; Hu, W. *J. of Chem. Cryst.* **2000**, *30,* 2,

41 Amantini, D.; Fringuelli, F.; Piermatti, O.; Pizzo, F.; Vaccaro, L. *J. Org. Chem.* **2003**, *68*, 9263.

42 Savelev, V. L.; Artamonova, O. S.; Makarov, A. V.; Troitskaya, V. S.; Antonova, A. V.; Vinokurov, V. G. *Khimiya Geterotsiklicheskikh Soedinenii*, **1989**, *9*, 1208.

43 Ventkatesh, C.; Singh, B.; Mahata, P. K.; Ila, H.; Junjappa, H. Org. Lett. **2005**, *7*, 2169.

44 Beccalli, E. M.; Contini, A.; Trimarco, P. *Eur. J. Org. Chem.* **2003**, *20*, 3976.

45 (a) Ballini, R.; Bosica, G.; Fiorini, D.; Palmieri, A. *Green Chem.* **2005**, *7,* 825.(b) Yan, M. C.; Jang, Y. J.; Kuo, W. Y.; Tu, Z.; Shen, K. H.; Cuo, T. S.; Ueng, C. H.; Yao, C. F. *Heterocycles* **2002**, *57,* 1033.

46 Rahman, A. H. A.; Hammouda, M. A. A.; Desoky, S. I. E. *Heteroatom Chem.* **2005**, *16,* 20.

47 (a) Balbi, A.; Ermili, A.; Mazzei, M.; Roma, G.; Braccio, M. D. *Farmaco* **1984**, *39,* 863.(b) Ibrahim, S. S. *Ind. Eng. Chem. Res.* **2001**, *40,* 37.

48 (a) Minami, T.; Okauchi, T.; Matsuki, H.; Nakamura, M.; Ichikawa, J.; Ishida, M. *J. Org. Chem.* **1996**, *61,* 8132.(b) Yahira, S.; Shibata, K.; Saito, T.; Okauchi, T.; Minami, T. *J. Org. Chem.* **2003**, *68,* 4947.

49 (a) Yuan, H. J.; Wang, M.; Liu, Y. J.; Liu, Q. *Adv. Synth. Catl.* **2009**, *351,* 112.(b) Bi, X; Dong, D.; Liu, Q.; Tan, J.; Li, B. *J. Am. Chem. Soc.* **2005**, *127,* 4578.(c) Liu, J.; Wang, M.; Li, B.; Liu, Q.; Zhao, Y. *J. Org. Chem.* **2007**, *72,* 4401.

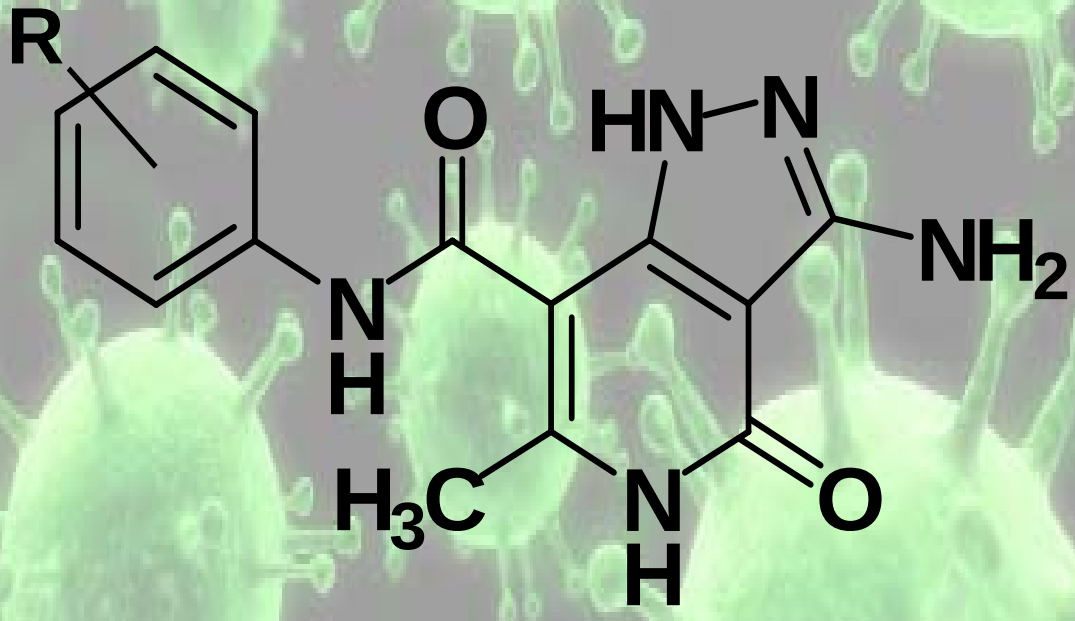
50 (a) Kumar, S.; Peruncheralathan, S.; Ila, H.; Junjappa, H. *Org. Lett.* **2008**, *10,* 965.(b) Mishra, N. C.; Panda, K.; Ila, H.; Junjappa, H. *J. Org. Chem.* **2007**, *72,* 1246.(c) Peruncheralathan, S.; Khan, T. A.; Ila, H.; Junjappa, H. *J. Org. Chem.* **2005**, *70,* 10030.(d) Roy, A.; Nandy, S.; Ila, H.; Junjappa, H. *Org. Lett.* **2001**, *3,* 229.(e) Syam Kumar, U. K.; Ila, H.; Junjappa, H. *Org. Lett.* **2001**, *3,* 4193.

51 (a) Rao, H. S. P.; Geetha, K. *Tet. Lett.* **2009**, *50,* 3836.(b) Rao, H. S. P.; Vasantham, K. *J. Org. Chem.* **2009**, *74,* 6847.(c) Rao, H. S. P.; Sivkumar, S. *J. Org. Chem.* **2006**, *71,* 8715.(d) Rao, H. S. P.; Sivakumar, S. *J. Org. Chem.* **2005**, *70,* 4524.

52 Kim, Y. W.; Mobley, J. A.; Brueggemeier, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13,* 1475.

CHAPTER 5

Synthesis, Characterization and Anti-Viral Screening of Novel 3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-aryl-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide Derivatives.



5.1 INTRODUCTION

Biaryls and heterobiaryls have attracted significant attention from the scientific community because of their relevance in medicinal chemistry. Heterobiaryls frequently can be observed in numerous bioactive small molecules, and in particular, heterobiaryls fused with various heterocycles, such as pyrazole, pyridine, and pyrimidine, have been used as key pharmacophores.[1] As shown in **Figure 1**, a blockbuster drug, sildenafil citrate (**1**),[2] and a potent anticancer agent (**2**),[3] contain heterobiaryls fused with privileged heterocycles as core skeletons. In addition, 1*H*-pyrazolo[3,4-*b*]pyridine is recognized as a privileged substructural motif of drug-like molecules and potential drugs. Compound **3**, which contains the heterobiaryl pyrazolopyridine substructure, stimulates soluble guanylate cyclase *via* a nitric oxide independent regulatory site and induces vasodilation.[4] 6-Aryl pyrazolo[3,4-*b*]pyridines are also reported as potentinhibitors of glycogen synthase kinase-3 (**4**).[5] These examples emphasize the importance of pyrazol-fused heterobiaryls, as well as pyrazolopyridines, as key pharmacophores in bioactive small molecules.

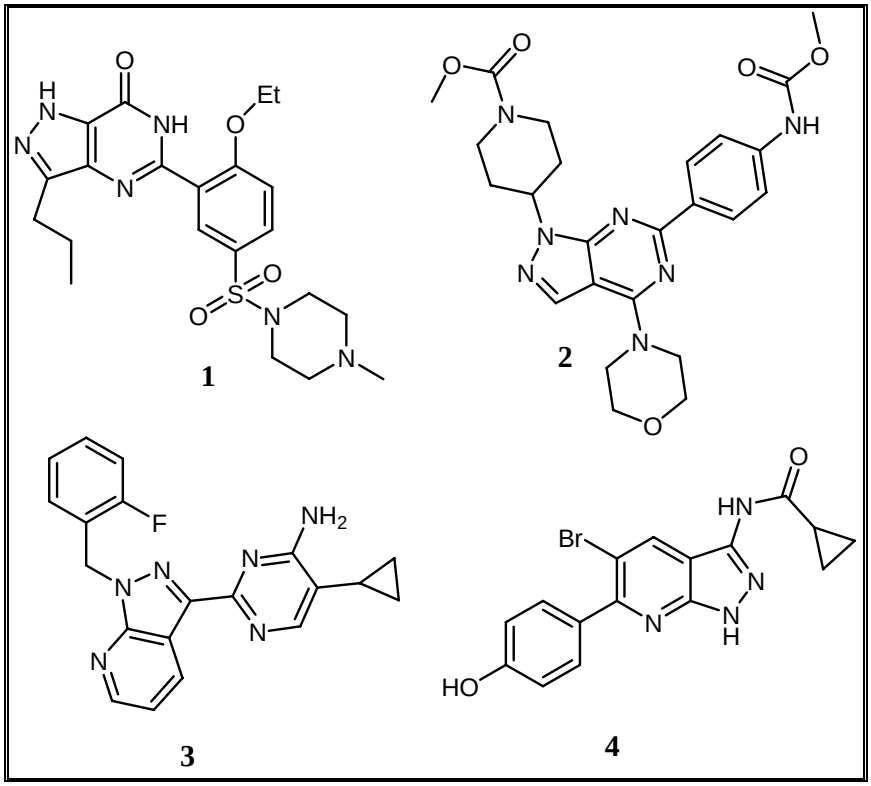
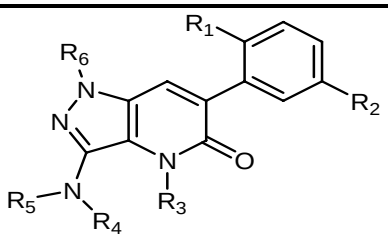


Figure 1

5.2 Biological activity of several fused pyrazolopyridine and pyrazolopyrimidine derivatives.

Several diverse biological activities have been reported for condensed polyazaaromatic ring systems which are described as below.

Mitogen-activated protein kinases (MAP) are a family of praline-directed serine/threonie kinases that activate their substrates by dual phosphorylation. The kinases are activated by a variety of signals including nutritional and osmotic stress, UV light, growth factors, endotoxin and inflammatory cytokines. One group of MAP kinases is the p38 kinase group that includes various isoforms (ex. p38α, p39β, p38γ and p38δ). The p38 kinases are responsible for phosphorylating and activating transcription factors as well as other kinases, and are activated by physical and chemical stress, pro-inflammatory cytokines and bacterial lipopolysaccharide. More importantly, the products of the p38 phosphorylation have been shown to mediate the production of inflammatory cytokines, including TNF and IL-1, and cyclooxygenae-2. Each of these cytokines has been implicated in numerous disease states and conditions. The inhibition of these cytokines by inhibition of the p38 kinases of benefit in controlling, reducing and alleviating many of this disease states. In this context, some novel substituted pyrazolopyridones (**Figure 2**) have been synthesized and found potent for the treatment of disease associated with p38 MAP kinase.[6]



R_1, R_2 = H, Me, Halo, Alkoxy, NO_2
R_3, R_4, R_5 = H, Me, Alkyl
R_6 = H, Me, Alkyl, Aryl

Figure 2

Recently, Yassin F. A.[7] has synthesized some pyrazolopyridine derivatives (**Figure 3**) and evaluated for their antimicrobial activity.

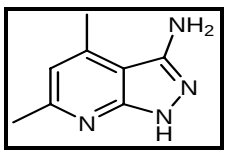


Figure 3

Echevarri A. et al[8] have developed three series of 4-anilino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic esters to study potential anti-*Leishmania* activity. These compounds were obtained by a condensation reaction of 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine with several aniline derivatives. Some of them were also obtained by an alternative pathway involving a Mannich-type reaction. They were determined the hydrophobic parameter, log *P*, by shake-flask methodology, and using the Hansch-Fujita addictive hydrophobic fragmental constants. Among them, compound (**Figure 4)** shown most promising activity (IC_{50}) 0.39 and 0.12 *t*M.

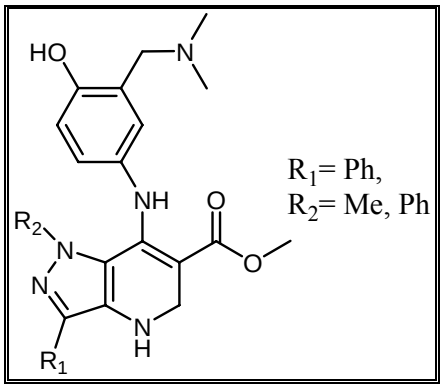


Figure 4

Green N. J. et al[9] have studied structure-activity relationship of a series of dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3-ones binding to the immune regulatory protein B7.1. The interaction of co-stimulatory molecules on T cells with B7 molecules on antigen presenting cells plays an important role in the activation of naive T cells. Consequently, agents that disrupt these interactions should have applications in treatment of transplant rejection as well as autoimmune diseases. They have identified several leads that prevented the interaction of B7.1 with CD28 with activities in the nanomolar to low micromolar range. One of these, the dihydrodipyrazolopyridinone (**Figure 5**), was subsequently shown to bind the V-like domain of human B7.1 at equimolar stoichiometry.

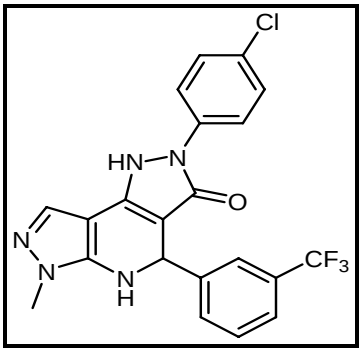
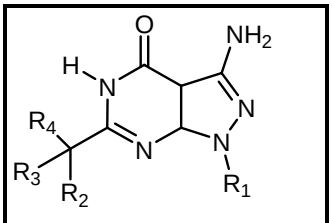


Figure 5

Phosphodiesterase 9A (PDE9A) is one member of the wide family of phosphodiesterases (PDE). These kinds of enzymes modulate the levels of the cyclic nucleotides 5'-3' cylic adenosine monophosphate (cAMP) and 5'-3' cyclic guanosine monophosphate (cGMP). These cyclic nucleotides (cAMP and cGMP) are important second messengers and therefore play a central role in cellular signal transduction cascades. Each of them reactivates inter alia, but not exclusively, protein kinases. The protein kinase activated by cAMP is called protein kinase A (PKA), and the protein kinase activated by cGMP is called protein kinase G (PKG). Activated PKA and PKG are able in turn to phosphorylate a number of cellular effector proteins. It is possible in this way for the second messengers cAMP and c GMP to control a wide variety of physiological processes in a wide variety of organs. However, the cyclic nucleotides are also able to act directly on effector molecules. Thus, it is known, for example, the cGMP is able to act directly on ion channels and thus is able to influence the cellular ion concentration. The phosphodiesterases are a control mechanism for controlling the activity of cAMP and cGMP and thus in turn for the corresponding physiological processes. Thus, several phyrazolopyrimidones (**Figure 6**) have been synthesized and found potent PDE inhibitors.[10]



R_1= H, alkyl, aryl,
R_2, R_3, R_4= Alkyl, aryl

Figure 6

Fossa P. et al[11] have synthesized substituted pyrazolopyridine and pyrazolopyrimidine derivatives and demonstrated its molecular modeling studies and pharmacological activity of selective A_1 receptor antagonists (**Figure 7**). They were applied an approach combining pharmacophore mapping, molecular alignment, and pseudoreceptor generation to derive a hypothesis of the interaction pathway between a set of A1 AR antagonists taken from a model of the putative A1 receptor. The pharmacophore model consists of seven features and represents an improvement of the N6-C8 model, generally reported as the most probable pharmacophore model for A1 AR agonists and antagonists. It was used to build up a pseudoreceptor model able to rationalize the relationships between structural properties and biological data. All the synthesized compounds were tested for their affinity toward A1, A2a, and A3 AR, showing interesting antagonistic activity and A1 selectivity.

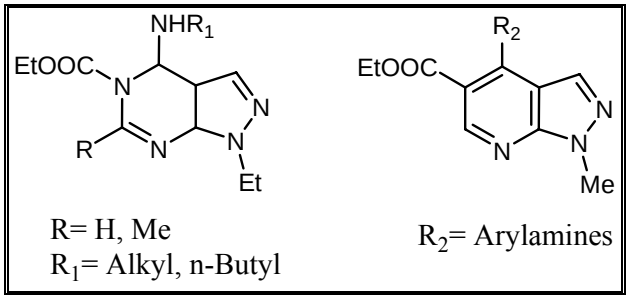


Figure 7

Moreover, pyrazolopyrimidinones and their salts are also (**Figure 8**) important heterocycles due to their application for the treatment of impotency.[12] Pyrazolopyrimidones are also useful in the treatment of such diseases and adverse conditions as angina, hypertension, congestive heart failure, reduced blood vessel patency, peripheral vascular disease, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, and gut motility (**Figure 8**).[13]

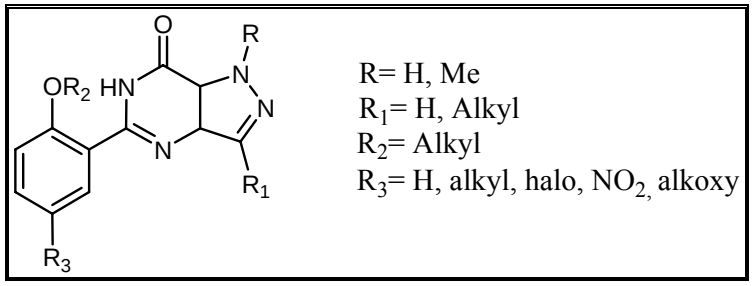


Figure 8

Das S. K. et al[14] have designed, synthesized and evaluated several dual PPAR *α*/*γ* agonists with three different heterocycles, viz. pyrazolo[4,3-*d*]pyrimidin-7-one, quinazolin-4-one and benzo[*e*][1,3]oxazine-4-one for the treatment of type 2 diabetes and associated dyslipidemia. Among them, compounds (**Figure 9)** were found to possess a potent dual PPAR *α*/*γ* agonist property. It significantly reversed diabetic hyperglycemia while improving overall lipid homeostasis in preclinical animal models.

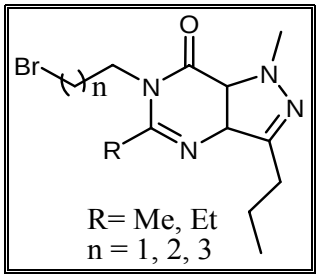


Figure 9

Voelter W. et al[15] have developed a simple high-yielding procedure for the synthesis of pyrazolopyrimidinones (**Figure 10**). They have also demonstrated its considerable utility for the production of intermediates for potential phosphodiesterase inhibitors.

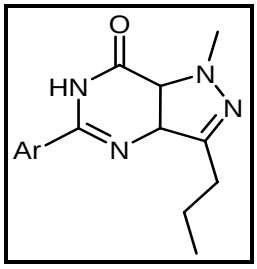


Figure 10

Dumaitre B. et al[16] have synthesized a series of 6-phenylpyrazolo[3,4-*d*]pyrimidones for inhibitors of cGMP specific (type V) phosphodiesterase. Enzymatic and cellular activity as well as *in vivo* oral antihypertensive activity is evaluated. They have found that a *n*-propoxy group at the 2-position of the phenyl ring is necessary for activity. This position can accommodate many unrelated groups. Amino derivatives were very potent but lacked metabolic stability. Substitution by carbon-linked small heterocycles provided both high levels of activity and stability. Cellular activity very often correlated with *in vivo* activity. Among the compounds, 1,3-dimethyl-6-(2-propoxy-5-methanesulfonamidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one and 1-ethyl- 3-methyl-6-(2-propoxy-5-(4-methylthiazol-2-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**Figure 11**) displayed outstanding *in vivo* activities at 5 mg/kg/os and good metabolic stabilities.

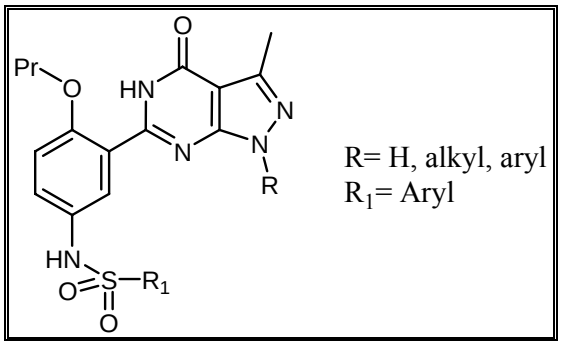


Figure 11

Synthesis of sildenafil analogues (**Figure 12**) from anacardic acid and their phosphodiesterase-5 inhibition activity have been reported by Rao S. A. and coworkers.[17] Anacardic acid (6-pentadecylsalicylic acid), a major component of cashew nut shell liquid, consists of a heterogeneous mixture of monoenes, dienes, and trienes. The enes mixture of anacardic acid was hydrogenated to a saturated compound. Using saturated anacardic acid as

a starting material, analogues of sildenafil [a potent phosphodiesterase-5 (PDE5) inhibitor and an orally active drug for the treatment of erectile dysfunction] were synthesized, to observe the effect of the pentadecyl side chain on PDE5 inhibition.

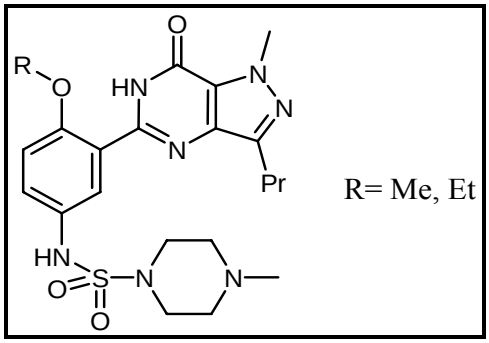


Figure 12

Magedov I. V. et al[18] have synthesized some 4-aza-2,3-didehydro podophyllotoxin analogues (**Figure 13**). They were implementing a bioisosteric replacement of the methylenedioxybenzene subunit with a pyrazole moiety to afford tetracyclic dihydropyridopyrazoles. Libraries of these structurally simple analogues were prepared by a straightforward one-step multicomponent synthesis and demonstrated to display antiproliferative properties in a number of human cancer cell lines. These new heterocycles potently induce apoptosis in cancerous Jurkat cells even after a short 24 h exposure. The ease of synthesis and encouraging biological activities make the presented library of dihydropyridopyrazoles promising new leads in anticancer drug design.

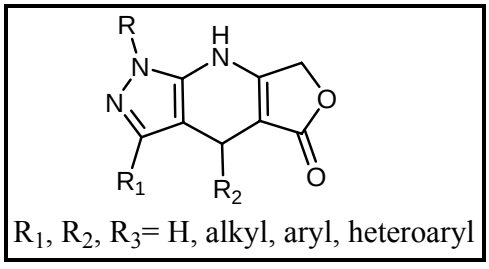


Figure 13

Claudia M. et al[19] have synthesized a series of ethyl-4-amino-1-(2-chloro-2-phenylethyl)-6-oxo-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates (**Figure 14**) as potential A1 adenosine receptor (A1 AR) ligands. Binding affinities of these compounds were determined for adenosine A1, A2A and A3 receptors. Among these, two molecules showed good affinity (Ki = 299 μM and 517 μM) and selectivity towards A1 AR, whereas some showed good affinity for A2A AR (Ki = 290 μM), higher than towards A1 AR (Ki = 1000 μM). The only

arylamino derivatives of the series displayed high affinity (Ki = 4.6 nM) and selectivity for A3 AR.

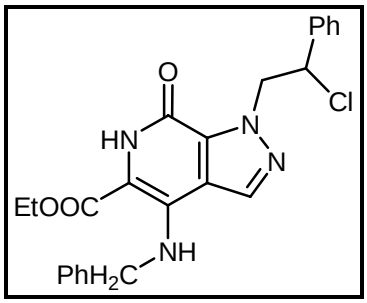


Figure 14

5.3 Various synthetic approaches for substituted pyrazolopyridines and pyrazolopyrmidines.

Condensed polyazaaromatic ring systems are present in a variety of biologically active compounds (both naturally-occurring and synthetic). Although a large number of methods for their synthesis have been documented in the literature, many of them require multistep procedures using intermediates which are not readily available. Among them, few methods are discussed here.

Adamo M. F. A. et al[20] have described the preparation of two novel heterocyclic nuclei isoxazolopyridone and pyrazolopyridone (**Figure 15**) starting from NO_2 substituted isoxazole, arylaldehydes and nitro methane. The syntheses were modular in nature and fast to execute. The title compounds were obtained pure without intervention of chromatography.

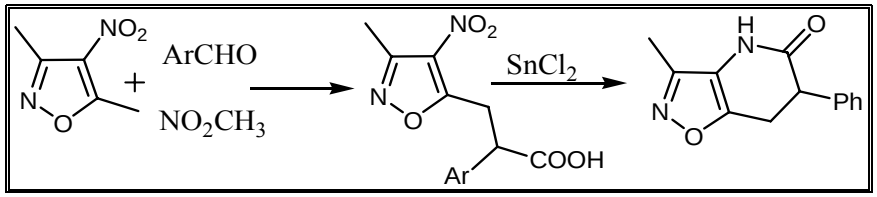


Figure 15

Sayed G. L. et al[21] have prepared bifunctional pyrazolopyridine (**2**) derivatives by the reaction of 2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**1**) with *p*-methoxybenzaldehyde, malononitrile in the presence of ammonium acetate (**Figure 16**). Further, compound **2** was used as the key intermediate to prepare the pyrazolo-pyrido-pyrimidine derivatives through its reaction with formic acid, formamide-formic acid-DMF,

ammonium thiocyanate or reaction with triethyl orthoformate followed by cyclization with hydrazine hydrate.

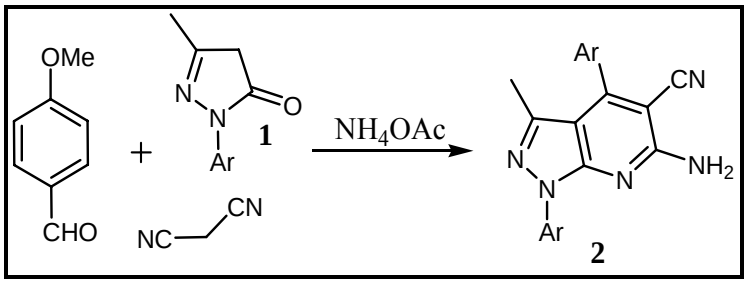


Figure 16

Attaby F. A. et al[22] have synthesized some pyrazolopyridone derivatives (**Figure 17**). The reaction of *α-β* unsaturated nitrile derivatives with *S*-methylisothiourea was afforded the propene derivatives **1**. Cyclization of **1** using ethanolic hydrochloric acid afforded the pyridine derivatives in good yields. This on reactions with hydrazine hydrate and of phenylhydrazine afforded the corresponding pyrazolopyridine derivatives **2**.

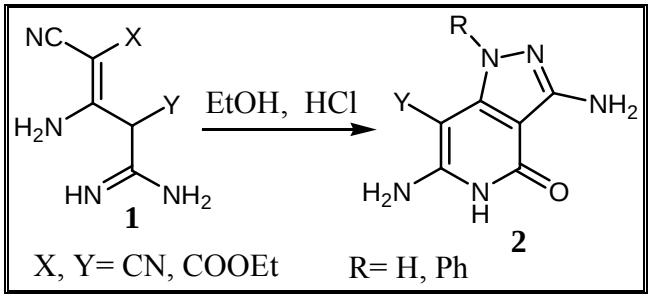


Figure 17

Junjappa H. et al[23] have developed a novel process for the synthesis of substituted *N*-methylpyrazolopyridones (**Figure 18**). The pyrazolopyridones were prepared by alkylation of the pyridones with dimethyl sulphate, followed by heating the mixture of *N*-methyl products with methyl iodide. Treatment of the pyridones (**1)** with hydrazine in refluxing propanol yielded the respective pyrazolo-[4,3-*c*]pyridone (**2)** derivatives in excellent yields.

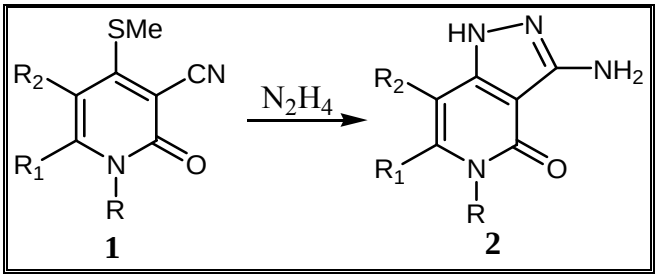


Figure 18

Moreover, a variety of novel *α*-cyanoketene *S*,*S*-acetals, were readily prepared by the reaction of cyanoacetanilides or cyanothioacetamide with carbon disulfide, followed by alkylation, react smoothly with nucleophiles to afford variously substituted methylthio derivatives of pyrazolepyridine (**Figure 19**).[24]

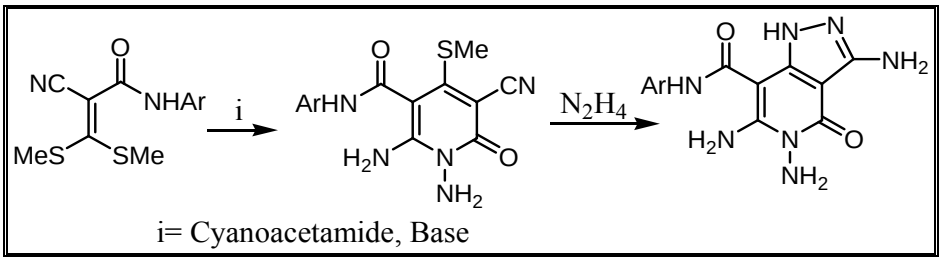


Figure 19

Additionally, a novel and efficient method for the synthesis of substituted 4-alkylthio-*N*-arylsulphonylamino-2-pyridons *via* the reaction of ketene-*S,S*-acetals with *N*-cyanoacetoarylsulfonylhydrazides has been developed by Elgemeie G. H. and coworkers.[25] The arylsulfonylamino-pyrazolo[3,4-*c*]pyridine-2(1*H*)-ones have also been prepared from the reaction of 4-alkylthio-N-arylsulfonylamino-2-pyridones with hydrazines (**Figure 20**).

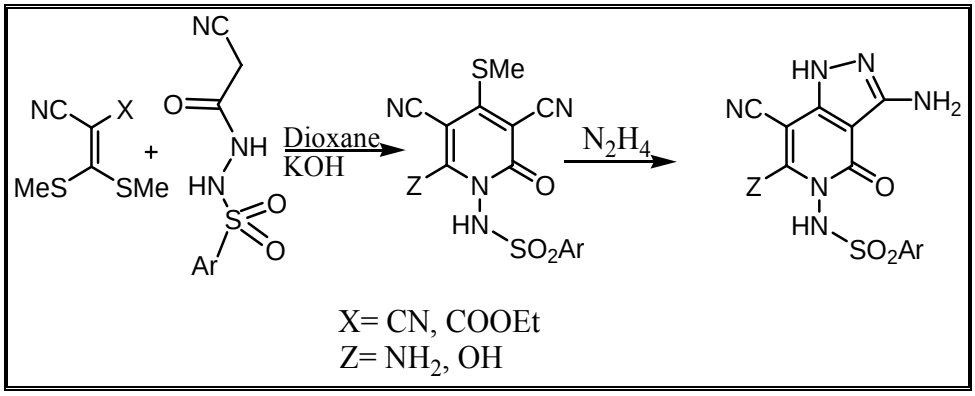


Figure 20

Lacova M. et al[26] have developed one-pot and facile preparations of 6-(2-hydroxy-5-*R*-benzoyl)-4-methyl-2-aryl-pyrazolo[3,4-*b*]pyridines (**Figure 21**), using the reaction of 3-formyl chromones **1** with 5-amino-1-aryl-pyrazoles **2**. An enamine-intermediate 2-ethyloxy-6-*R*-3-(3-methyl-1-phenylpyrazol-5-ylaminomethylene)chroman-4-one **3** was isolated at lower temperatures. They were observed that reactions under microwave irradiation proceeded significantly faster and with high yields.

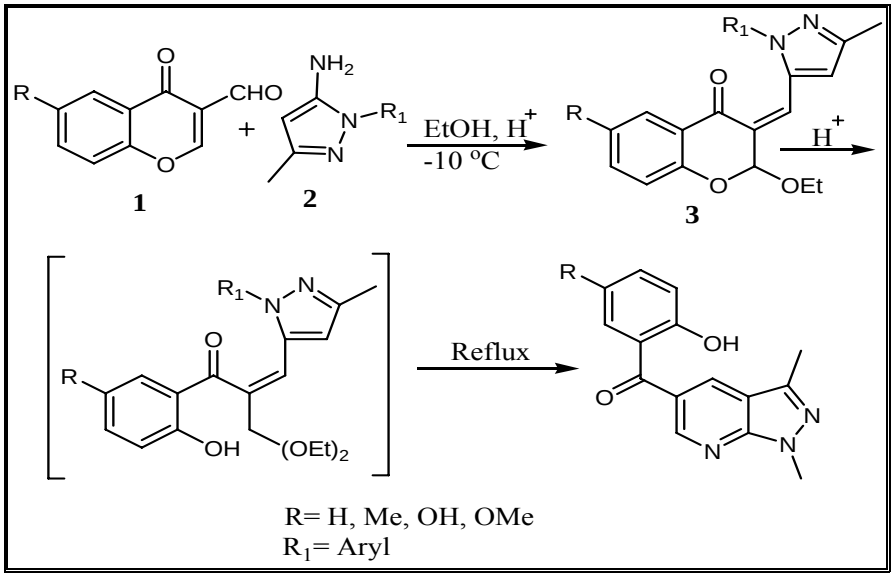


Figure 21

Abass M. has synthesized several fused pyrazolopyrimidones (**Figure 22**) with quinolone scaffold. He has described the synthesis of amino-ester, its hydrolysis and chloroacetylatio, which were utilized for the synthesis of pyrazoloyridones.[27]

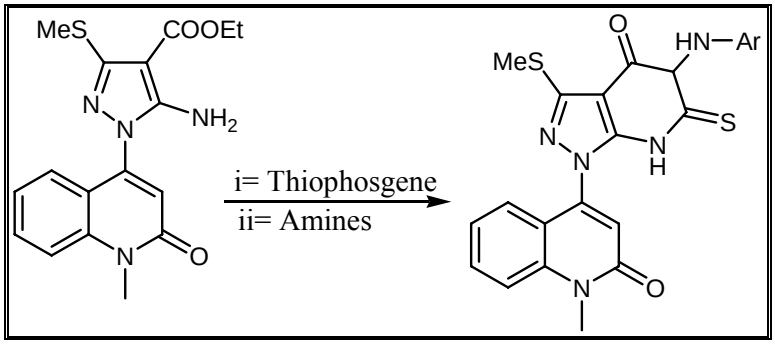


Figure 22

Hassanein E. M. et al[28] have synthesized some pyrazolopyridone derivatives *via* the reaction of compound **1** with ketene dithioacetal **2,** yielded compound **3** in good yields (**Figure 23**). Further, the reaction of **3** with hydrazine afforded pyrazolopyridones **4** in high yield.

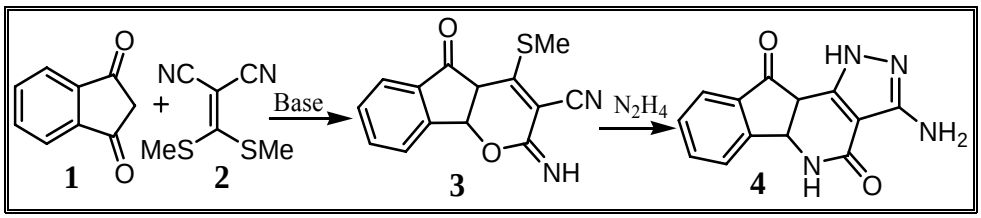


Figure 23

A mild one-step synthetic method to access privileged pyrazolearylpyrazole[3,4-*b*]pyridines (**Figure 24**) from indole-3-carboxaldehyde derivatives and a variety of aminopyrazoles has

been developed by Park S. B. and coworker.[29] This novel method constructs heterobiaryls with the wide scope of substrate generality and excellent regioselectivity *via* indole ring opening.

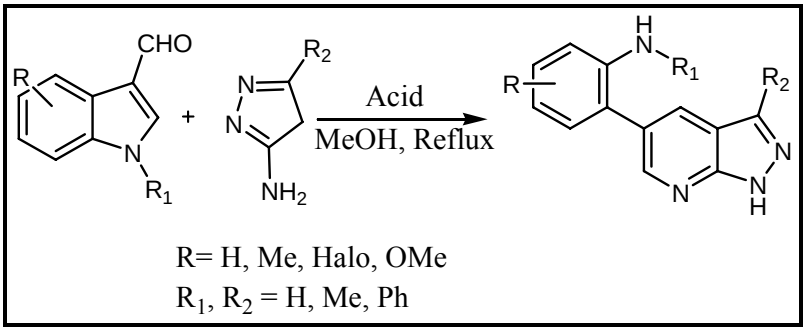


Figure 24

Rodrigues L. M. et al[30] have synthesized some pyrazolopyridine derivatives (**Figure 25**). The reaction of *N*-substituted-5-amino-4-cyanopyrazoles with malononitrile occurs with formation of 6-substituted pyrazole[3,4-*b*]pyridines respectively.

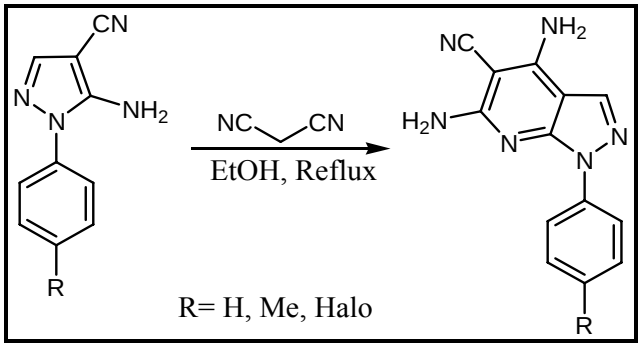


Figure 25

Recently, Yao H. et al[31] have developed a synthesis of indeno[2',1':5,6]pyrido[2,3-*d*]pyrazoles by the three-component reaction of aldehyde, 5-amino-3-methyl-1-phenyl-pyrazole and 1,3-indenedione in the presence of SDS in aqueous media (**Figure 26**).

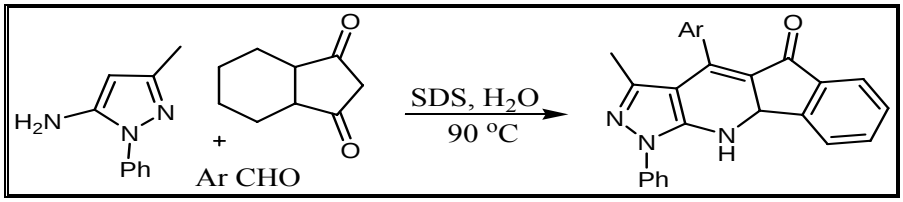


Figure 26

Li J. R. et al[32] have developed synthesis of pyrazolopyrimidinones under microwave irradiation (**Figure 27**). They have demonstrated that the direct reaction of *o*-aminopyrazocarbonitriles and carbonyl compounds afforded pyrazolopyrimidinones under microwave irradiation with high yields.

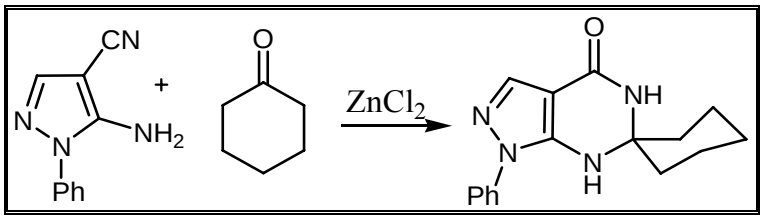


Figure 27

Mekheimer R. et al[33] have synthesized some benzoannulated pyrazolopyridones **2** by the reaction of **1** with hydrazine hydrate (**Figure 28**).

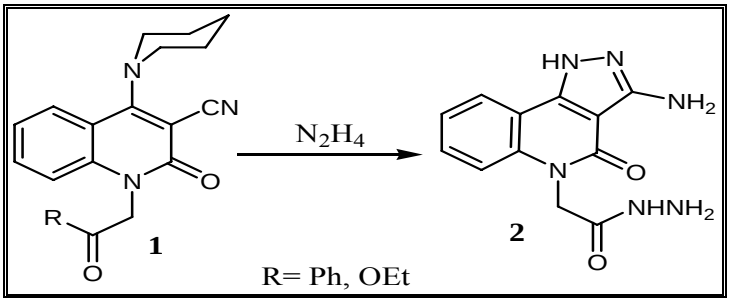


Figure 28

Swett L. R. et al[34] have synthesized two isomeric pyrazolopyridones (**Figure 29**) which were identified as their tetrahydropyrazolopyridine derivatives by the reaction of 5-amino-1,3-dimethylpyrazole with ethyl acetoacetate.

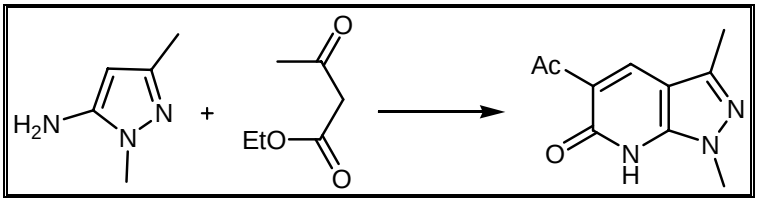


Figure 29

5.4 CURRENT RESEARCH WORK

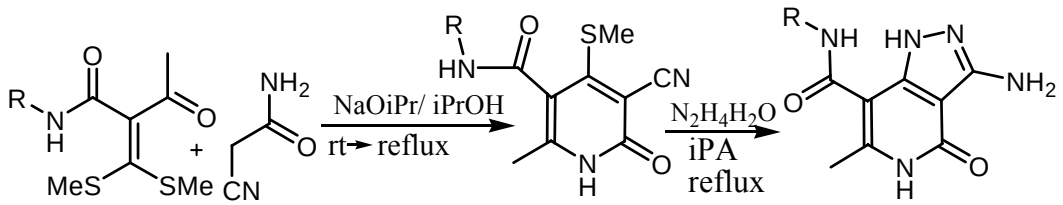
The pyrazolopyridine and pyrazolpyrimidine derivatives have considerable chemical and pharmacological importance because of a broad range of biological activities displayed by these classes of molecules. As we demonstrated, the tremendous biological potential of pyrazolopyridine derivatives encouraged us to synthesize some pyrazolopyridine derivatives. Various methodologies have been described for the synthesis of pyrazolopyridone derivatives. However, the existing methods are suffer with some drawbacks, such as; yield, time, product isolation, isomer formation.

During the course of our ongoing interest on the synthesis of various heterocyclic compounds using ketene dithioacetals, we observed that ketene dithioacetals are versatile intermediate for the synthesis of pyrazolopyridone derivatives. Thus, to synthesized target molecules, the reaction of various ketene dithioacetals with cyanoacetamide in the presence of base was afforded pyridones. Further, the pyridones on reaction with hydrazine hydrated in isopropyl alcohol furnished the novel pyrazolopyridone derivatives in excellent yields. The synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for *in vitro* anti-viral activity against HIV-1 III_B and ROD strains.

5.5 RESULTS AND DISCUSSION

Initially, the reaction of 2-(bis(methylthio)methylene)-3-oxo-*N*-phenylbutanamide (**1a)** with cyanoacetamide **2** was carried out using sodium methoxide in methanol. The reaction of **1a** with **2** in sodium methoxide was afforded the product **3a** in 75 % yield with long reaction time (Table 1). To optimize the reaction condition for the synthesis of compound **3a**, various sodium alkoxides were utilized in respective alcohol. As a result, we found the reaction of **1a** with **2** was faster and afforded the pyridone **3a** in good yield in the presence of sodium isopropoxide and isopropyl alcohol.

Scheme 1: Synthesis of substituted pyrazolopyridones using ketene dithioacetals.



1a-w **2** **3a-w** **PPMS 1-23**

Table 1: Reaction of 1a with 2 using various bases.

Entry	Base	Time h	Yield %
1	NaOMe	7	75
2	NaOEt	6	82
3	NaOiPr	4	88

The resulting pyridones **3a-w** were further reacted with hydrazine hydrate in isopropyl alcohol to afford the pyrazolopyridone derivatives in excellent yield with short reaction time. The results are gathered in table 2. The synthesized compounds were confirmed by IR, Mass, ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their *in vitro* anti-viral activity using HIV-1 III_B and ROD strains.

Table 2: Synthesis of various pyrazolpyridones PPMS 1-23.

Entry	R	Time min	Yield %	mp ^{o}C
PPMS-1	Ph	50	96	312-314
PPMS-2	4-CH_3Ph	65	95	> 320
PPMS-3	4-OCH_3Ph	40	96	> 320
PPMS-4	4-FPh	45	97	> 320
PPMS-5	2-OCH_3Ph	55	88	> 320
PPMS-6	2-CH_3	65	89	> 320
PPMS-7	4-ClPh	60	91	> 320
PPMS-8	4-EtPh	45	93	> 320
PPMS-9	4-NO_2Ph	50	95	> 320
PPMS-10	3-Cl,4-FPh	60	85	> 320
PPMS-11	5-Cl,2-OCH_3Ph	65	87	> 320
PPMS-12	2,5-diClPh	70	88	> 320
PPMS-13	2,5-diCH_3Ph	60	89	> 320
PPMS-14	4-Cl,2-CH_3Ph	70	83	> 320
PPMS-15	3,4-diFPh	75	92	> 320
PPMS-16	2-ClPh	75	84	> 320
PPMS-17	2-FPh	60	85	> 320
PPMS-18	4-BrPh	55	92	> 320
PPMS-19	3,4-diClPh	60	94	> 320
PPMS-20	3-NO_2Ph	55	84	> 320
PPMS-21	3-CH_3Ph	65	88	> 320
PPMS-22	2,3-diCH_3Ph	65	89	> 320
PPMS-23	2-OCH_3,4-NO_2Ph	70	91	> 320

In mechanism, the cyanoacetamide on the treatment with base generate an anion at active methylene group which attack on β carbon of ketene dithioacetal. The amine nucleophile attack on carbonyl carbon and form sodium salt of pyridine moiety by removal of methylthio and water molecule. The sodium salt on acidification affords pyridone. The binucleophile hydrazine hydrate on reaction with pyridone form pyrazolopyridone.

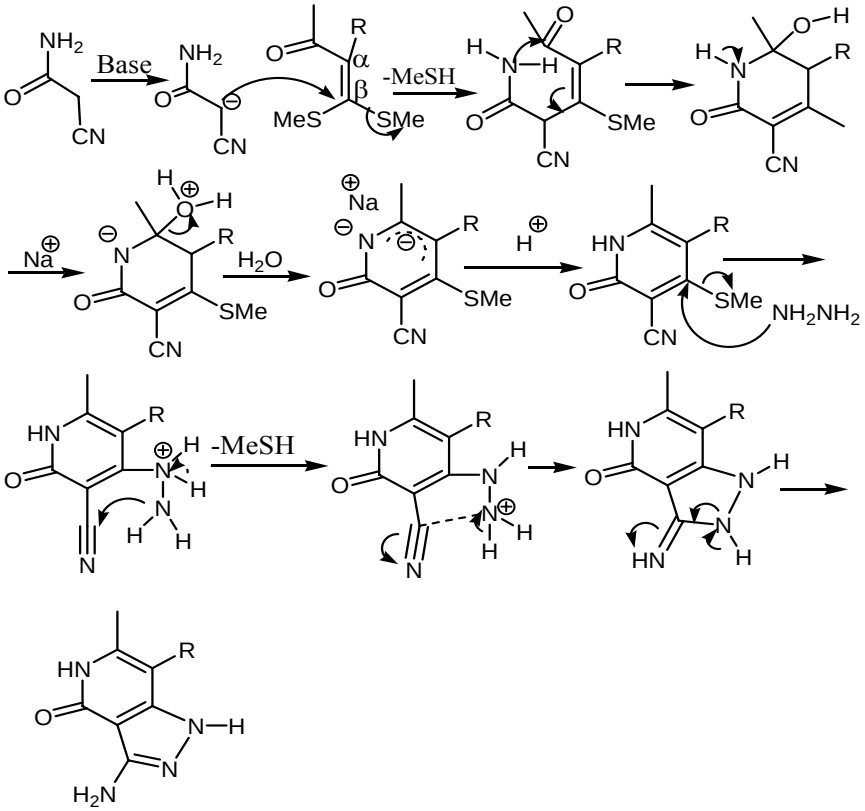


Figure 30: Proposed mechanism for the formation of pyrazolopyridone.

5.6 ANTI-VIRAL SCEREENING

Antiviral activity was examined by Tetrazolim-based colorimetric (MTT) assay[35] using the human T-lymphotropic virus type I (HTLV-I)-transformed MT-4 cell line7. The method is based on HIV-induced cytopathogenic effect (CPE) and measures the degree of cell killing on HIV infection. The replication of HIV in MT-4 cells was monitored 5 days after infection. Stock solutions of compounds were made in DMSO (generally at 10 mg/ ml). Final DMSO concentration in the test should not exceed 1% (v/v). Nevirapine was used as reference standard.

➢ Materials and reagents

- 'Complete medium': RPMI-1640 medium with 20 mM HEPES buffer, supplemented with 10% (v/v) heat-inactivated FCS, 2 mM L-glutamine, 0.1% sodium bicarbonate and 20 μg/ml gentamicin.
- Virus stock (see Steps 1–13)
- A solution of 30 ml Triton X-100 and 2 ml methanesulfonic acid or 2 ml concentrated hydrochloric acid in 500 ml isopropanol.

➢ Equipments

- Titertek multidrop dispenser (ThermoFisher Scientific)
- Biomek 3000 robot (Beckman)
- Microplate washer (Biotek EL404, Beun-de Ronde and Serlabo)
- Multiskan Ascent reader (ThermoFisher Scientific)
- An invert light microscope (magnification: ocular ×10 and objective × 20)

➢ Reagent setup

MTT 7.5mg/ml of MTT in PBS was prepared as follows: put 10 g MTT (powder) in a 2-liter bottle, add 1.333 ml of PBS and cover the bottle with tin foil (to protect against light). Sonicated it until MTT was dissolved. The solution was filtered over an easy flow filter (0.22 mm cellulose acetate membrane for tissue culture applications) under reduced pressure and stored it in dark-brown plastic bottles (±150 ml). The bottles of cleared MTT were placed inside a -20 ^{o}C freezer.

(**Note**: Do not fill the bottles completely as the aqueous solution will expand during the freezing process)

- **Preparation of the stock solution of test compounds**

DMSO, water, buffer solutions (e.g., PBS) and, a mixture of these solvents were used as solvents to make homogeneous aliquots (solution, suspension or emulsion) of the test compounds.

- **Growing virus stock**

1| The quality of the cells was examined by microscopically.

2| Placed the 300,000 number of cells per ml of final culture in a 50-ml tube.

3| PBS was added to the tube to obtain a final volume of 40 ml.

4| Pellet the cells by centrifugation (5 min, 220g at room temperature).

5| The cells were resuspended by adding the required volume of an adequate culture in the complete medium.

6| The required volume of virus stock was added to the cell suspension.

7| The treated cells were transferred in an appropriate culture flask.

8| Incubated at 37 ^{o}C, 5% CO_2 (≥95% relative humidity (RH)).

9| The cell culture was inspected microscopically everyday for the presence of cytopathogenic effect.

10| The content of the culture flask was transferred to a 50-ml tube.

11| The cells were pellet by centrifugation (5 min, 220g).

12| Carefully labeled the required number of cryotubes.

13| Dispensed the supernatant (virus stock) in aliquots of 0.5 or 1 ml in cryotubes.

- **Titration of virus stock**

14| The infected MT-4 cells were cultivated in a humidified atmosphere (≥95% RH) at 37 ^{o}C and 5% CO_2 in air. Subcultivated the cells every 3 to 4 d. Seed the cells at 6×10^5 cells per ml before starting the experiment.

15| 96-well microtiter plates were filled with 100 ml of complete medium.

16| Virus stock was added in 25-µl volume to the six middle cups (2B–G) of the microtiter plate. The actual volume in the cups 2B–G was 125 µl.

17| The wells 2B–G were diluted using the Biomek 3000 robot or a multichannel pipette.

18| The exponentially growing MT-4 cells were centrifuged for 5 min at 220g and discarded the supernatant by pouring into bleach solution.

19| Resuspended the cells at 6×10^5 cells per ml in complete medium in a flask that was connected to an autoclaved dispensing cassette of a Titertek multidrop dispenser.

20| Dispensed 50 ml of cell suspension to the microtiter plate wells.

21| The microtiter plates were incubated in a humidified atmosphere (≥95% RH) at 37 ^{o}C and 5% CO_2 in air for 5 d.

22| After the 5-d incubation, the cells were examined by microscopically for eventual HIV-induced CPE. A well was scored positive if any trace of CPE was observed. The 50% cell culture infective dose ($CCID_{50}$) value was calculated using the Reed and Muench method[36]. The calculation is as follows: M ¼ inv log{x1 + [(x2 _ x1)((y1 _ 50) / y1 _ y2)]} where y1 ¼ percent of wells scored positive closest to, but higher than, 50% at a certain virus dilution, y2 ¼ percent of wells scored positive closest to, but lower than, 50% at a certain virus dilution, x1 ¼ the log(dilution of the virus where y1 was observed), x2 ¼ the log(dilution of the virus where y2 was observed) and M ¼ dilution of virus stock for 1 $CCID_{50}$

- **Assessing the anti-HIV activity and cytotoxicity of compounds**

23| The MT-4 cells were cultivated in a humidified atmosphere (≥95% RH) at 37 ^{o}C and 5% CO_2. Subcultivated the cells every 3–4 d, seeding at 6×10^5 cells per ml.

24| 96-well microtiter plates were filled with 100 ml of complete medium.

25| The stock solutions of compounds were added in 25-ml volumes to the six middle cups of the second column of the microtiter plate (2B–G). The actual volume in the cups 2B–G was 125 µl.

26| 50 ml of HIV was added at 100–300 $CCID_{50}$ and medium, respectively.

27| The exponentially growing MT-4 cells were centrifuged for 5 min (220g) and discarded the supernatant.

28| Resuspended the MT-4 cells at 6×10^5 cells per ml in complete medium in a flask which was connected with an autoclavable dispensing cassette of a Titertek multidrop.

29| 50 ml of cell suspension was dispensed to the microtiter plate wells.

30| The plates were incubated at 37 ^{o}C in a humidified atmosphere of 5% CO_2 in air.

31| Five days after infection, the viability of HIV and mock infected cells was examined spectrophotometrically by the MTT method.

- **MTT assay**

32| The 20 ml of MTT (7.5 mg/ml) solution (warmed to 37 ^{o}C) was added to each well of the microtiter plates using the Titertek multidrop dispenser.

33| The trays were incubated at 37 ^{o}C in a CO_2 incubator for 1 h.

34| A constant volume of medium was removed (e.g., 150 ml) from each cup using a multichannel pipette or a microplate washer (Biotek EL404, a double, aspiration and

dispensing, 96-channel washer) without disturbing the MT-4 cell clusters containing the formazan crystals.

35| Lyse the cells (and infectious virus) and solubilize the formazan crystals by adding 100 ml of the acidified Triton X-100 isopropanol solution to each cup using the microplate washer (or a multichannel pipette).

36| The formazan crystals were completely dissolved by placing the plates on a vibrating platform shaker for 10 min.

37| The absorbance was examined in an eight-channel computer-controlled Multiskan Ascent reader and stacker at two wavelengths (540 and 690 nm). Subtract the absorbance measured at 690 nm from the absorbance at 540 nm to eliminate the effects of scattering by cell debris.

38| Calculated the 50% cytotoxic concentration (CC_{50}) and 50% inhibitory concentration (IC_{50}); CC_{50} is the concentration of compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%.

Calculated the protection achieved by the compounds in HIV-infected cells using the formula: $\mathbf{((OD_T)_{HIV} - (OD_C)_{HIV}) / ((OD_C)_{mock} - (OD_C)_{HIV}) \times 100}$.

where $(OD_T)_{HIV}$ is the OD measured with a given concentration of the test compound in the HIV-infected cells; $(OD_C)_{HIV}$ is the OD measured for the control untreated, HIV-infected cells, which stands for 100% infection-related CPE; and $(OD_C)_{mock}$ is the OD measured for the control untreated, mock-infected cells, which stands for 0% infection-related CPE. The concentration achieving 50% protection according to the above formula is defined as IC_{50}.

The OD ratio, defined as $(OD_C)_{mock}/(OD_C)_{HIV}$, should be at least 5. The OD ratio is low when either the cells are not in optimal condition or when not enough virus is added.

Table 3: The *in-vitro* anti-viral activity against HIV-1 III_B and ROD strains using MTT method.

Code Name	Strain	Exp.no.	IC_{50}(µg/ml)	CC_50(µg/ml)	SI	Max Prot (%)	Appr.	Average IC_{50}(µg/ml)	Average CC_{50}(µg/ml)	SD	SI	Remarks
PPMS-01	III_B	P3.4842	>101	=101	<1	8	1					
		P3.4848	>125	>125	X1	0	1	>94.70	≥94.70		≤1	
	ROD	P3.4843	>94.7	=94.7	<1	9	1					
		P3.4849	>125	>125	X1	0	1	>94.70	≥94.70		≤1	
PPMS-02	III_B	P3.4842	>125	>125	X1	12	1					Cryst.
		P3.4848	>125	>125	X1	9	1	>125.00	>125.00		X1	observ. at
	ROD	P3.4843	>125	>125	X1	4	1					125 µg/ml
		P3.4849	>125	>125	X1	1	1	>125.00	>125.00		X1	
PPMS-03	III_B	P3.4842	>88.8	=88.8	<1	11	1			19.78		Cryst.
		P3.4848	>113	=113	<1	0	1	>103.93	91.87		<1	observ. at
	ROD	P3.4843	>73.8	=73.8	<1	2	1			19.78		125 µg/ml
		P3.4849	>125	>125	X1	0	1	>103.93	91.87		<1	
PPMS-04	III_B	P3.4842	>89	=89	<1	8	1			18.27		Cryst.
		P3.4848	>107	=107	<1	4	1	>100.98	100.98		<1	observ. at
	ROD	P3.4843	>83.9	=83.9	<1	6	1			18.27		25 µg/ml
		P3.4849	>124	=124	<1	14	1	>100.98	100.98		<1	
PPMS-05	III_B	P3.4842	>125	>125	X1	16	1					Cryst.
		P3.4848	>125	>125	X1	2	1	>125.00	>125.00		X1	observ. at
	ROD	P3.4843	>125	>125	X1	6	1					125 µg/ml
		P3.4849	>125	>125	X1	0	1	>125.00	>125.00		X1	

PPMS-06	III_B	P3.4842	>83.3	=83.3	<1	12	1			6.76		Cryst.
		P3.4848	>92.7	=92.7	<1	0	1	>92.73	92.73		<1	observ. at
	ROD	P3.4843	>96.1	=96.1	<1	11	1			6.76		125 µg/ml
		P3.4849	>98.8	=98.8	<1	10	1	>92.73	92.73		<1	
PPMS-07	III_B	P3.4842	>92.6	=92.6	<1	8	1			9.80		
		P3.4848	>110	=110	<1	0	1	>109.50	98.70		<1	
	ROD	P3.4843	>93.5	=93.5	<1	6	1			9.80		
		P3.4849	>125	>125	X1	1	1	>109.50	98.70		<1	
PPMS-08	III_B	P3.4842	>125	>125	X1	10	1					Cryst.
		P3.4848	>125	>125	X1	10	1	>125.00	>125.00		X1	observ. at
	ROD	P3.4843	>125	>125	X1	3	1					25 µg/ml
		P3.4849	>125	>125	X1	5	1	>125.00	>125.00		X1	
PPMS-09	III_B	P3.4842	>56.8	=56.8	<1	8	1			23.60		Cryst.
		P3.4848	>103	=103	<1	0	1	>99.83	77.10		<1	observ. at
	ROD	P3.4843	>71.5	=71.5	<1	7	1			23.60		25 µg/ml
		P3.4849	>125	>125	X1	3	1	>99.83	77.10		<1	
PPMS-10	III_B	P3.4842	>31.4	=31.4	<1	11	1			15.42		Cryst.
		P3.4848	>57.2	=57.2	<1	6	1	>36.30	36.30		<1	observ. at
	ROD	P3.4843	>20.4	=20.4	<1	19	1			15.42		125 µg/ml
		P3.4849	>36.2	=36.2	<1	23	1	>36.30	36.30		<1	
MMS-11	III_B	P3.4842	>75.5	=75.5	<1	9	1			9.92		
		P3.4848	>73.1	=73.1	<1	3	1	>69.60	69.60		<1	
	ROD	P3.4843	>54.8	=54.8	<1	6	1			9.92		
		P3.4849	>75	=75	<1	5	1	>69.60	69.60		<1	
PPMS-12	III_B	P3.4842	>51.1	=51.1	<1	8	1			15.02		Cryst.

		P3.4848	>68	=68	<1	0	1	>66.95	66.95		<1	observ. at
	ROD	P3.4843	>61.8	=61.8	<1	7	1			15.02		125 µg/ml
		P3.4849	>86.9	=86.9	<1	13	1	>66.95	66.95		<1	
PPMS-13	III_B	P3.4842	>55.6	=55.6	<1	10	1			7.46		
		P3.4848	>63.1	=63.1	<1	0	1	>58.55	58.55		<1	
	ROD	P3.4843	>49.5	=49.5	<1	3	1			7.46		
		P3.4849	>66	=66	<1	2	1	>58.55	58.55		<1	
PPMS-14	III_B	P3.4842	>75	=75	<1	5	1			6.12		Cryst.
		P3.4848	>68.6	=68.6	<1	5	1	>68.65	68.65		<1	observ. at
	ROD	P3.4843	>60.4	=60.4	<1	2	1			6.12		25 µg/ml
		P3.4849	>70.6	=70.6	<1	3	1	>68.65	68.65		<1	
PPMS-15	III_B	P3.4842	>100	=100	<1	16	1			1.67		
		P3.4848	>100	=100	<1	0	1	>99.03	99.03		<1	
	ROD	P3.4843	>97.1	=97.1	<1	27	1			1.67		
		P3.4849	>125	>125	X1	24	1	>99.03	99.03		<1	
PPMS-16	III_B	P3.4842	>58.7	=58.7	<1	11	1			8.99		Cryst.
		P3.4848	>65.9	=65.9	<1	7	1	>62.65	62.65		<1	observ. at
	ROD	P3.4843	>52.6	=52.6	<1	3	1			8.99		25 µg/ml
		P3.4849	>73.4	=73.4	<1	13	1	>62.65	62.65		<1	
PPMS-17	III_B	P3.4842	>98.9	=98.9	<1	8	1			8.55		Cryst.
		P3.4848	>101	=101	<1	0	1	>92.88	92.88		<1	observ. at
	ROD	P3.4843	>82.9	=82.9	<1	15	1			8.55		25 µg/ml
		P3.4849	>88.7	=88.7	<1	0	1	>92.88	92.88		<1	
PPMS-18	III_B	P3.4842	>116	=116	<1	13	1			11.82		Cryst.
		P3.4848	>94.6	=94.6	<1	9	1	>108.20	108.20		<1	observ. at

	ROD	P3.4843	>114	=114	<1	7	1			11.82	5 µg/ml
		P3.4849	>125	>125	X1	13	1	>108.20	108.20	<1	
PPMS-19	III_B	P3.4842	>78.1	=78.1	<1	14	1			6.53	
		P3.4848	>74	=74	<1	0	1	>71.08	71.08	<1	
	ROD	P3.4843	>62.9	=62.9	<1	2	1			6.53	
		P3.4849	>69.3	=69.3	<1	2	1	>71.08	71.08	<1	
PPMS-20	III_B	P3.4842	>106	=106	<1	5	1				Cryst.
		P3.4848	>125	>125	X1	7	1	>106.00	≥106.00	≤1	observ. at
	ROD	P3.4843	>106	=106	<1	7	1				25 µg/ml
		P3.4849	>125	>125	X1	4	1	>106.00	≥106.00	≤1	
PPMS-21	III_B	P3.4842	>80.1	=80.1	<1	6	1			5.85	
		P3.4848	>67.2	=67.2	<1	0	1	>72.05	72.05	<1	
	ROD	P3.4843	>68.3	=68.3	<1	2	1			5.85	
		P3.4849	>72.6	=72.6	<1	3	1	>72.05	72.05	<1	
PPMS-22	III_B	P3.4842	>50.8	=50.8	<1	8	1			11.09	
		P3.4848	>67.7	=67.7	<1	6	1	>57.85	57.85	<1	
	ROD	P3.4843	>46	=46	<1	7	1			11.09	
		P3.4849	>66.9	=66.9	<1	10	1	>57.85	57.85	<1	
PPMS-23	III_B	P3.4844	>116	=116	<1	5	1			21.57	Cryst.
		P3.4848	>86.7	=86.7	<1	0	1	>102.20	102.20	<1	observ. at
	ROD	P3.4845	>125	=125	<1	8	1			21.57	25 µg/ml
		P3.4849	>81.1	=81.1	<1	5	1	>102.20	102.20	<1	

5.7 CONCLUSION

In summary, we have described the synthesis substituted pyrazolopyridone derivatives in excellent yields. The reaction of various ketene dithioacetals with cyanoacetamide was afforded the pyridone derivatives with good yields in the presence of base. Sodium isopropoxide was found as an efficient base for the synthesis of pyridones. The pyridones were further reacted with hydrazine hydrate to furnished pyrazolopyridones in excellent yields with short reaction time. Unfortunately, the synthesized compounds were found inactive against HIV-1 III_B and ROD strains.

5.8 EXPERIMENTAL SECTION

The solvents and chemicals were analytical grade. Analytical thin layer chromatography (TLC) was performed on 0.2-mm precoated plates of Silica Gel 60 F_{254} precoated plates. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO, and TMS was used as an internal reference on a Bruker AVANCE II spectrometer. Mass spectra were determined using direct inlet probe on a GCMSQP2010 mass spectrometer. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. Melting points were measured in open capillaries and are uncorrected.

- **General procedure for the synthesis of pyridones 3a-w.**

To a well stirred mixture of cyanoacetamide (10 mmol) and sodium isopropoxide (10 mmol) in isopropyl alcohol was added the solution of ketene dithioacetals **1a-w** (10 mmol) in isopropyl alcohol within 10-15 min. The resulting reaction mixture was further stirred at rt for 15 min. Then, reflux the reaction mixtures for 4-5 h on water bath. After completion of the reaction, the solvent was evaporated under *vacuuo* and the resulting solid was treated with dilute HCl solution. Thus, the obtained solid was filtered, wash with water and dried at rt to afford analytically pure products. The solid products were used for next step without further purification.

- **General procedure for the synthesis of pyrazolopyridones PPMS 1-23.**

The mixture of substituted pyridones **3a-w** (5 mmol) and hydrazine hydrate (10mmol) in isopropyl alcohol was refluxed for appropriate time on water bath (Table 2). After completion of the reaction, solid product was appeared in the reaction. Cool the reaction mixture upto rt and filter the separated product washed with iPA and dried at rt to furnished analytically pure products.

3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-phenyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-1): Creamish solid; IR (KBr): 3367, 3138, 2897, 1667, 1494, 1342, 1159, 854 cm^{-1}; ^{1}H NMR: δ 2.59 (s, 3H, CH_3), 5.60 (s, 2H, NH_2), 7.06-7.71 (m, 5H, ArH), 9.93 (s, 1H, NH), 11.15 (s, 1H, NH), 11.68 (s, 1H, NH); ^{13}C NMR: δ 18.60, 94.71, 110.01, 119.45, 123.07, 125.62, 128.76, 139.20, 148.51, 159.93, 163.53; MS (*m/z*): 283 (M^+); Anal. Calcd for $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72; Found: C, 59.29; H, 4.54; N, 24.64.

3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-*p*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-2): Creamish solid; IR (KBr): 3425, 3369, 2899, 1664, 1506, 1354, 1157, 839 cm^{-1}; ^1H NMR: δ 2.22 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 5.16 (s, 2H, NH_2), 7.17 (d, J = 8.16, 2H, ArH), 7.51 (d, J = 7.4, 2H, ArH), 10.16 (s, 1H, NH), 11.02 (s, 1H, NH), 11.78 (s, 1H, NH); MS (*m/z*): 297 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56; Found: C, 60.49; H, 5.01; N, 23.49.

3-amino-4,5-dihydro-*N*-(4-methoxyphenyl)-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-3): Creamish solid; IR (KBr): 3379, 3024, 2902, 1651, 1516, 1332, 1166, 954 cm^{-1}; ^1H NMR: δ 2.58 (s, 3H, NH), 3.78 (s, 3H, OCH_3), 5.78 (s, 2H, NH_2), 6.85 (d, J = 8.92, 2H, ArH), 7.57 (d, J = 8.88, 2H, ArH), 10.39 (s, 1H, NH), 11.00 (s, 1H, NH), 11.94 (s, 1H, NH); ^{13}C NMR: δ 14.49, 55.14, 95.11, 113.83, 121.10, 132.29, 148.51, 155.36, 159.85, 164.93; MS (*m/z*): 313 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35; Found: C, 57.41; H, 4.74; N, 22.29.

3-amino-*N*-(4-fluorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-4): Creamish solid; IR (KBr): 3347, 3104, 2842, 1681, 1496, 1331, 1152, 857 cm^{-1}; ^1H NMR: δ 2.54 (s, 3H, CH_3), 5.18 (s, 2H, NH_2), 7.14 (d, J = 8.92, 2H, ArH), 7.66 (d, J = 8.12, 2H. ArH), 10.24 (s, 1H, NH), 11.07 (s, 1H, NH), 11.82 (s, 1H, NH); MS (*m/z*): 301 (M^+); Anal. Calcd for $C_{14}H_{12}FN_5O_2$: C, 55.81; H, 4.01; N, 23.25; Found: C, 55.74; H, 3.94; N, 23.19.

3-amino-4,5-dihydro-*N*-(2-methoxyphenyl)-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-5): Creamish solid; IR (KBr): 3309, 3014, 2908, 1671, 1563, 1287, 1246, 927 cm^{-1}; MS (*m/z*): 313 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_3$: C, 57.50; H, 4.83; N, 22.35; Found: C, 57.43; H, 4.73; N, 22.26.

3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-*o*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-6): Creamish solid; IR (KBr): 3406, 3115, 2902, 1660, 1514, 1280, 1178, 952 cm^{-1}; MS (*m/z*): 297 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56; Found: C, 60.51; H, 5.02; N, 23.48.

3-amino-*N*-(4-chlorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-7): Creamish solid; IR (KBr): 3330, 3142, 2830, 1688, 1489, 1241, 1118, 827 cm^{-1}; MS (*m/z*): 317 (M^+); Anal. Calcd for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.81; N, 22.04; Found: C, 52.84; H, 3.74; N, 21.96.

3-amino-*N*-(4-ethylphenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-8): Creamish solid; IR (KBr): 3324, 3143, 2812, 1675, 1532, 1298, 1114, 897 cm^{-1}; MS (*m/z*): 311 (M^+); Anal. Calcd for $C_{16}H_{17}N_5O_2$: C, 61.72; H, 5.50; N, 22.49; Found: C, 61.64; H, 5.44; N, 22.41.

3-amino-4,5-dihydro-6-methyl-*N*-(4-nitrophenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-9): Creamish solid; IR (KBr): 3369, 3020, 2897, 1685, 1573, 1494, 1157, 854 cm^{-1}; MS (*m/z*): 328 (M^+); Anal. Calcd for $C_{14}H_{12}N_6O_4$: C, 51.22; H, 3.68; N, 25.60; Found: C, 51.19; H, 3.61; N, 25.49.

3-amino-*N*-(3-chloro-4-fluorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-10): Creamish solid; IR (KBr): 3341, 3158, 2957, 1687, 1471, 1282, 1206, 956 cm^{-1}; MS (*m/z*): 335 (M^+); Anal. Calcd for $C_{14}H_{11}ClFN_5O_2$: C, 50.09; H, 3.30; N, 20.86; Found: C, 50.01; H, 3.21; N, 20.81.

3-amino-*N*-(5-chloro-2-methoxyphenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-11): Creamish solid; IR (KBr): 3317, 3142, 2870, 1671, 1418, 1266, 1107, 897 cm^{-1}; MS (*m/z*): 347 (M^+); Anal. Calcd for $C_{15}H_{14}ClN_5O_3$: C, 51.81; H, 4.06; N, 20.14; Found: C, 51.76; H, 3.94; N, 20.09.

3-amino-*N*-(2,5-dichlorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-12): Creamish solid; IR (KBr): 3347, 3031, 2810, 1681, 1546, 1322, 1183, 974 cm^{-1}; MS (*m/z*): 352 (M^+); Anal. Calcd for $C_{14}H_{11}Cl_2N_5O_2$: C, 47.75; H, 3.15; N, 19.89; Found: C, 47.69; H, 3.04; N, 19.81.

3-amino-4,5-dihydro-6-methyl-*N*-(2,5-dimethylphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-13): Creamish solid; IR (KBr): 3358, 3108, 2920, 1698, 1543, 1248, 1198, 854 cm^{-1}; MS (*m/z*): 311 (M^+); Anal. Calcd for $C_{16}H_{17}N_5O_2$: C, 61.72; H, 5.50; N, 22.49; Found: C, 61.62; H, 5.45; N, 22.40.

3-amino-*N*-(4-chloro-2-methylphenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-14): Creamish solid; IR (KBr): 3342, 3017, 2902, 1616, 1471, 1236, 1087, 874 cm^{-1}; MS (*m/z*): 331 (M^+); Anal. Calcd for $C_{15}H_{14}ClN_5O_2$: C, 54.30; H, 4.25; N, 21.11; Found: C, 54.14; H, 4.18; N, 21.08.

3-amino-*N*-(3,4-difluorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-15): Creamish solid; IR (KBr): 3369, 3124, 2837, 1685, 1426, 1212, 1106, 854 cm^{-1}; MS (*m/z*): 319 (M^+); Anal. Calcd for $C_{14}H_{11}F_2N_5O_2$: C, 52.67; H, 3.47; N, 21.94; Found: C, 52.59; H, 3.41; N, 21.87.

3-amino-*N*-(2-chlorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-16): Creamish solid; IR (KBr): 3369, 3124, 2802, 1657, 1566, 1432, 1216, 874 cm^{-1}; MS (*m/z*): 317 (M^+); Anal. Calcd for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.81; N, 22.04; Found: C, 52.86; H, 3.76; N, 21.96.

3-amino-*N*-(2-fluorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-17): Creamish solid; IR (KBr): 3352, 3124, 2922, 1658, 1506, 1323, 1066, 827 cm^{-1}; MS (*m/z*): 301 (M^+); Anal. Calcd for $C_{14}H_{12}FN_5O_2$: C, 55.81; H, 4.01; N, 23.25; Found: C, 55.71; H, 3.93; N, 23.17.

3-amino-*N*-(4-bromophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-18): Creamish solid; IR (KBr): 3369, 3104, 2824, 1657, 1416, 1232, 1096, 924 cm^{-1}; MS (*m/z*): 362 (M^+); Anal. Calcd for $C_{14}H_{12}BrN_5O_2$: C, 46.43; H, 3.34; N, 19.34; Found: C, 46.36; H, 3.28; N, 19.29.

3-amino-*N*-(3,4-dichlorophenyl)-4,5-dihydro-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-19): Creamish solid; IR (KBr): 3298, 2949, 2810, 1678, 1511, 1336, 1174, 852 cm^{-1}; MS (*m/z*): 352 (M^+); Anal. Calcd for $C_{14}H_{11}Cl_2N_5O_2$: C, 47.75; H, 3.15; N, 19.89; Found: C, 47.68; H, 3.05; N, 19.80.

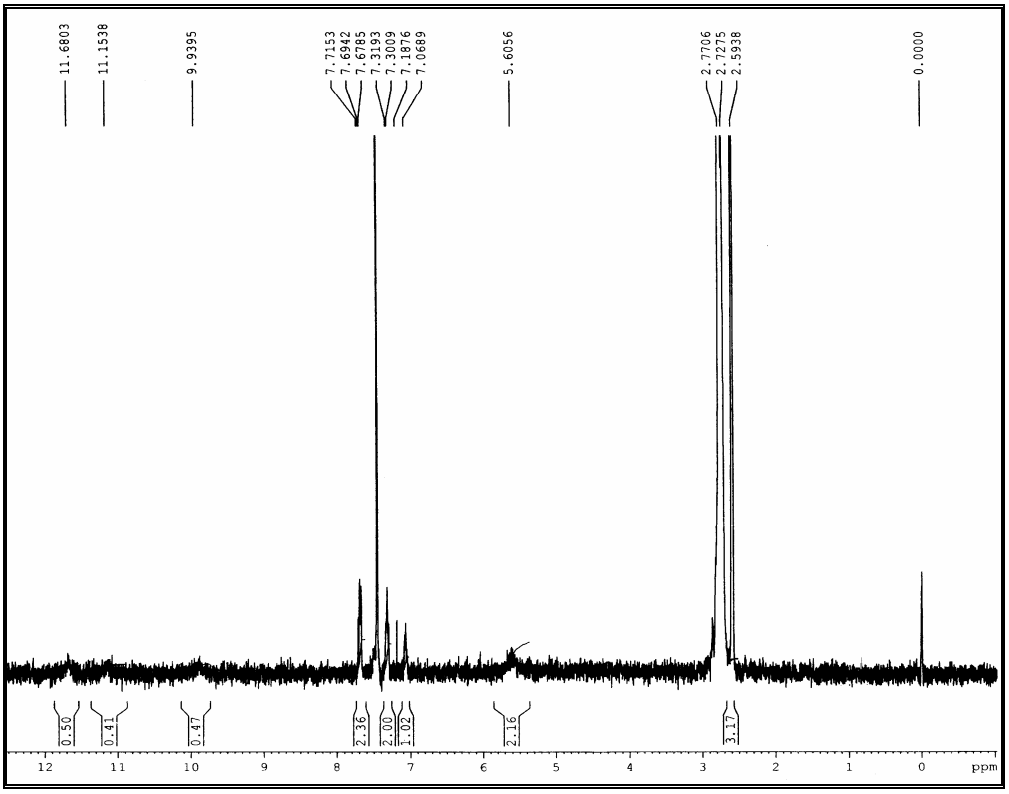
3-amino-4,5-dihydro-6-methyl-*N*-(3-nitrophenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-20): Creamish solid; IR (KBr): 3391, 2902, 2802, 1692, 1596, 1473, 1176, 974 cm^{-1}; MS (*m/z*): 328 (M^+); Anal. Calcd for $C_{14}H_{12}N_6O_4$: C, 51.22; H, 3.68; N, 25.60; Found: C, 51.17; H, 3.58; N, 25.51.

3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-*m*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PPMS-21): Creamish solid; IR (KBr): 3364, 2927, 1674, 1533, 1281, 1112, 873 cm^{-1}; MS (*m/z*): 297 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56; Found: C, 60.52; H, 4.98; N, 23.49.

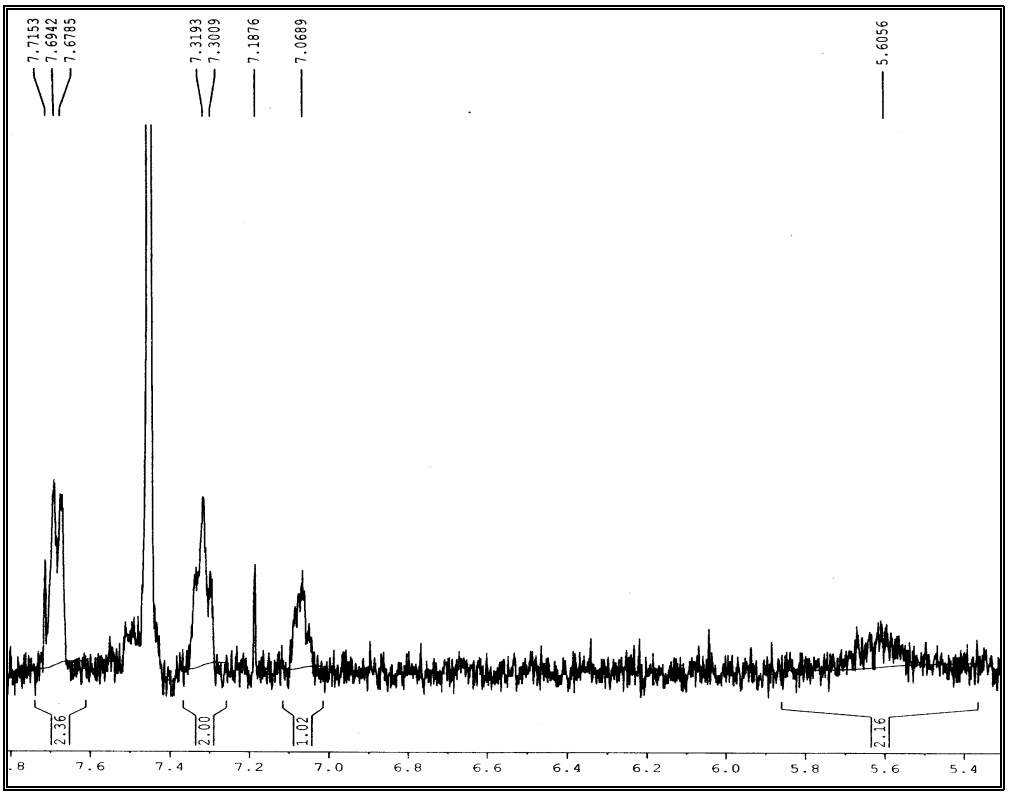
3-amino-4,5-dihydro-6-methyl-*N*-(2,3-dimethylphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-22): Creamish solid; IR (KBr): 3288, 3038, 2992, 1698, 1584, 1224, 1186, 854 cm^{-1}; MS (*m/z*): 311 (M^+); Anal. Calcd for $C_{16}H_{17}N_5O_2$: C, 61.72; H, 5.50; N, 22.49; Found: C, 61.64; H, 5.44; N, 22.41.

3-amino-4,5-dihydro-*N*-(2-methoxy-4-nitrophenyl)-6-methyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PPMS-23): Creamish solid; IR (KBr): 3375, 3024, 2802, 1684, 1516, 1395, 1236, 894 cm^{-1}; MS (*m/z*): 358 (M^+); Anal. Calcd for $C_{15}H_{14}N_6O_5$: C, 50.28; H, 3.94; N, 23.45; Found: C, 50.21; H, 3.87; N, 23.39.

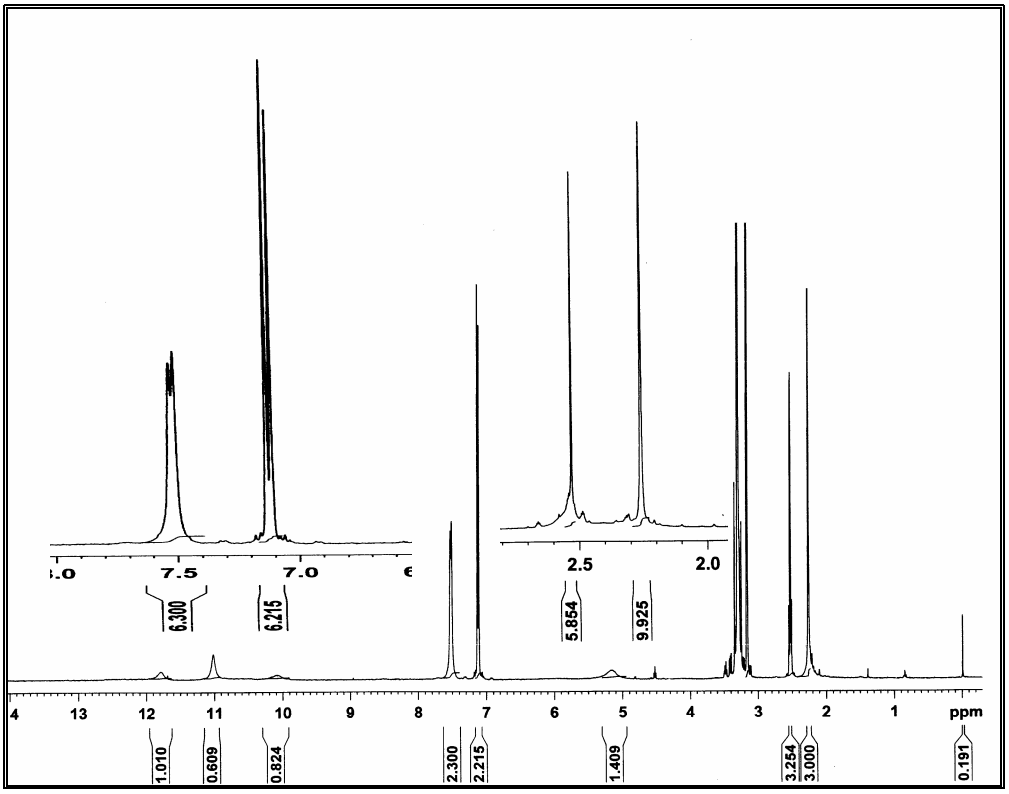
^{1}H NMR spectrum of compound PPMS-1



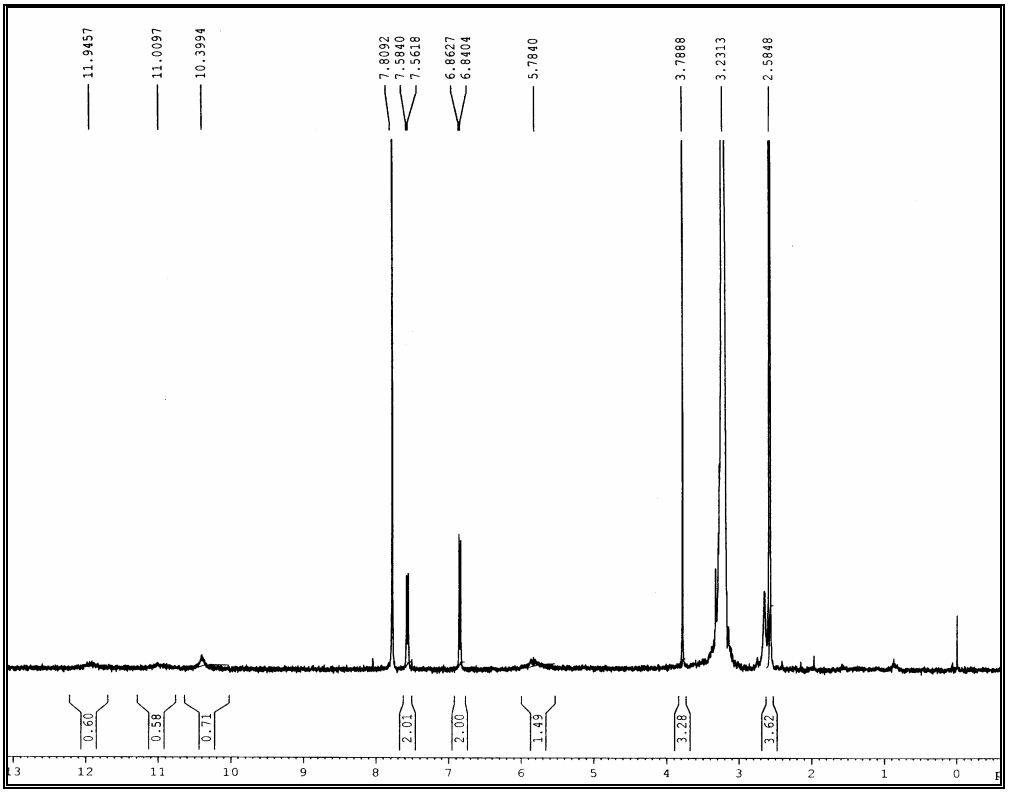
Expanded ^{1}H NMR spectrum of compound PPMS-1



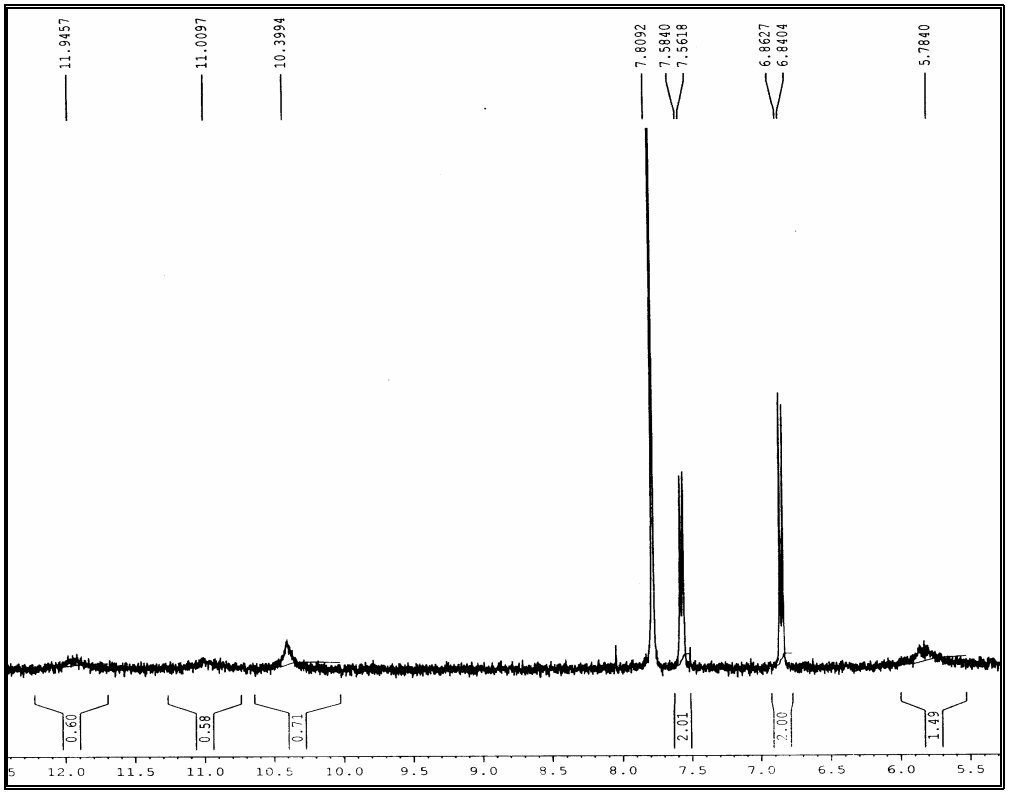
^{1}H NMR spectrum of compound PPMS-2



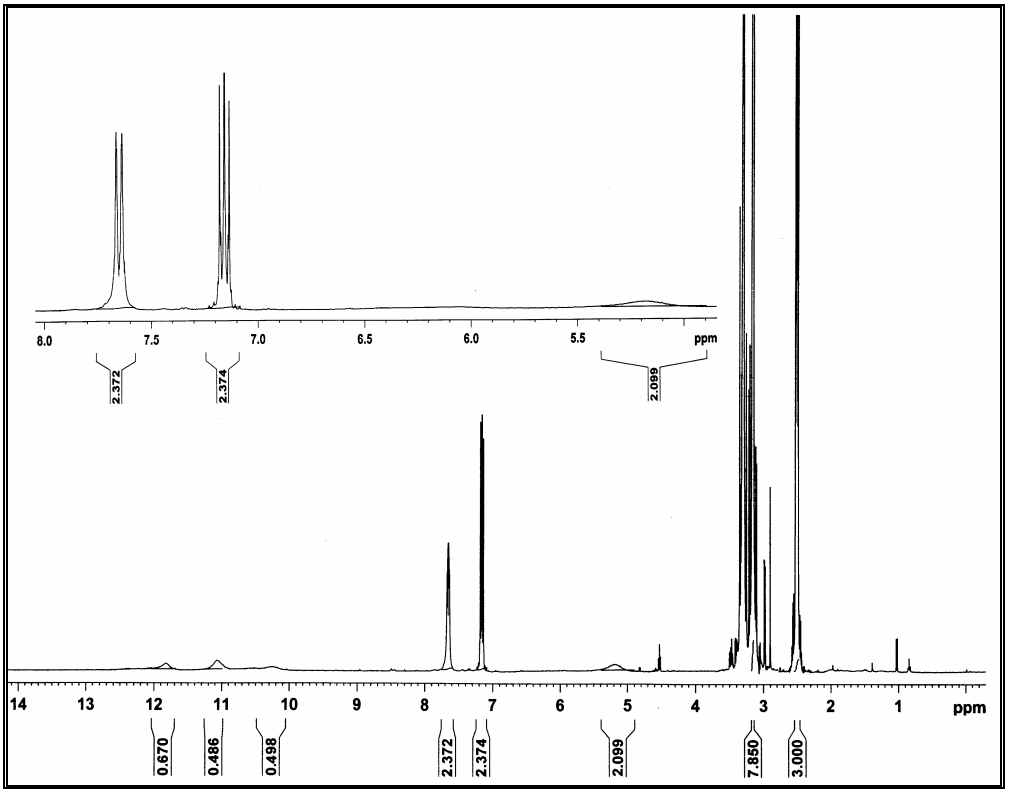
^{1}H NMR spectrum of compound PPMS-3



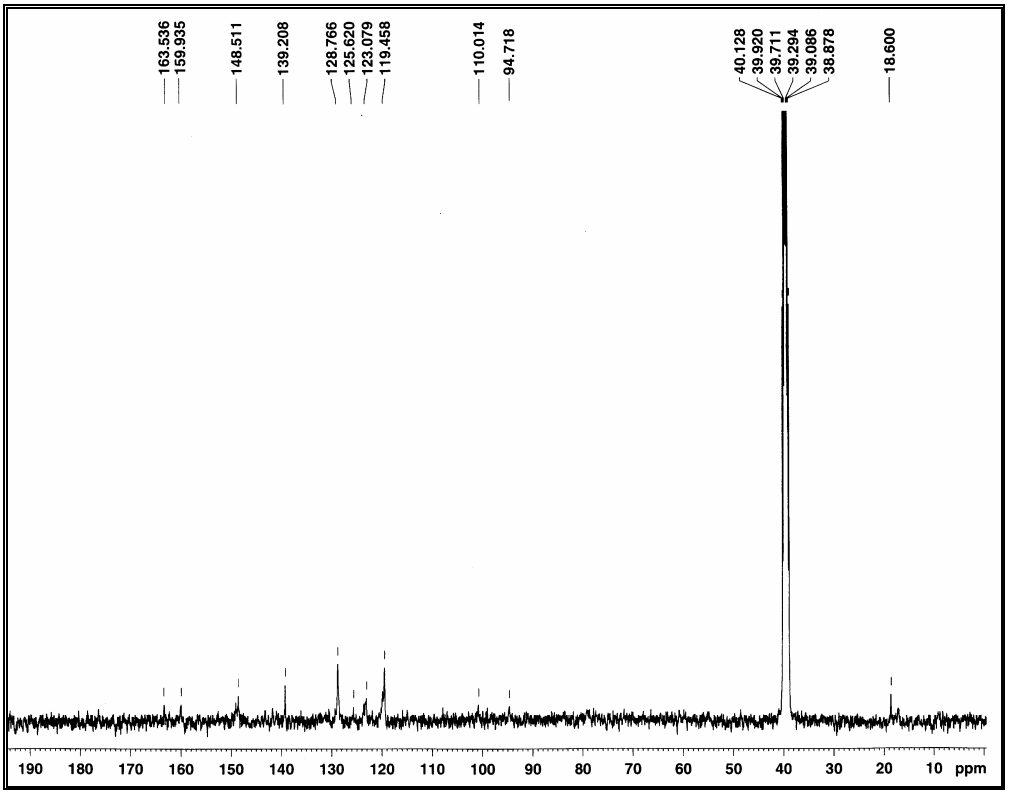
Expanded ^{1}H NMR spectrum of compound PPMS-3



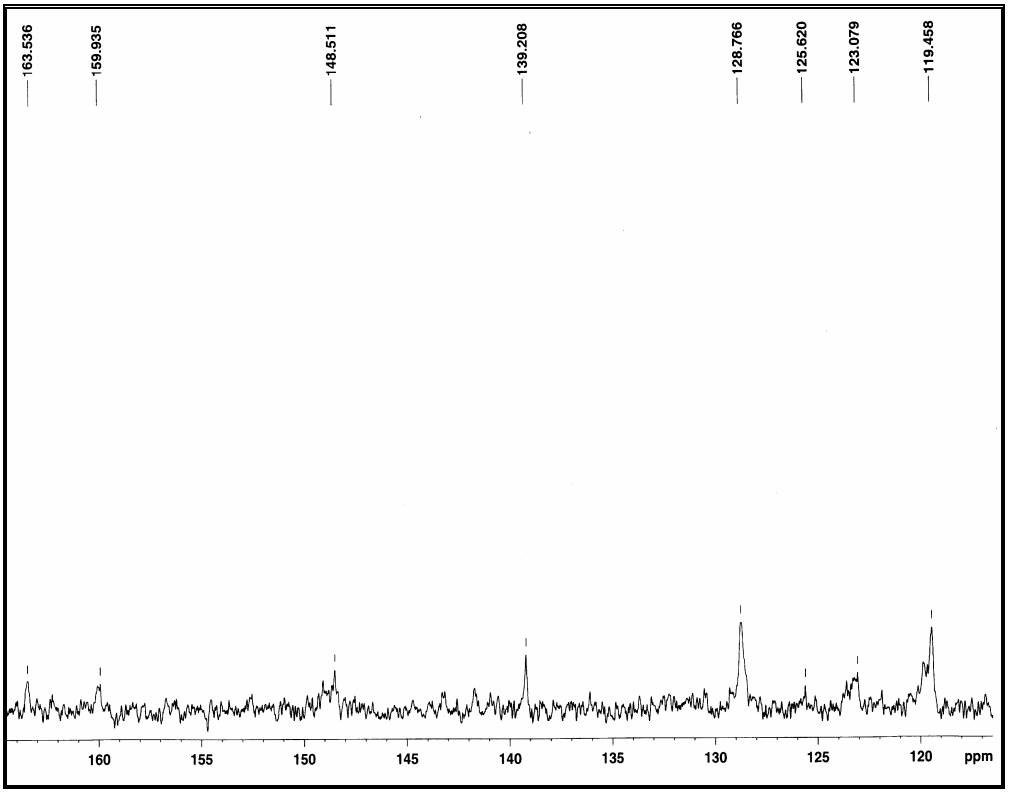
^{1}H NMR spectrum of compound PPMS-4



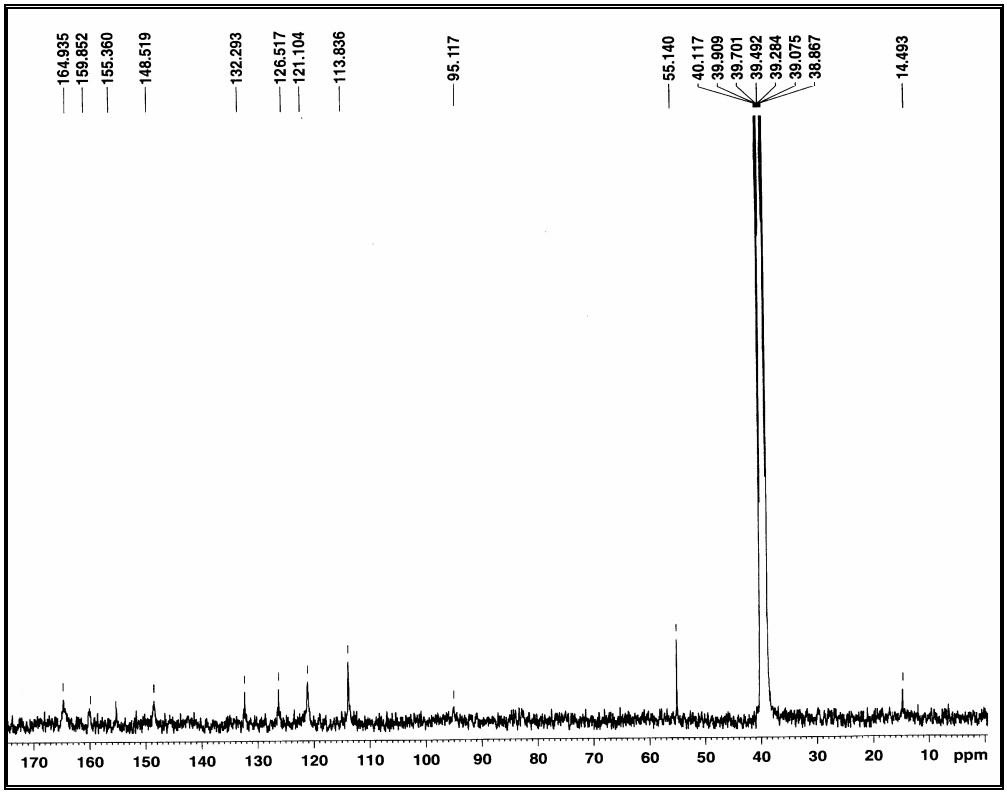
^{13}C NMR spectrum of compound PPMS-1



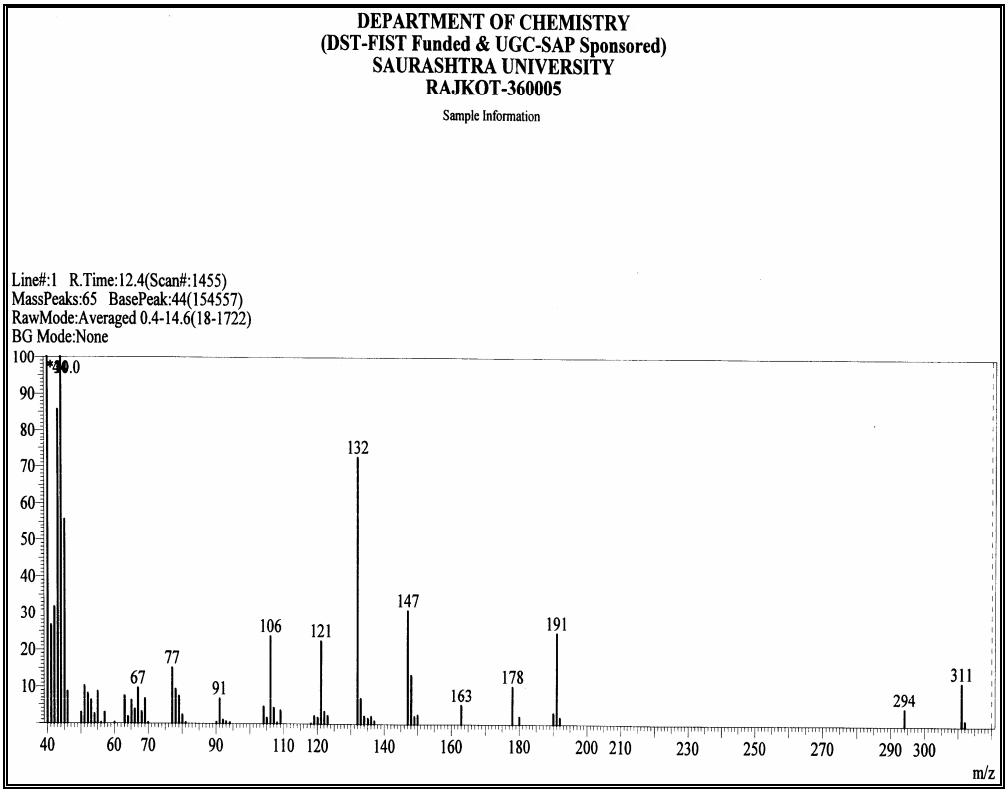
Expanded ^{13}C NMR spectrum of compound PPMS-1



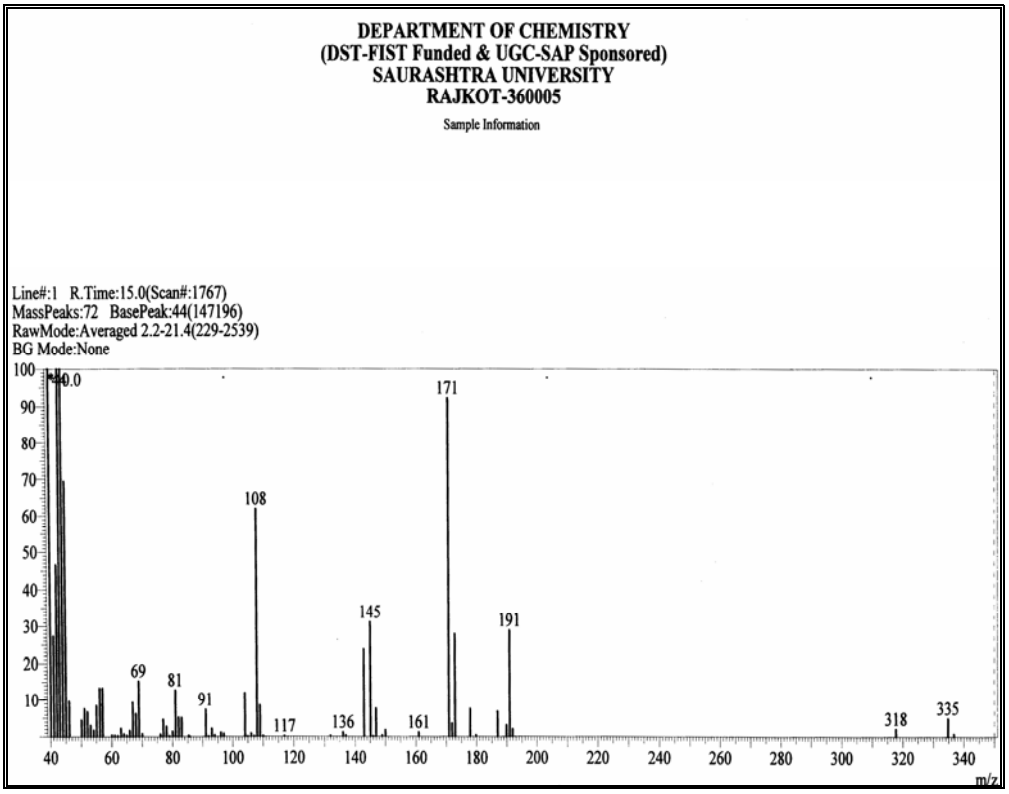
^{13}C NMR spectrum of PPMS-3



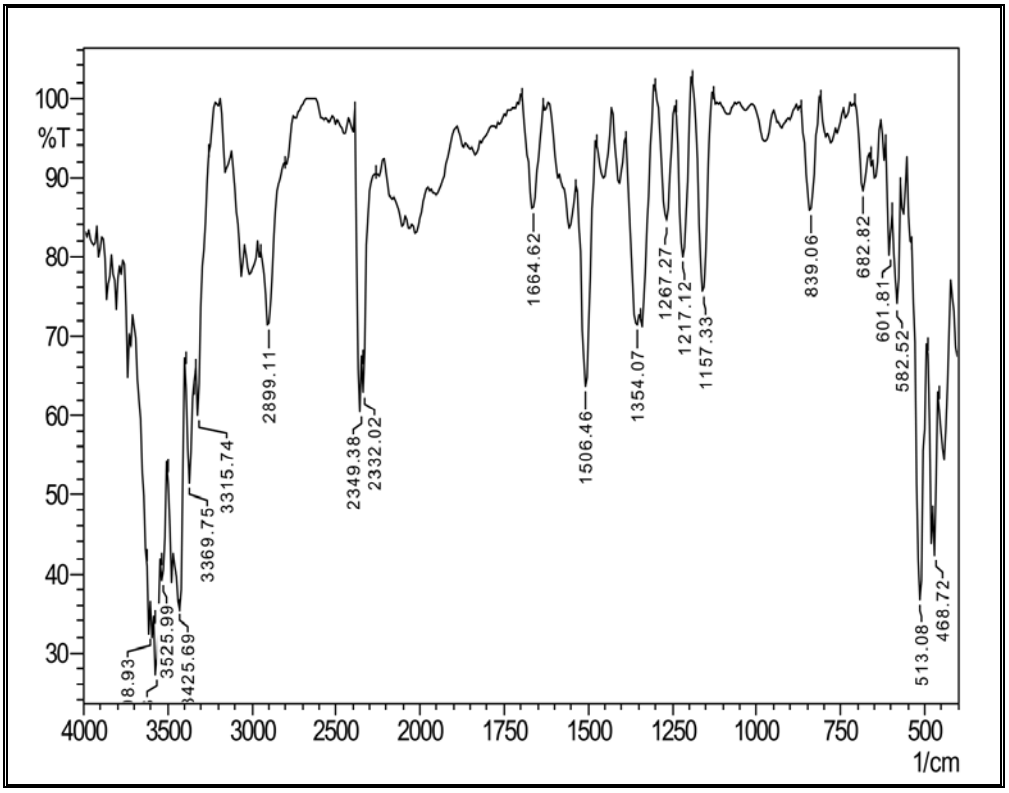
Mass spectrum of compound PPMS-8



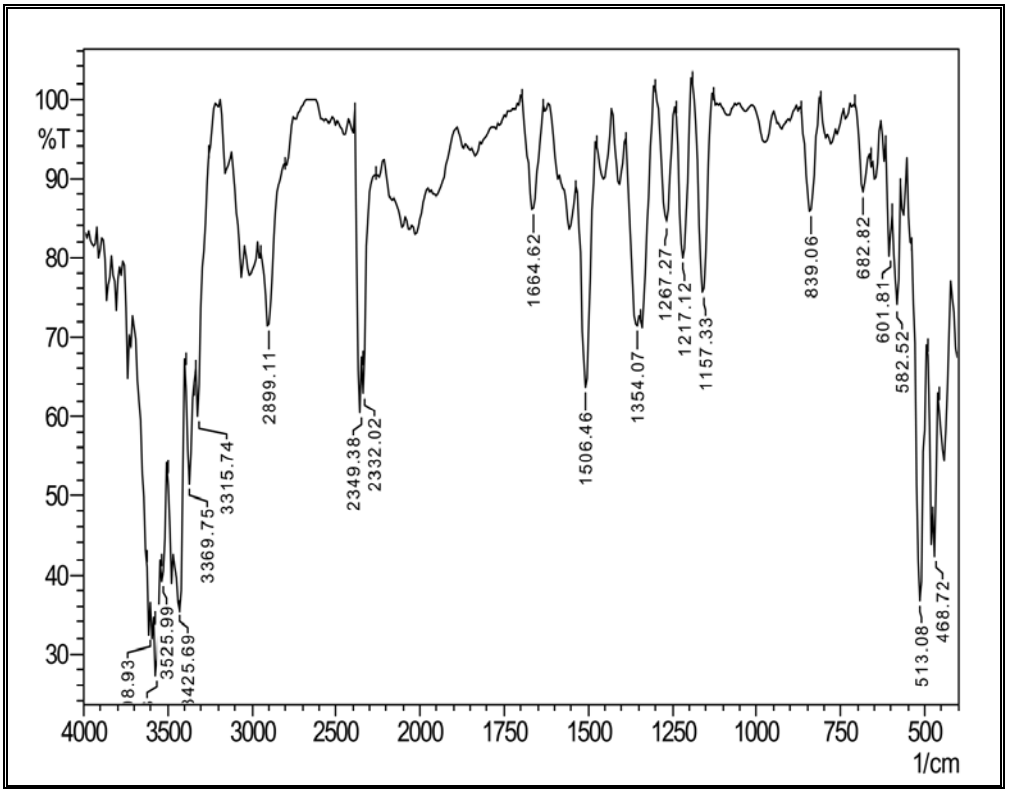
Mass spectrum of compound PPMS-10



IR spectrum of compound PPMS-2



IR spectrum of compound PPMS-3



5.9 REFERENCES

1. PCT Int. Appl., 2007, WO 2007076092 A2 20070705.

2. Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819.

3. Yu, K.; Toral B. L.; Shi, C.; Zhang, W. G.; Lucas, J.; Shor, B.; Kim, J.; Verheijen, J.; Curran, K.; Malwitz, D. J.; Cole, D. C.; Ellingboe, J.; Ayral K. S.; Mansour, T. S.; Gibbons, J. J.; Abraham, R. T.; Nowak, P.; Zask, A. *Cancer Res.* **2009**, *69*, 6232.

4. Stasch, J. P.; Becker, E. M.; Alonso A. C.; Apeler, H.; Dembowsky, K.; Feurer, A.; Gerzer, R.; Minuth, T.; Perzborn, E.; Pleiss, U.; Schroder, H.; Schroeder, W.; Stahl, E.; Steinke, W.; Straub, A.; Schramm, M. *Nature* **2001**, *410*, 212.

5. Witherington, J.; Bordas, V.; Gaiba, A.; Garton, N. S.; Naylor, A.; Rawlings, A. D.; Slingsby, B. P.; Smith, D. G.; Takle, A. K.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3055.

6. Gabriel, T.; Yan, L. Patent, WO2008/104473 A2.

7. Yassin, F. A. *Chem. of Hetero. Comp*. **2009**, *45*, 1253,

8. Mello, H. D.; Echevarria, A.; Bernardino, A. M.; Cavalheiro, M. C.; Leon, L. L. *J. Med. Chem.* **2004,** *47,* 5427.

9. Green, N. J.; Xiang, J.; Chen, J.; Chen, L.; Davies, A. M.; Erbe, D. Tam, S.; Tobin, J. F. *Bioorg. & Med. Chem*. **2003**, *11*, 2991.

10. Riccardo, G.; Clossek, D.; Christian, E.; Dennis, F. Patent WO 2009/121919.

11. Bondavalli, F.; Botta, M.; Bruno, O.; Ciacci, A.; Corelli, F.; Fossa, P.; Lucacchini, A.; Manetti, F.; Martini, C.; Menozzi, G.; Mosti, L.; Ranise, A.; Schenone, S.; Tafi, A.; Trincavelli, M. L. j. Med. Chem. **2002**, *45*, 4875.

12. Peter, E; Terrett, N. K. Patent WO 1994/28902.

13. Bell, A. S.; Terrett, N. K. Patent 1995, 5426107.

14. Das, S. K.; Abbineni, C.; Rao, K. V. L. N.; Iqbal, J.; Krishna Babu, R.; Chakrabarti, R. *Lett. in Drug Des. & Disc.* **2007**, *4,* 27.

15. Jalil, A. R.; Khanfar, M.; Abu-Safieh, K.; Al-Gharabli, S.; El-Abadelah, M; Voelter, W.; *Monatshefte Fur Chemie*, **2005**, *136*, 619.

16. Dumaıtre, B.; Dodic, N. *J. Med. Chem.* **1996,** *39,* 1635.

17. Paramashivappa, R.; Phanikumar, P.; Subba Rao, P. V.; Rao, S. A.; *J. Agric. Food Chem.* **2002,** *50,* 7709.

18 Magedov, I. V.; Manpadi, M.; Slambrouck, S. V.; Steelant, W. F. A.; Rozhkova, E.; Przhevalskii, N. M.; Rogelj, S.; Kornienko, A. *J. Med. Chem.* **2007,** *50,* 5183.

19. Paola, F.; Marco, P.; Giulia, M.; Luisa, M.; Silvia, S.; Angelo, R.; Francesco, B.; Letizia, T. M.; Antonio, L.; Claudia, M. *Org. & Biomol. Chem.* **2005**, *3*, 2262.

20. Adamo, M. F. A.; Duffy, E. F.; Donati, D.; Fantoni, P. S. *Tet.*, **2007**, *63*, 2047.

21. El-Assiery, S. A.; Sayed, G. H.; Fouda, A. *Acta Pharm.* **2004**, *54,* 143.

22. Attaby, F. A.; Eldin, S. M. *Arch. Pharm. Res.* **1990**, *13*, 274.

23. Kumar, A.; Ila, H.; Junjappa, H.; *J. Chem. Soc. Perkin I*, **1978**, 857.

24. Elgemeie, G. H.; Elghandour, A. H.; Elzanate, A. M.; Ahmed, S. A. *J. Chem. Soc., Perkin Trans. 1,* **1997,** 3285.

25. Elgemeie, G. H.; Ali, H. A.; Elghandour, A. H.; Abdel-aziz, H. M. *Phosphorus, Sulfur, and Silicon and the Related Elements*, **2001**, *170*, 171.

26. Lacova, M.; Puchala, A.; Solcanyova, E.; Lac, J.; Kois, P.; Chovancova, J.; Rasala, D. *Molecules* **2005**, *10*, 809.

27. Abass, M. *Phosphorus, Sulfur, and Silicon and the Related Elements,* **2003**, *178,* 1413.

28. Hassanein, A. Z. A. E. B. *Syn. Comm*. **2000**, *30*, 3883.

29. Lee, S.; Park, S. B. *Org. Lett.,* **2009**, *11*, 5214.

30. Salaheldin, A. M.; Oliveira-Campos, A. M. F.; Rodrigues, L. M. *Syn. Comm*. **2009**, *39*, 1186.

31. Shi, D. Q.; Shi, J. W.; Yao, H. *J. Chin. Chem. Soc.,* **2009**, *56,*504.

32. Zhang, L. J.; Shi, D. X.; Li, J. R. *Syn. Comm*. **2009**, *39*, 4010.

33. Mekheimer, R.; Ahmed, E. K.; Khattab, A. F. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2936.

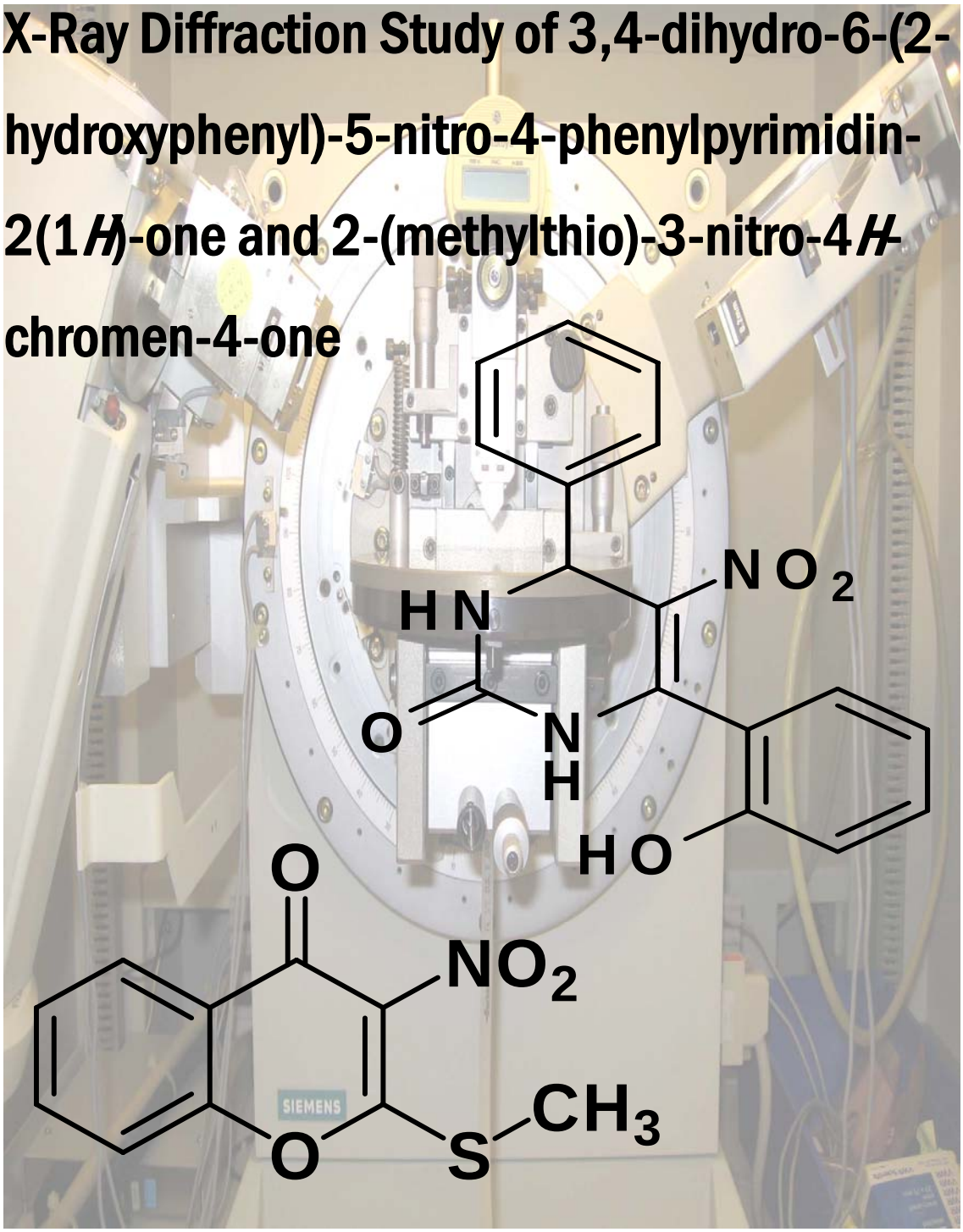
34. Ratajczyk, J. D.; Swett, L. R. *J. of Hetero. Chem.* **1975**, *12*, 517**.**

35. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nature Protocols* **2008**, *3*, 1.

36. Reed, L.J.; Muench, H. A. *Am. J. Hyg.* **1983**, *27*, 493.

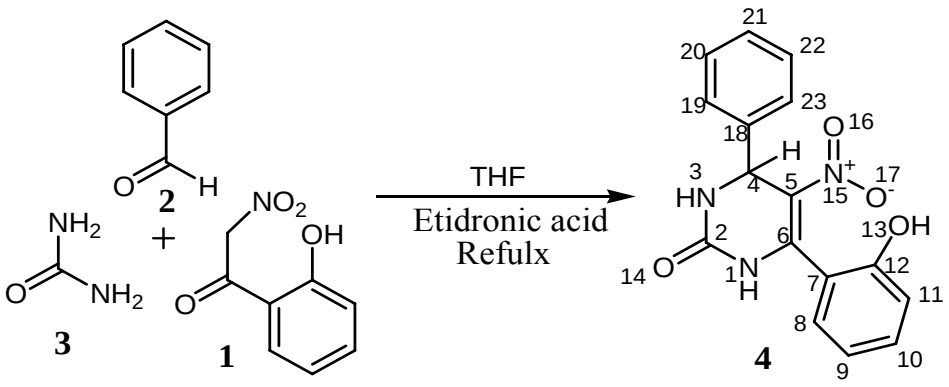
CHAPTER 6

X-Ray Diffraction Study of 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one and 2-(methylthio)-3-nitro-4*H*-chromen-4-one



6.1 Crystal and Molecular Structure of 3,4-dihydro-6-(2-hydroxy phenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one

SCHEME



❖ **Growth and Characterization of 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one.**

Dihydropyrimidines, especially NO_2 functionalized pyrimidine finds applications in medicinal chemistry due to their important pharmacological and therapeutic properties[1-3]. Due to the medicinal properties of pyrimidine derivatives, the crystal growth of organic material compound **4** has been carried out.

6.2 Procedure for the development of single crystals.

In the present study, the pure, single spot (on TLC) compound was taken in glacial acetic acid and heated with stirring till it dissolved. A small quantity of charcoal was added for decolorizing. The solution was then heated to boiling and immediately filtered while hot in corkable 50 ml conical flask using Whatmann filter paper. The flask was corked and kept for several days. The crystals thus grown by thin film evaporation technique were isolated and washed with chilled methanol. The constitution of 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one was supported by IR, ^{1}H & ^{13}C NMR and Mass spectral studies.

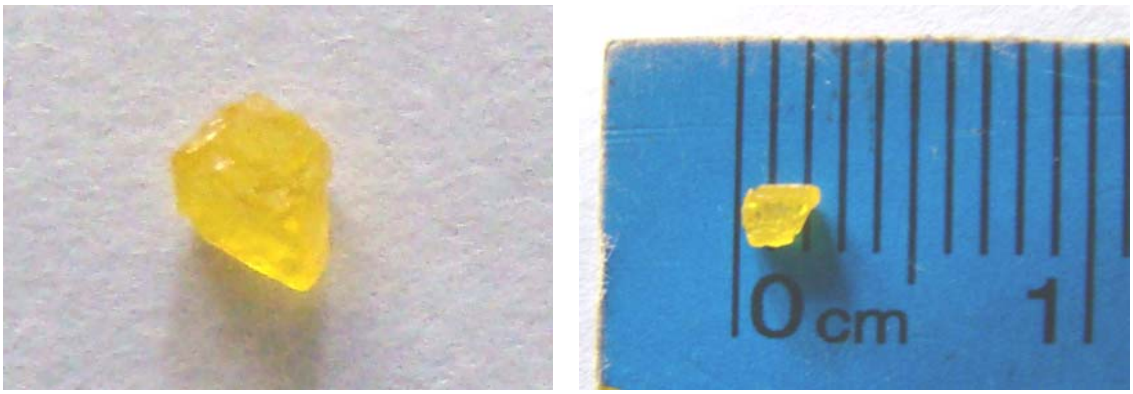


Figure 1: Photographs of the grown crystal of DHPM 4

Good quality single crystals with maximum dimension 0.2 cm X 0.2 cm were obtained. Figures 1 show the types of crystals grown. The crystals were lemon yellow in color.

6.3 SINGLE CRYSTAL X-RAY DIFFRACTION ANALYSIS

Single crystal X-ray diffraction is the most common experimental method for obtaining a detailed picture of a small molecule that allows resolution of individual atoms. It is performed by analyzing the diffraction of x-rays from an ordered array of many identical molecules. Many molecular substances, including proteins, polymers and other solidify in to crystals under the proper conditions. When solidifying in to the crystalline state, these individual molecules typically adapted as one of only a few possible orientations. A crystal is a three dimensional array of those molecules that are held together by Van der Waals and noncovalent bonding. The smallest representative unit of this crystal is referred to as the unit cell. Understanding the unit cell of these arrays simplifies the understanding of a crystal as a whole.

❖ Single Crystal X-ray Diffraction and Structure Determination

A single crystal of suitable size was chosen for X-ray diffraction studies. The data were collected at room temperature on a DIPLabo Image Plate system with graphite monochromated radiation MoK_α. Each exposure of the image plate was set to a period of 400 s. Thirty-six frames of data were collected in the oscillation mode with an oscillation range of 5˚ and processed using Denzo.[4] The reflections were merged with Scalepack. All the frames

could be indexed using a primitive monoclinic lattice. The structure was solved by direct methods using SHELXS-97[5]. Least-squares refinement using SHELXL-97[5] with isotropic displacement parameters for all the non-hydrogen atoms converged the residual to 0.1402. Subsequent refinements were carried out with anisotropic thermal parameters for the non-hydrogen atoms. After eight cycles of refinement the residuals converged to 0.0470. The hydrogen atoms were fixed at chemically acceptable positions and were allowed to ride on their parent atoms. The details of crystal data and refinement are given in Table 1. The bond lengths and bond angles of all the non-hydrogen atoms (Table 2) are in good agreement with the standard values[6]. Figure 1 represents the ORTEP[7] diagram of the molecule with thermal ellipsoids drawn at 50% probability.

In the title compound $C_{16}H_{13}N_3O_4$, the heterocyclic ring adopts a flattened boat conformation, with a puckering amplitude[8] Q=0.3833(2)Å, θ=106.2(3)˚ and Φ=352.1(3)˚. The phenyl ring **1** (C7-C8-C9-C10-C11-C12) adopts an axial conformation with the heterocyclic ring whereas the phenyl ring **2** (C18-C19-C20-C21-C22-C23) adopts an equatorial conformation, as indicated by the dihedral angle values of 39.38(8)˚ and 81.12(1)˚ respectively. The nitro group C6-C5-N15-O17 is almost coplanar with the pyrimidine ring as indicated by the torsion angle value of 12.6(3)˚. The carbonly group C2=O14 is oriented in +*anti-periplanar* conformation, as indicated by the torsion angle value of 163.58(1)˚ for C6-N1-C2-O14. The hydroxyl group of phenyl ring **1** makes an angle of 121.74(2)° with the C11 and 119.25(2)° with the C7 atoms. The observed bond length of O13 atom of hydroxyl group with C12 was 1.343(2). The hydrogen atom H13 of hydroxyl group makes intramolecular hydrogen bond with O14 of heterocyclic ring with the bond length 2.799(2)Å and bond angle 172˚. The bond lengths N15-O16, N15-O17, N1-C6, N1-C2, N3-C2, N3-C4 are comparable with other reported compounds.[9] The structure exhibits intermolecular hydrogen bonds of the type N-H…O and O-H…O, which bind the molecules into one-dimensional polymeric chains. The observed hydrogen bonds are listed in Table 3. The packing of the molecules down *b*-axis is shown in the Figure 2.

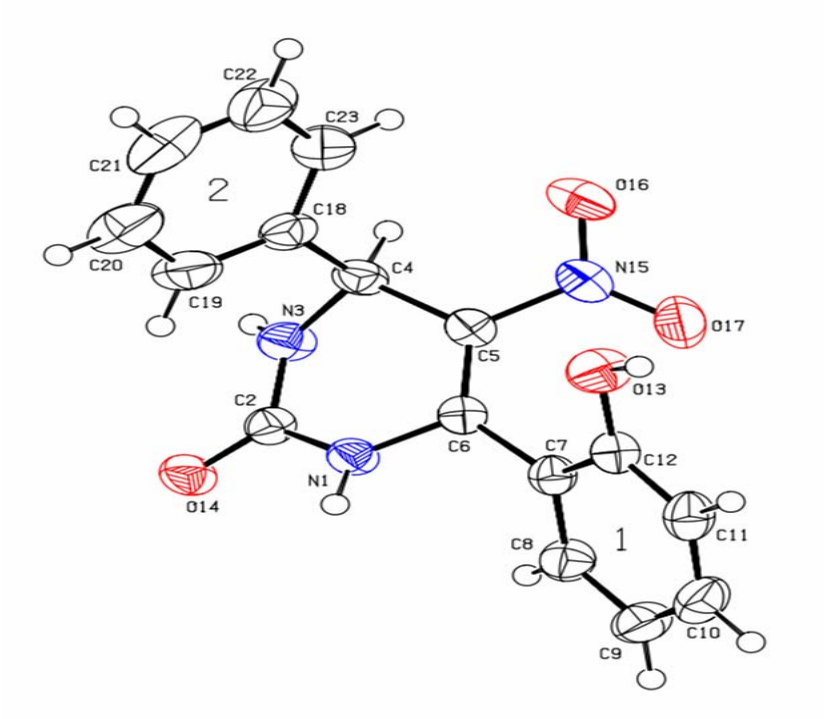


Figure 2: ORTEP of the molecule with thermal ellipsoids drawn at 50% probability.

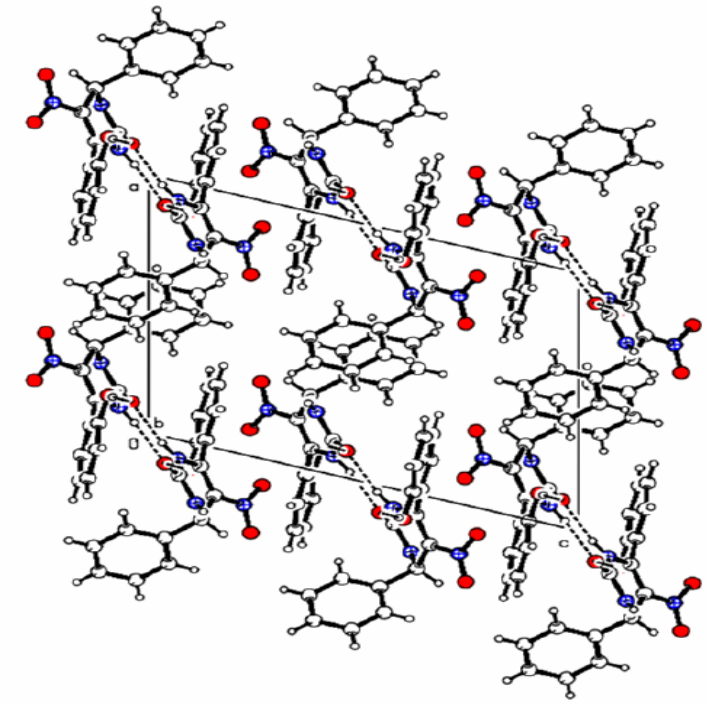


Figure 3: Packing of the molecules when viewed down the *b*-axis. The dashed lines represent the hydrogen bonds.

Table 1: Experimental details and other measurement data

Empirical formula	$C_{16}H_{13}N_3O_4$
Formula weight	311.29
Temperature	293 k
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Cell dimensions	a = 11.1070(10)Å
	b = 8.8210(4)Å
	c = 15.1110(13)Å
	β=106.193(2)˚
Volume	1421.76(2)Å^3
Z	4
Density (calculated)	1.454 Mg/m^3
Absorption coefficient	0.107 mm^{-1}
F_{000}	648
Crystal size	0.270 x 0.250 x 0.230 mm
Theta range for data collection	2.70 to 25.03˚
Index ranges	-13<=h<=13, -9<=k<=9, -17<=l<=17
Reflections collected	4326
Independent reflections	2351
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2351 / 0 / 209
Goodness-of-fit on F^2	1.023
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0470, wR_2 = 0.1344
R indices (all data)	R_1 = 0.0526, wR_2 = 0.1422
Extinction coefficient	0.045(8)
Largest diff. peak and hole	0.238 and -0.265 e.Å^{-3}
Deposition number	CCDC 743221

Table 2: Various Bond lengths (Å) and Bond angles (˚)

Atoms	Length (Å)	Atoms	Length (Å)
N1-C2	1.376(2)	C9-C10	1.380(3)
N1-C6	1.382(2)	C10-C11	1.377(3)
C2-O14	1.239(2)	C11-C12	1.388(3)
C2-N3	1.332(2)	C12-O13	1.343(2)
N3-C4	1.460(2)	N15-O17	1.223(2)
C4-C5	1.513(2)	N15-O16	1.229(2)
C4-C18	1.528(2)	C18-C23	1.381(3)
C5-C6	1.352(2)	C18-C19	1.386(3)
C5-N15	1.435(2)	C19-C20	1.384(3)
C6-C7	1.477(2)	C20-C21	1.383(4)
C7-C8	1.387(3)	C21-C22	1.371(4)
C7-C12	1.407(2)	C22-C23	1.382(3)
C8-C9	1.376(3)		
Atoms	**Angle(˚)**	**Atoms**	**Angle(˚)**
C2-N1-C6	123.67(1)	C8-C9-C10	119.27(2)
O14-C2-N3	123.97(1)	C11-C10-C9	120.69(2)
O14-C2-N1	120.70(1)	C10-C11-C12	120.57(2)
N3-C2-N1	115.32(1)	O13-C12-C11	121.74(2)
C2-N3-C4	122.82(1)	O13-C12-C7	119.25(2)
N3-C4-C5	106.58(1)	C11-C12-C7	118.98(2)
N3-C4-C18	112.46(1)	O17-N15-O16	122.50(1)
C5-C4-C18	113.67(1)	O17-N15-C5	120.23(1)
C6-C5-N15	121.88(1)	O16-N15-C5	117.21(1)
C6-C5-C4	120.65(1)	C23-C18-C19	118.20(2)
N15-C5-C4	117.36(1)	C23-C18-C4	119.40(2)
C5-C6-N1	116.27(1)	C19-C18-C4	122.40(2)
C5-C6-C7	130.54(1)	C20-C19-C18	120.9(2)
N1-C6-C7	113.19(1)	C21-C20-C19	119.9(2)
C8-C7-C12	119.16(2)	C22-C21-C20	119.6(2)
C8-C7-C6	118.23(1)	C21-C22-C23	120.3(2
C12-C7-C6	122.36(1)	C18-C23-C22	121.1(2)

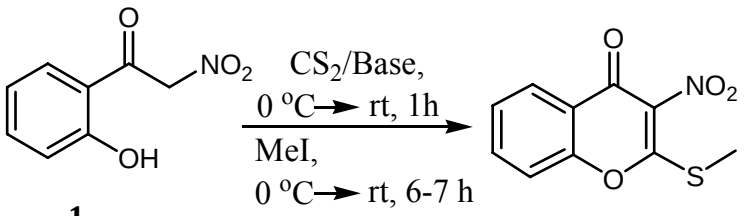
C9-C8-C7	121.31(2)

Table 3: Geometry of intermolecular hydrogen interactions

Atoms	Length (Å)	Angle(˚)	Symmetry codes
N1-H1…O14	2.892(2)	170	*2-x,1-y,-2*
O13-H13…O14	2.799(2)	172	*x,1+y,z*

6.4 Crystal and Molecular Structure of 2-(methylthio)-3-nitro-4*H*-chromen-4-one

SCHEME



1 **2**

6.5 Procedure for the development of single crystals.

In the present study, the pure, single spot (on TLC) compound was taken in chloroform and heated with stirring till it dissolved. A small quantity of charcoal was added for decolorizing. The solution was then heated to boiling and immediately filtered while hot in corkable 50 ml conical flask using Whatmann filter paper. The flask was corked and kept for several days. The crystals thus grown by thin film evaporation technique were isolated and washed with chilled methanol. The constitution of 2-(methylthio)-3-nitro-4*H*-chromen-4-one was supported by IR, ^{1}H & ^{13}C NMR and Mass spectral studies.

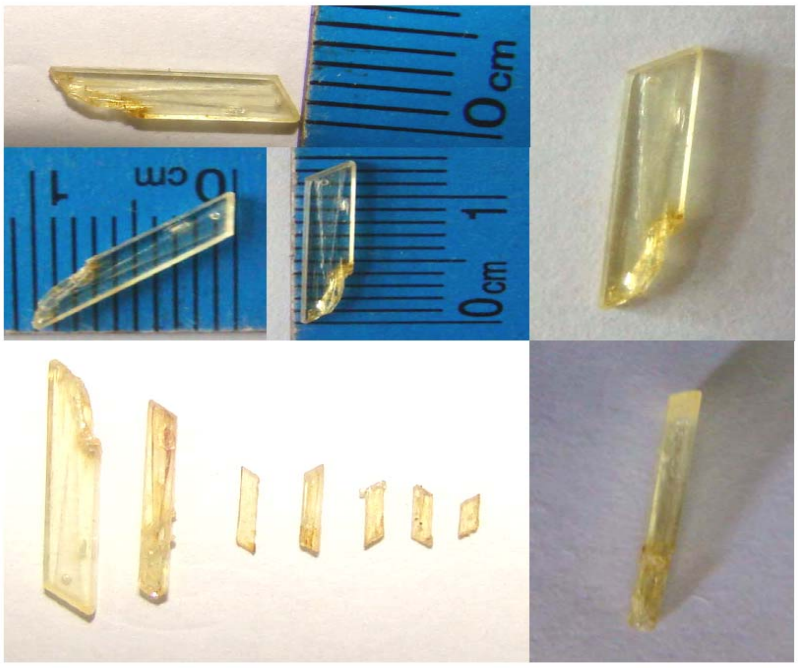


Figure 1: Photograph of the grown crystals of compound 2

Good quality single crystals with maximum dimension 1.0 cm X 0.2 cm were obtained. **Figures 1** show the types of crystals grown. The crystals were yellowish in color.

6.6 Single Crystal X-ray Diffraction and Structure Determination

A single crystal of the title compound with dimensions 0.30 x 0.25 x 0.25 mm was chosen for the X-ray diffraction study. The data were collected on a DIPLabo Image Plate system equipped with a normal focus, 3KW sealed X-ray source (graphite monochromated MoK_α). The crystal to detector distance was fixed at 120 mm with the detector area of 441 x 240 mm^2. Thirty six frames of data were collected at room temperature by the oscillation method. Each exposure of the image plate was set to 400 seconds. Successive frames were scanned in steps of 5º per minute with an oscillation range of 5º. Image processing and data reduction were done using Denzo.[4] The reflections were merged with Scalepack.[13] All the frames could be indexed using a monoclinic lattice. Absorption correction was not applied. The structure was solved by direct methods using SHELXS-97.[14] Least-squares refinement using SHELXL-97[15] with isotropic temperature factors for all the non-hydrogen atoms converged the residual R1 to 0.0528. Subsequent refinements were carried out with anisotropic thermal parameters for non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms which were placed at chemically acceptable positions. The hydrogen

atoms were allowed to ride on their parent atoms. After eight cycles of refinement the residual converged to 0.0446. The details of crystal data and refinement are given in Tables 1. Tables 2 give the list of bond lengths and bond angles respectively which are in good agreement with the standard values. Table 3 gives atomic coordinates and equivalent thermal parameters of the non-hydrogen atoms and Table 4 gives hydrogen-bonding geometry. The ORTEP of the molecule with thermal ellipsoids drawn at 50% probability is shown in **Fig. 2** and **Fig. 3** shows packing of the molecules down along *b*-axis.

The title compound shows planar conformation. The dihedral angle between the least squares planes O1-C2-C3-C4-C5-C10 and C5-C6-C7-C8-C9-C10 is 1.13(1)º. Total puckering amplitude *Q* for ten membered ring O1-C2-C3-C4-C5-C6-C7-C8-C9-C10 is 0.045(2)Å. The torsion angles about C3-C2-S11-C12 and C2-C3-C4-O16 being 168(2)º and 179.3(2)º show *anti-periplanar* and *anti-periplanar* conformations. The molecule exhibits inter-molecular hydrogen bonds of the type C-H...O. The inter-molecular hydrogen bonds C9-H9...O15 and C12-H12A...O16, have lengths of 3.341(3)Å and 3.332(3)Å, with angles of 161º and 144º respectively, with the symmetry codes x, y, 1+z and -x, 1-y, -z.

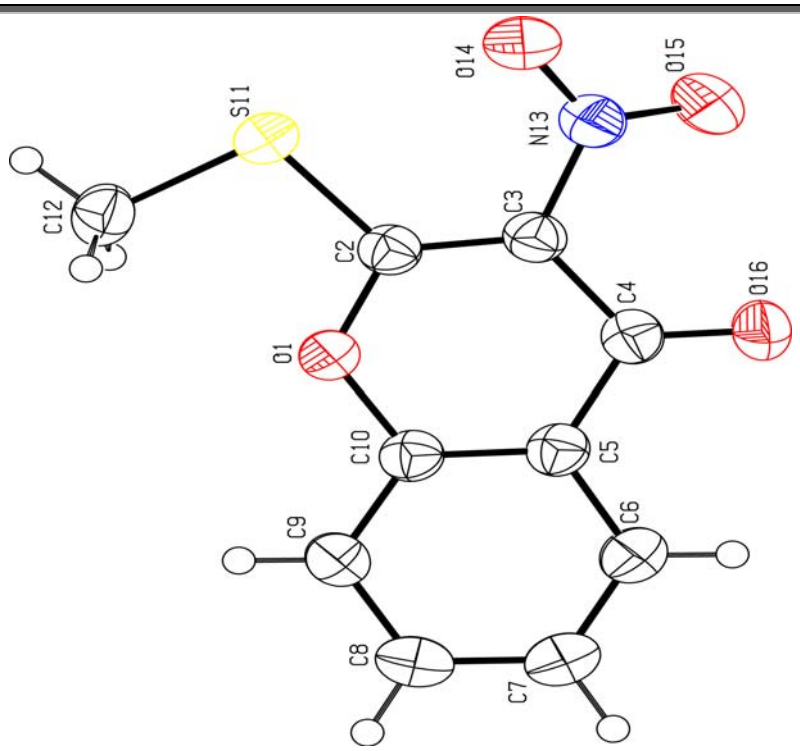


Figure 2: ORTEP of the molecule with thermal ellipsoids drawn at 50% probability.

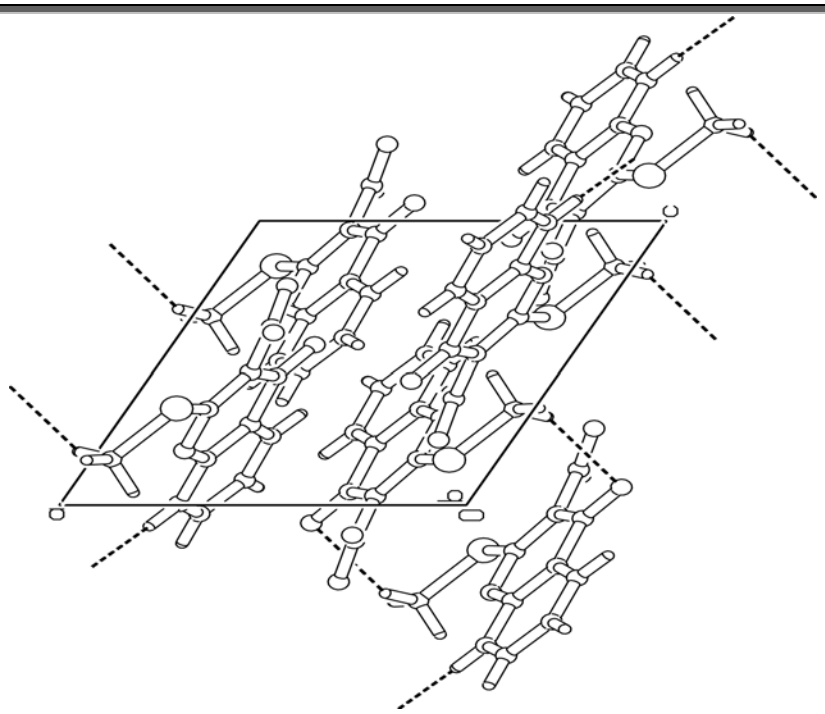


Figure 3: Packing of the molecules when viewed down the *b*-axis. The dashed lines represent the hydrogen bonds.

Table 1: Experimental details and other measurement data

Empirical formula	$C_{10}H_7NO_4S$
Formula weight	237.23
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Spacegroup	$P2_1/c$
Cell dimensions	a = 7.7940(8)Å b = 17.3990(16)Å c = 8.1600(7)Å β = 117.998(6)°
Volume	977.05(16)Å^3
Z	4
Density(calculated)	1.613 Mg/m ^{3}
Absorption coefficient	0.328 mm $^{-1}$
F_{000}	488
Crystal size	0.3 x 0.25 x 0.25 mm
θ range for data collection	2.34° to 25°
Index ranges	$-9 \le h \le 9$ $-20 \le k \le 20$ $-9 \le l \le 8$
Reflections collected	2713
Independent reflections	1530 [$R_{int} = 0.0239$]
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1530 / 0 / 147
Goodness-of-fit on F^2	1.23
Final R indices [I>2σ(I)]	R1 = 0.0446, wR2 = 0.1162
R indices (all data)	R1 = 0.0528, wR2 = 0.1312
Extinction coefficient	0.43(4)
Largest diff. peak and hole	0.412 and -0.376 e.Å^{-3}
CCDC Deposition	760004

Table 2: Various Bond lengths (Å) and Bond angles (˚)

Atoms	Length (Å)	Atoms	Length (Å)
O1-C2	1.338(2)	C5-C6	1.404(3)
O1-C10	1.387(2)	C6-C7	1.371(3)
C2-C3	1.375(3)	C7-C8	1.392(4)
C2-S11	1.734(2)	C8-C9	1.380(3)
C3-N13	1.441(3)	C9-C10	1.381(3)
C3-C4	1.467(3)	S11-C12	1.797(3)
C4-O16	1.218(2)	N13-O15	1.220(2)
C4-C5	1.473(3)	N13-O14	1.233(2)
C5-C10	1.381(3)		
Atoms	**Angle(˚)**	**Atoms**	**Angle(˚)**
C2-O1-C10	120.83(2)	C6-C5-C4	121.08(2)
O1-C2-C3	121.13(2)	C7-C6-C5	120.3(2)
O1-C2-S11	112.59(1)	C6-C7-C8	120.4(2)
C3-C2-S11	126.27(2)	C9-C8-C7	120.6(2)
C2-C3-N13	118.22(2)	C8-C9-C10	117.9(2)
C2-C3-C4	122.83(2)	C5-C10-C9	123.15(2)
N13-C3-C4	118.95(2)	C5-C10-O1	121.17(2)
O16-C4-C3	125.45(2)	C9-C10-O1	115.67(2)
O16-C4-C5	121.89(2)	C2-S11-C12	101.66(1)
C3-C4-C5	112.66(2)	O15-N13-O14	123.00(2)
C10-C5-C6	117.63(2)	O15-N13-C3	119.17(2)
C10-C5-C4	121.29(2)	O14-N13-C3	117.83(2)

Table 3: Atomic coordinates and equivalent thermal parameters of the non-hydrogen atoms.

Atom	x	y	z	U_{eq}
O1	0.2126(2)	0.5367(7)	0.3081(2)	0.0404(4)
C2	0.1982(3)	0.5767(1)	0.1622(3)	0.0357(5)
C3	0.2395(3)	0.5435(1)	0.0319(3)	0.0370(5)
C4	0.3097(3)	0.4642(1)	0.0473(3)	0.0368(5)
C5	0.3291(3)	0.4251(1)	0.2152(3)	0.0370(5)
C6	0.3984(3)	0.3494(1)	0.2565(3)	0.0465(5)
C7	0.4119(3)	0.3134(1)	0.4116(4)	0.0522(6)
C8	0.3586(3)	0.3517(1)	0.5304(3)	0.0499(6)
C9	0.2923(3)	0.4265(1)	0.4947(3)	0.0436(5)
C10	0.2789(3)	0.4614(1)	0.3371(3)	0.0365(5)
S11	0.1206(8)	0.6704(3)	0.1594(8)	0.0443(3)
C12	0.0475(4)	0.6672(1)	0.3379(4)	0.0501(6)
N13	0.2147(3)	0.5892(1)	-0.1251(3)	0.0444(5)
O14	0.2283(3)	0.6596(9)	-0.1058(3)	0.0668(6)
O15	0.1811(3)	0.5571(1)	-0.2701(2)	0.0649(6)
O16	0.3500(2)	0.4323(8)	-0.0631(2)	0.0467(4)

Table 4: Geometry of intermolecular hydrogen interactions

Atoms	D-H	H-A	Length (Å)	Angle(˚)	Symmetry codes
C9-H9...O15	0.93	2.45	3.341(3)	161	x, y, 1+z
C12-H12A...O16	0.96	2.51	3.332(3)	144	-x,1-y,-z

6.7 CONCLUSION

We have demonstrated the crystal and molecular structure of newly synthesized compounds 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one and 2-(methylthio)-3-nitro-4*H*chromen-4-one by the singly crystal x-ray diffraction technique. In compound 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one, the pyrimidine ring adopts a flattened boat conformation and the phenyl ring **1** (C7-C8-C9-C10-C11-C12) and phenyl ring **2** (C18-C19-C20-C21-C22-C23) adopts an axial and equatorial conformations with the pyrimidine ring, respectively. The nitro group is almost coplanar with the pyrimidine ring. The structure exhibits intermolecular hydrogen bonds of the type N-H…O and O-H…O, which bind the molecules into one-dimensional polymeric chains. The compound 2-(methylthio)-3-nitro-4*H*chromen-4-one shows planar conformation and molecule exhibits inter-molecular hydrogen bonds of the type C-H...O.

6.8 REFERENCES

1 Sedova, V. F.; Voevoda, T. V.; Tolstikova, T. G.; Shkurko, O. P. *Khim Farm Zh* **2002**, *36,* 4.

2 Remennikov, G. Y.; Shavaran, S. S.; Boldyrev, I. V.; Kurilenko, L. K.; Klebanov, B. M.; Kukhar, V. P. *Khim Farm Zh*, **1991**, *25*, 35.

3 Remennikov, G. Y.; Shavaran, S. S.; Boldyrev, I. V.; Kapran, N. A.; Kurilenko, L. K.; Shevchuk, V. G.; Klebanov, B. M.; *Khim Farm Zh,* **1994,** *28*, 25.

4 Otwinowski, Z.; Minor, W. Methods in Enzymology, 276, C.W. Carter Jr., R.M. Sweet (Eds), p. 307, Academic Press, New York, **1997**.

5 Sheldrick, G. M. *Acta. Cryst.* A, **2008**, *64*, 112.

6 Allen, F. H.; Kennard, O.; Watson, D. G.; Brummer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc. Perkin Trans II,* **1987**, *12*, S1.

7 Spek, A. L.; *J. Appl. Cryst.* **2003,** *36*, 7.

8 Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354.

9 Rybalvo, T. V.; Sedova, V. F.; Gatilov, Y. V.; Shkurko, O. P. *Zh Strukt Khim* **2004,** *45*, 287.

10 Sottofatoori, E.; Anzaldi, M.; Mazzei, M.; Meile, M.; Balbi, A.; Pyshnyi, D. S.; Zakhrovab, O. D.; Abramovab, T. V. *Bioorg. Med. Chem.* **2005**, *13,* 1515.

11 Perrella, F. W.; Chen, S. F.; Behrens, D. L.; Kaltenbach, R. F.; Seitz, S. P. *J. Med. Chem.* **1994**, *37,* 2232.

12 Yan, M. C.; Jang, Y. J.; Kuo, W. Y.; Tu, Z.; Shen, K. H.; Cuo, T. S.; Ueng, C. H.; Yao, C. F. *Heterocycles* **2002**, *57,* 1033

13 Mackay, S.; Gilmore, C. J.; Edward, C.; Stewart, N.; Shankland, K.; maXus Computer Program for the Solution and Refinement of Crystal Structures, Bruker Nonius, The Netherlands, **1999**.

14 Sheldrick, G. M. SHELXS97, Program for Crystal Structure Solution, Germany; University of G"ottingen, **1997**.

15 Sheldrick, G. M. SHELXL97, Program for Crystal Structure Solution, Germany; University of G"ottingen, **1997**.

Summary

The work presented in the Thesis entitled “Studies on Bioactive Heterocycles” can be summarized as below.

Chapter 1, we have demonstrated the utility of *ω*-nitro acetophenone for the synthesis of pyrimidine heterocycle *via* cyclocondensation using bisphosphonic acid (EDA) as a catalyst. We have examined the catalytic utility of various bisphosphonic acids and found the etidronic acid was efficient for the synthesis of nitro bearing pyrimidines. The use of etidronic acid was well tolerated with a range of aldehydes. This protocol is general and provides dihydropyrimidines in good to excellent yields depending on the reactivity of arylaldehydes. Thus, the present synthesis of pyrimidines will serve as an exclusive method of preparative importance for this class of compounds. However, the newly synthesized compounds were inactive against HIV-1 III_B and ROD strains.

Chapter 2 and 3, we have exhibited a solution-phase library of pyrazoles/isoxazoles functionalized with methyl, sulfone and carboxamide groups in two steps with excellent yield and chemical purity for biological interest. Water was emerged as an efficient and green solvent in the condensation reaction of various ketene dithioacetals with binucleophile such as; hydrazine hydrate and hydroxyl amine. Further, the oxidation of sulfide was achieved by sodium perborate to synthesized sulfone group containing pyrazoles/isoxazoles. Sodium perborate is more efficient and effective for oxidation of sulfide to sulfone in aqueous medium. This procedure offers a good scope for the synthesis of a wide variety of pyrazoles containing caboxamide and sulfone in two steps with excellent yield, purity and simple isolation of products. The biological screening of the synthesized compounds is under process.

Chapter 4, we have demonstrated a novel synthetic strategy for the synthesis of substituted 3-nitro-4*H*-chromen-4-ones through [5+1] heteroannulation of readily accessible 2-hydroxy-*ω*-nitro acetophenone with carbon disulfide and followed by straightforward nucleophilic addition through elimination of methylthio with various amines. The direct C-N bond formation reaction at C2 was achieved by the presence of nitro functionality at C3 position of chromenone. The newly developed methodology allows direct access to 2-substituted-3-nitro chromenone in excellent yield and high chemical purity. The presence of nitro functionality further makes them useful substrates for various transformations for biological

interest. Thus, the ω-nitro acetophenone has been useful for the synthesis of nitro group containing chromenones. The biological screening of the synthesized compounds is under process.

Chapter 5, we have described the synthesis substituted pyrazolopyridone derivatives in excellent yields. The reaction of various ketene dithioacetals with cyanoacetamide was afforded the pyridone derivatives in the presence of base with good yields. Sodium isopropoxide was found as an efficient base for the synthesis of pyridones. The pyridones were further reacted with hydrazine hydrate to furnished pyrazolopyridones in excellent yields with short reaction time. The synthesized compounds were found inactive against HIV-1 III_B and ROD strains.

Chapter 6, We have demonstrated the crystal and molecular structure of newly synthesized compounds 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one and 2-(methylthio)-3-nitro-4*H*-chromen-4-one by the singly crystal x-ray diffraction technique. In compound 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one, the pyrimidine ring adopts a flattened boat conformation and the phenyl ring **1** (C7-C8-C9-C10-C11-C12) and phenyl ring **2** (C18-C19-C20-C21-C22-C23) adopts an axial and equatorial conformations with the pyrimidine ring, respectively. The nitro group is almost coplanar with the pyrimidine ring. The structure exhibits intermolecular hydrogen bonds of the type N-H…O and O-H…O, which bind the molecules into one-dimensional polymeric chains. The compound 2-(methylthio)-3-nitro-4*H*-chromen-4-one shows planar conformation and molecule exhibits inter-molecular hydrogen bonds of the type C-H...O.

List of Publications

- **Mahesh M. Savant**, Akshay M. Pansuriya, Chirag V. Bhuva, Naval Kapuriya, Anil S. Patel, Vipul B. Audichya, Piyush V. Pipaliya and Yogesh T. Naliapara*. *Journal of Combinatorial Chemistry,* **2010**, *12*, 176-180.

- **Mahesh M. Savant**, Akshay M. Pansuriy, Chirag V. Bhuva, Naval Kapuriya, Yogesh T. Naliapara* *Catalysis Letters,* **2009**, *132*, 281-284.

- **Mahesh M. Savant**, Akshay M. Pansuriya, Chirag V. Bhuva, J. V. Padaliya, H. H. Bhat, Yogesh T. Naliapara* *Organic Chemistry: An Indian journal*, **2009,** *5(2)*, 237-242,

- **Mahesh M. Savant**, Akshay M. Pansuriya, Chirag V. Bhuva, Naval Kapuriya, Anil S. Patel, Vipul B. Audichya, Piyush V. Pipaliya and Yogesh T. Naliapara*. *Der Pharmacia Lettre*. **2009***, 1 (2),* 277-285.

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V. Bhuva, Jyoti Singh, and Yogesh T. Naliapara* *ARKIVOC,* **2009,** *12*, 254-260.

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V, Bhuva, Jyoti singh and Yogesh T. Naliapara*. *ARKIVOC*, **2009,** *7*, 79-85.

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V. Bhuva, Naval Kapuriya, Jyoti Singh, Yogesh T. Naliapara*. *Letters in Organic Chemistry,* **2009,** *8*, 619-623.

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V. Bhuva, Jyoti Singh, Naval Kapuriya, Yogesh T. Naliapara*. *Journal of Heterocyclic chemistry*, (Accepted)

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V. Bhuva, Jyoti Singh, Yogesh T. Naliapara*. *E-Journal of Chemistry*, (Accepted)

- Akshay M. Pansuriya, **Mahesh M. Savant**, Chirag V. Bhuva, Naval Kapuriya, Piyush Pipaliya, Anil Patel, Vipul Audichya, Yogesh T. Naliapara*. *E-Journal of Chemistry*, (Accepted)

- Synthesis, Characterization, Crystal and Molecular Structure Analysis of 3,4-dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenylpyrimidin-2(1*H*)-one
  Mahesh M. Savant, Lakshminarayana B. gowda, Akshay M. Pansuriya, Chirag V. Bhuva, Sridhar M. Anandalwar, J. Shashidhara Prasad, Anamik Shah, Yogesh T. Naliapara* *Journal of Chemical Crystallography* (Under review)

- A novel concise synthetic strategy to functionalized chromenones via [5+1] heteroannulation and facile C-N/C-S/C-O bond formation with various nucleophiles.
  Mahesh M. Savant, Neetha S., Akshay M. Pansuriya, Chirag V. Bhuva, Naval Kapuriya, Sridhar M. A., Shashidhara Prasad J., Anamik Shah, Yogesh T. Naliapara* *The Journal of organic chemistry* (Under review)

- Synthesis and anti-viral screening of some novel 3-amino-4,5-dihydro-6-methyl-4-oxo-*N*-aryl-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide derivatives.(Manuscript under preparation)

Conferences participated

- Two days seminar on "Entrepreneurship development & industrial opportunities in Gujarat" held at yogidham campus at Rajkot during 20th & 21st Sept. **2003** by sarvodaya kelavani samaj.

- UGC sponsored National workshop on "E-Resources in chemical synthesis and natural products" heat at DOC, Saurashtra University, Rajkot during 2nd and 3rd March **2006**.

- "International conference on the interface of chemistry-biology in biomedical research" held at Birla institute of Technology and Science, Pilani, during 22nd to 24th February **2008**.

- UGC sponsored National workshop on "Management and use of chemistry databases and patent literature" held at DOC, Saurashtra University, Rajkot during 27th to 29th February **2008**.
- "Frontier Lectures Series in Chemistry" organized by CSMCRI-Bhavnagar and JNCASR-Bangalore at CSMCRI, Bhavnagar on **2008**.

- 11th CRSI National Symposium in Chemistry held at NCL, Pune, Feb, 6-8, **2009**

- National Workshop on Updates in Process & Medicinal Chemistry held at Saurashtra University, Rajkot, March, 3-4, **2009**.

- National Conference on Stereochemistry and Spectroscopy held at Saurashtra University, Rajkot, March, 18-20, **2009**.

Water Mediated Construction of Trisubstituted Pyrazoles/Isoxazoles Library Using Ketene Dithioacetals

Mahesh M. Savant, Akshay M. Pansuriya, Chirag V. Bhuva, Naval Kapuriya, Anil S. Patel, Vipul B. Audichya, Piyush V. Pipaliya, and Yogesh T. Naliapara*

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A small molecule library of alkyl, sulfone, and carboxamide functionalized pyrazoles and isoxazoles has been developed via a rapid sequential condensation of various α-acylketene dithioacetals (**1a**−**o**) with hydrazine hydrate or hydroxylamine hydrochloride, followed by oxidation of sulfide to sulfone using water as the reaction medium. An efficient and safe oxidation of sulfides (**4/5a**−**o**) to the corresponding sulfones (**6/7a**−**o**) using sodium per borate system in aqueous medium is reported. The concise and two step synthesis of trisubstituted pyrazoles and isoxazoles was investigated under variety of reaction condition. The newly developed methodology has the advantage of excellent yield and chemical purity with short reaction time using water as a solvent.

Introduction

In recent decades, combinatorial chemistry tools have enabled the rapid synthesis of a large number of heterocyclic small molecule libraries and it is recognized now as a key element of early drug discovery.[1] The main advantage of the combinatorial technique is the speed at which diverse types of organic compounds can be synthesized, formulated, and tested for a particular application. Moreover, in combinatorial study the quantity of required material is less in comparison to conventional methods, which makes it more suitable when the materials are expensive.[2]

The development of new methods for the synthesis of five member heterocyclic compound libraries, both in solution and in solid phase, is an ever-expanding area in combinatorial chemistry. Specifically, those containing the pyrazole and isoxazole nucleus have been widely used as key building blocks for pharmaceutical agents. Its derivatives are endowed with high pharmacological properties, for example, hypoglycemic, analgesic, anti-inflammatory, antibacterial, anti-HIV, and anticancer activity,[3] as well as useful activities in conditions like schizophrenia, hypertension, and Alzheimer's disease.[4] In addition, they also have agrochemical properties including herbicidal and soil fungicidal activity; thus, they have been used as pesticides and insecticides.[5] Recently, pyrazoles containing aryl substituted emerged as p38 Kinase inhibitors, antiparasitic activities.[6]

Among these, pyrazoles and isoxazoles bearing sulfone and carboxamide moieties demonstrated to have significant pharmacological applications. For examples, cyclooxygenase-2 (COX-2) selective inhibitors, celecoxib (**1**),[7] rofecoxib (**2**),[8] and valdecoxib (**3**)[9] are currently prescribed for the treatment of arthritis and inflammatory diseases (Figure 1, **1**−**3**). These COX-2 inhibitors exhibited anti-inflammatory activity with reduced gastrointestinal side effects. Oxacillin and its derivatives are useful compounds because of their narrow spectrum anti biotic properties[10] (Figure 1, **4**). Recently, pyrrolyl aryl sulfones have been reported by Silvestri et al.[11] and Artico et al.[12] as a new class of human immunodeficiency virus type 1 (HIV-1) RT inhibitors acting at the non-nucleoside binding site of this enzyme. Haruna et al.,[13] have synthesized the propargylic sulfones with various planar molecules and evaluated their DNA binding properties and DNA cleavage activity. Moreover, the 1-(4-methylsulfonyl)benzene and 4-(4-methylsulfonyl)benzene substituted pyrazole compounds containing a nitric oxide donating group at the 3-position of the pyrazole ring, respectively, have been synthesized and evaluated for their ability to inhibit COX isoenzymes in human whole blood.[14] Pyrazoles containing a sulfone group at *N* position have been exhibited promising antimicrobial activity.[15] Furthermore, amide groups linked with isoxazole derivatives are found to

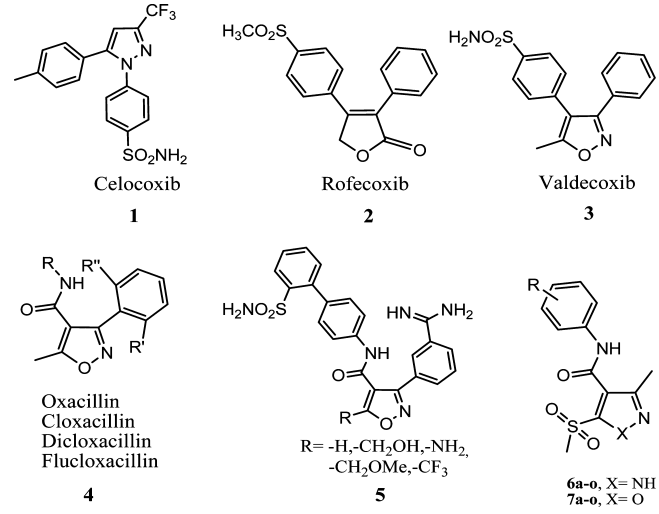

Figure 1. Biologically active pyrazoles and isoxazoles containing alkyl, sulfone, and carboxamide groups (**1**−**5**).

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have combined α_2-adrenoceptor antagonistic and serotonine reuptake inhibiting activities.[16] The isoxazoles containing aryl and carboxamide (Figure 1, **5**) were also shown to have potent in vivo antithrombotic efficacy.[17]

As described above, the tremendous biological potential of the sulfone group and carboxamide group bearing pyrazole and isoxazole scaffolds have attracted many chemists to synthesize this class of molecules. The classical methods to synthesized pyrazoles and isoxazoles involves the condensation of a 1,3-dicarbonyl compound or its synthetic equivalent with hydrazine in appropriate organic solvent.[18] On the other hand, functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the construction of substituted heterocycles such as pyrazoles and isoxazole. The nucleophilic displacement of one of the alkylthio groups from ketene dithioacetals either in an organic solvents or using microwave irradiation which followed by cyclization to afforded the heterocycles.[19] The sulfone group containing synthesis of pyrazoles and isoxazoles library from 2-sulfonylacetonitriles using solid-phase strategy is reported. However, it required a long reaction time, 40 h, and a lengthy workup process.[20] Thus, the practical synthesis of structurally diverse isoxazole/pyrazole based small molecules is of great significance.

Nowadays, a great deal of effort has been focused on the field of green chemistry in adopting methods and processes. As a part of this "green" concept, toxic and/or flammable organic solvents are replaced by alternative non-toxic and nonflammable media. In this context, many efforts have been made to use aqueous media. Among alternative green solvents, water has been the solvent of choice for a variety of transformations.[21] Given the importance of sulfone and carboxamide group containing pyrazoles and isoxazoles, we set out to prepare a small molecule library of 3-methyl-5-(methylsulfonyl)-*N*-aryl-1*H*-pyrazole/isoxazole-4-carboxamide derivatives using ketene dithioacetals in aqueous medium (Figure 1, **6/7a**−**o**).

Herein, we wish to report a novel synthesis of alkyl, methylsulfonyl, and carboxamide functionalized pyrazole or isoxazole heterocyles via condensation of α-acylketene dithioacetals (α-AKDTAs) with hydrazine hydrate or hydroxyl amine hydrochloride and followed by oxidation of sulfide to sulfone using sodium per borate (SPB) in aqueous medium. To our knowledge, this is the first attempt to construct 3-methyl-5-(methylsulfonyl)-*N*-aryl-1*H*-pyrazole/isoxazole-4-carboxamide in solution phase.[19a,22]

Results and Discussions

A series of various α-AKDTAs **1a**−**o** was prepared by some modification in reported procedure.[23] Initially, condensation of α-AKDTA **1a** with hydrazine hydrate **2** took place smoothly in isopropyl alcohol reflux to afford the 3-methyl-5-(methylsulfonyl)-*N*-phenyl-1*H*-pyrazole-4-carboxamide **4a** in good yield (Scheme 1; Entry 1, Table 1). The condensation of **1a** with **2** to generate pyrazole **4a** was investigated using a variety of solvents, as a part of the "green chemistry" concept and to optimize the yield, and the results are summarized in Table 1.

Scheme 1. Synthesis of Trisubstituted Pyrazoles and Isoxazoles in Aqueous Medium

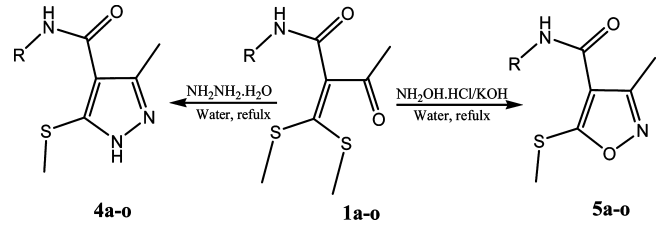


Table 1. Synthesis of 3-Methyl-5-(methylthio)-*N*-phenyl-1*H*-pyrazole-4-carboxamide **4a** Using Variety of Solvents

entry[a]	solvents	time, h	yield[b] %
1	^{i}PrOH	2.8	85
2	MeOH	4.0	81
3	EtOH	3.5	83
4	THF	4.5	79
5	CH_3CN	4.0	75
6	dioxane	3.5	80
7	water	3.0	97

[a] All solution-phase reactions were conducted at reflux temperature of the solvent used. [b] Isolated yield after purification.

The condensation reaction was clean in water, and the yield of desired product was higher (Entry 7, Table 1). On the other hand, the reaction was relatively fast when ^{i}PrOH was used as a solvent with 12% lower yield (Entry 1, Table 1). The yield of desired product was reasonable when MeOH, EtOH, and dioxane were used as a solvent (Entry 2,3,6, Table 1). The other solvents, tetrahydrofuran (THF) and acetonitrile, gave lower yield with higher reaction time (Entry 4,5, Table 1). Thus, it is clear from the aforementioned experiments that the best yield of pyrazoles **4a** could be obtained by employing water as a solvent without using any phase transfer catalyst.

To test the generality of the condensation and to realize the synthesis of a small combinatorial library of substituted pyrazoles and isoxazoles, **15** α-AKDTAs **1a**−**o** were reacted with hydrazine hydrate **2** or hydroxyl amine hydrochloride **3** and potassium hydroxide to furnish pyrazoles **4a**−**o** and isoxazoles **5a**−**o** in excellent yield using water as a solvent (Scheme 1, Table 2). The synthesized compounds were characterized by spectral data. The ^{1}H NMR spectra of compound **4c** displayed characteristic singlet for methyl, mehtylthio, and methoxy hydrogen, respectively, at δ 2.54, 2.64, and 3.92. The two singlets appeared for pyrazole *NH* at δ 10.12 and amide hydrogen at δ 9.62 which revealed the formation of pyrazole ring. However, in ^{1}H NMR of isoxazole **5b** a characteristic singlet for amide proton appeared at δ 9.19 and hydrogen of methylthio group displayed a singlet at δ 2.63.

Because of the remarkable utility of sulfone group in pharmaceuticals and to develop a library of pyrazole and isoxazole functionalized with alkyl, carboxamide, and sulfone, we next planned to oxidize the sulfides to sulfones. Although sulfides can be easily oxidized by a wide variety of oxidizing reagents, unfortunately some of these reagents are not satisfactory for the oxidation of sulfide to sulfone because of low yields of products, toxicity, and expensive reagents or catalysts.[24] The reaction condition for oxidation of sulfide to sulfone was optimized with a variety of oxidizing agent in various solvent (Table 3, Scheme 2).

Table 2. 3-Methyl, 5-Methylthio, 4-Carboxamide Substituted Pyrazoles and Isoxazoles

entry	R	time, h	yield[a] %	mp, °C
4a	Ph	3.0	97	120−122
4b	4-CH_3Ph	3.5	95	125−127
4c	4-CH_3OPh	3.0	96	118−120
4d	4-FPh	2.8	94	132−134
4e	2-CH_3OPh	2.9	92	126−128
4f	2-CH_3	3.2	93	122−124
4g	4-ClPh	3.8	94	128−130
4h	4-EtPh	3.5	95	130−132
4i	4-NO_2Ph	2.5	91	135−137
4j	3-Cl,4-FPh	3.2	90	128−130
4k	5-Cl,2-CH_3OPh	3.0	93	136−138
4l	2,5-diClPh	3.4	89	126−128
4m	2,5-diCH_3Ph	3.2	91	122−124
4n	4-Cl,2-CH_3Ph	2.9	94	121−123
4o	3,4-diFPh	3.2	93	130−132
5a	Ph	2.5	94	135−137
5b	4-CH_3Ph	2.8	92	141−142
5c	4-CH_3OPh	3.0	92	128−130
5d	4-FPh	3.2	90	142−144
5e	2-CH_3OPh	2.6	88	136−138
5f	2-CH_3	3.0	87	133−135
5g	4-ClPh	2.9	89	145−147
5h	4-EtPh	3.3	90	147−148
5i	4-NO_2Ph	2.5	87	149−151
5j	3-Cl,4-FPh	3.4	88	134−136
5k	5-Cl,2-CH_3OPh	3.6	90	151−153
5l	2,5-diClPh	2.8	89	142−144
5m	2,5-diCH_3Ph	2.9	98	136−138
5n	4-Cl,2-CH_3Ph	3.0	87	137−139
5o	3,4-diFPh	3.2	91	146−148

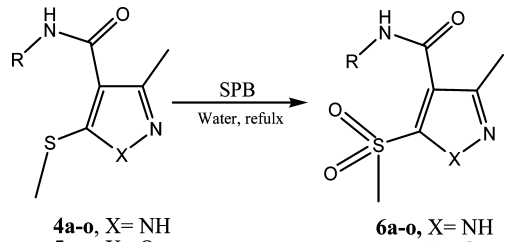
[a] Isolated yield after purification.

Table 3. Optimization of the Reaction Condition for Oxidation of **4a** and **5a** to Its Sulfone

entry[a]	oxidant[b]	solvent	yield[c] % **6a: 7a**	time, min
1	mCPBA	CH_2Cl_2	74:76	125
2	mCPBA	acetone	56:60	95
3	mCPBA	water	65:64	75
4	SPB	CH_2Cl_2	79:82	110
5	SPB	acetone	62:64	95
6	SPB	water	91:94	60
7	SPC	CH_2Cl_2	59:60	120
8	SPC	acetone	52:55	90
9	SPC	water	75:77	60

[a] All solution phase reactions were heated at reflux temperature of the solvent used. [b] Oxidant: mCPBA-2 equiv, SPB-3 equiv, and SPC-3 equiv. [c] Isolated yields after purification.

Scheme 2. Water Mediated Synthesis of Pyrazoles and Isoxazoles Containing Methyl, Sulfone, and Carboxamide Groups



4a-o, X= NH
5a-o, X= O

6a-o, X= NH
7a-o, X= O

The results gathered in Table 3 indicate that when dichloromethane was used as a solvent the yield of sulfone was higher with *m*-chloroperbenzoic acid (mCPBA) as compared to sodium per carbonate (SPC) and SPB, but it required high reaction time (Entry 1,4,7, Table 3). The yields of desired products were very poor when acetone was used as solvent, and the products were isolated using column chromatography (Entry 2,5,8, Table 3). The best results were obtained when water was used as solvent with the SPB, and the sulfide underwent oxidation to the corresponding sulfone in 45 min with excellent yield (Entry 6, Table 3). However, an excess amount of SPC did not improve yield. When the amount of SPB was reduced, the yield of desired product was lower. The above results indicate, the cheap, environmentally friendly and effective oxidizing agent in water was SPB and gave quantitatively yield of product without use of any activator. With this oxidizing system, all the synthesized compounds **4/5a−o** were oxidized to generate sulfone containing pyrazoles and isoxazole based small molecule library using solution phase synthesis, and the results are gathered in Table 4. The chemical purity of all newly synthesized compounds was examined using UPLC at 254 nm. Among all the final compounds, compounds **6k** and **7l** shown less than 95% chemical purity and other showed more than 95% chemical purity (Figure 2). The ^{1}H NMR spectrum of pyrazole **6j** displayed two characteristic singlets for the methyl and mehtylthio proton, respectively, at δ 2.53 and 3.64. However, two singlets appeared for pyrazole *NH* at δ 12.97 and amide hydrogen at δ 9.61. Compound **7a** displayed a characteristic singlet for amide proton at δ 9.88 and two singlets for methyl and mehtylthio hydrogen, respectively, at δ 2.69 and 3.33. The overall study indicates that this is the simple and facile methodology to introduce sulfone and caboxamide group to pyrazole and isoxazole scaffold in excellent yield and chemical purity.

Table 4. 3-Methyl, 5-Sulfone, 4-Carboxamide Functionalized Library of Pyrazoles and Isoxazoles

entry	R	time, min	yield[a] %	mp, °C
6a	Ph	60	91	168−170
6b	4-CH_3Ph	45	92	172−174
6c	4-CH_3OPh	55	89	166−168
6d	4-FPh	50	88	175−177
6e	2-CH_3OPh	60	90	170−172
6f	2-CH_3	50	94	165−167
6g	4-ClPh	55	89	173−175
6h	4-EtPh	65	92	177−179
6i	4-NO_2Ph	55	92	181−183
6j	3-Cl,4-FPh	50	91	176−178
6k	5-Cl,2-CH_3OPh	50	93	186−188
6l	2,5-diClPh	65	88	176−178
6m	2,5-diCH_3Ph	60	87	170−172
6n	4-Cl,2-CH_3Ph	55	89	171−173
6o	3,4-diFPh	50	85	181−183
7a	Ph	60	94	186−188
7b	4-CH_3Ph	50	95	192−194
7c	4-CH_3OPh	60	93	188−190
7d	4-FPh	55	91	191−193
7e	2-CH_3OPh	65	94	184−186
7f	2-CH_3	55	96	179−181
7g	4-ClPh	60	92	185−187
7h	4-EtPh	65	95	192−194
7i	4-NO_2Ph	60	93	196−198
7j	3-Cl,4-FPh	55	92	188−190
7k	5-Cl,2-CH_3OPh	55	91	195−197
7l	2,5-diClPh	60	90	188−190
7m	2,5-diCH_3Ph	55	91	181−183
7n	4-Cl,2-CH_3Ph	50	88	187−189
7o	3,4-diFPh	65	89	189−191

[a] Isolated yield after purification.

Conclusion

In summary, we have synthesized a solution-phase library of pyrazoles and isoxazoles functionalized with methyl,

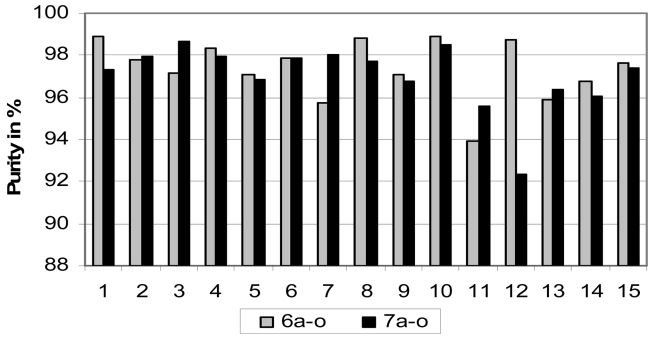


Figure 2. Chemical purity of trifunctionalized pyrazoles and isoxazoles using UPLC at 254 nm.

sulfone and carboxamide moieties in two steps with excellent yield and chemical purity for medicinally interesting molecules. Water emerged as an efficient and green solvent in the condensation reaction of various ketene dithioacetals with hydrazine hydrate or hydroxyl amine hydrochloride. Further, the facile synthesis of sulfone containing pyrazoles and isoxazoles was achieved via oxidation of sulfide to sulfone. A comparative study of various oxidants has been performed, and revealed that SPB is more efficient and effective for oxidation of sulfide to sulfone in aqueous medium. This procedure offers a good scope for the synthesis a wide variety of pyrazoles and isoxazoles containing caboxamide and sulfone in two steps. The present procedure is significant over the existing methods to develop this class of molecules with excellent yield, purity, and simple isolation of products. Currently, we are engaged to make further diversification of pyrazoles and isoxazoles at the C-3 position.

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Supporting Information Available. General experimental procedures for the synthesis of **1-o**, **4/5a−o**, and **6/7a−o**, analytical and spectral characterization data along with IR, Mass, UPLC purity, ^{1}H and ^{13}C NMR spectral copies. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (b) Booth, S.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1998**, *54*, 15385.

(2) (a) Devlin, J. P. *High Throughput Screening: The Discovery of Bioactive Substances*; Marcel Dekker: New York, 1997. (b) Gordon, E. M.; Kerwin, J. F., Jr. *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Wiley: New York, 1998.

(3) (a) Shin, K. D.; Lee, M. Y.; Shin, D. S.; Lee, S.; Son, K. H.; Koh, S.; Paik, Y. K.; Kwon, B. M.; Han, D. C. *J. Biol. Chem.* **2005**, *280*, 41439. (b) Demers, J.; Hageman, W.; Johnson, S.; Klaubert, D.; Look, R.; Moore, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2451. (c) Simoni, D.; Roberti, M.; Paolo, I. F.; Rondanin, R.; Baruchello, R.; Malagutti, C.; Mazzali, A.; Rossi, M.; Grimaudo, S.; Capone, F.; Dusonchet, L.; Meli, M.; Raimondi, M. V.; Landino, M.; D'Alessandro, N.; Tolomeo, M.; Arindam, D.; Lu, S.; Benbrook, D. M. *J. Med. Chem.* **2001**, *44*, 2308. (d) Liu, X. H.; Cui, P.; Song, B. A.; Bhadury, P. S.; Zhu, H. L.; Wang, S. F. *Bioorg. Med. Chem.* **2008**, *16*, 4075. (e) Velaparthi, S.; Brunsteiner, M.; Uddin, R.; Wan, B.; Franzblau, S. G.; Petukhov, P. A. *J. Med. Chem.* **2008**, *51*, 1999. (f) Magedov, I. V.; Manpadi, M.; Van slambrouck, S.; Steelant, W. F. A.; Rozhkova, E.; Przheval'skii, N. M.; Rogelj, S.; Kornienko, A. *J. Med. Chem.* **2007**, *50*, 5183.

(4) (a) Rowley, M.; Broughton, H. B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Ragan, C. I.; Freedman, S. B.; Leeson, P. D. *J. Med. Chem.* **1996**, *39*, 1943. (b) Wittenberger, S. J. *J. Org. Chem.* **1996**, *61*, 356. (c) Dannhardt, G.; Dominiak, P.; Laufer, S. *Arznei-Forschung.* **1993**, *43*, 441.

(5) (a) Pinho, E. M.; Teresa, M. V. D. *Curr. Org. Chem.* **2005**, *9*, 925. (b) Colliot, F.; Kukorowski, K. A.; Hawkins, D. W.; Roberts, D. A. *Brighton Crop Prot. Conf. Pests Dis.* **1992**, *1*, 29. (c) Chen, H. S.; Li, Z. M.; Han, Y. F. *J. Agric. Food. Chem.* **2000**, *48*, 5312. (d) Vicentini, C. B.; Romagnoli, C.; Reotti, E.; Mares, D. *J. Agric. Food Chem.* **2007**, *55*, 10331. (e) Vicentini, C. B.; Mares, D.; Tartari, A.; Manfrini, M.; Forlani, G. *J. Agric. Food Chem.* **2004**, *52*, 1898.

(6) (a) Graneto, M. J.; Kurumbail, R. G.; Vazquez, M. L.; Shieh, H. S.; Pawlitz, J. L.; Williams, J. M.; Stallings, W. C.; Geng, L.; Naraian, A. S.; Koszyk, F. J.; Stealey, M. A.; Xu, X. D.; Weier, R. M.; Hanson, G. J.; Mourey, R. J.; Compton, R. P.; Mnich, S. J.; Anderson, G. D.; Monahan, J. B.; Devraj, R. *J. Med. Chem.* **2007**, *50*, 5712. (b) Kuettel, S.; Zambon, A.; Kaiser, M.; Brun, R.; Scapozza, L.; Perozzo, R. *J. Med. Chem.* **2007**, *50*, 5833.

(7) Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.

(8) Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromilish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Wong, E.; Xu, L. J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.

(9) Talley, J. A.; Brown, D. L.; Carter, J. S.; Masferrer, M. J.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.

(10) (a) Lawrence, S. A.; Roth, V.; Slinger, R.; Toye, B.; Gaboury, I.; Lemyre, B. *BMC Pediatr* **2005**, *5*, 49. (b) Miranda, N. G.; Leanos, M. B. E.; Vilchis, P. M.; Solorzano, S. F. *Ann. Clin. Microbiol. Antimicrob.* **2006**, *5*, 25. (c) Sutherland, R.; Croydon, E. A.; Rolinson, G. N. *Br. Med. J.* **1970**, *4*, 455.

(11) Silvestri, R.; Artico, M.; Regina, G. L.; Martino, G. D.; Colla, M. L.; Loddo, R.; Colla, P. L. *Il Farmaco* **2004**, *59*, 201.

(12) (a) Artico, M.; Silvestri, R.; Stefancich, G.; Massa, S.; Pagnozzi, E.; Musiu, D.; Scintu, F.; Pinna, E.; Tinti, E.; Colla, P. L. *Arch. Pharm.* **1995**, *328*, 223. (b) Artico, M.; Silvestri, R.; Massa, S.; Loi, A. G.; Corrias, S.; Piras, G.; Colla, P. L. *J. Med. Chem.* **1996**, *39*, 522. (c) Artico, M.; Silvestri, R.; Pagnozzi, E.; Bruno, B.; Novellino, E.; Greco, G.; Massa, S.; Ettorre, A.; Loi, A. G.; Scintu, F.; Colla, P. L. *J. Med. Chem.* **2000**, *43*, 1886.

(13) Haruna, K.; Kanezaki, H.; Tanabe, K.; Daib, W. M.; Nishimotoa, S. *Bioorg. Med. Chem.* **2006**, *14*, 4427.

(14) (a) Ezawa, M.; Garvey, D. S.; Janero, D. R.; Khanapure, S. P.; Letts, L. G.; Martino, A.; Ranatunge, R. R.; Schwalb, D. J.; Young, D. V. *Lett. Drug Des. Discovery* **2005**, *2*, 40. (b) Ranatunge, R. R.; Augustyniak, M.; Bandarage, U. K.; Earl, R. A.; Ellis, J. L.; Garvey, D. S.; Janero, D. R.; Letts, L. G.; Martino, A. M.; Murty, M. G.; Richardson, S. K.; Schroeder, J. D.; Shumway, M. J.; Tam, S. M.; Trocha, A. M.; Young, D. V. *J. Med. Chem.* **2004**, *47*, 2180.

(15) Bonacorso, H. G.; Wentz, A. P.; Lourega, R. V.; Cechinel, C. A.; Moraes, T. S.; Coelho, H. S.; Zanatta, N.; Martins, A. P.; Hoerner, M.; Alves, S. H. *J. Fluorine Chem.* **2006**, *127*, 1066.

(16) Andre, I.; Alcazar, J.; Alonso, J. M.; Lucas, A. I. D.; Iturrino, L.; Biesmansb, I.; Megens, A. A. *Bioorg. Med. Chem.* **2006**, *14*, 4361.

(17) Pruitt, J. R.; Pinto, D. J.; Estrella, M. J.; Bostrom, L. L.; Knabb, R. M.; Wong, P. C.; Wright, M. R.; Wexler, R. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 685.

(18) (a) Makino, K. H. S.; Kurasawa, Y. *J. Heterocycl. Chem.* **1998**, *35*, 489. (b) Grotjahn, D. B.; Van, S.; Combs, D.; Lev, D. A.; Schneider, C.; Rideout, M.; Meyer, C.; Hernandez, G.; Mejorado, L. *J. Org. Chem.* **2002**, *67*, 9200.

(19) (a) Junjappa, H.; Ila, H; Asokan, C. V. *Tetrahedron* **1990**, *46*, 5432. (b) Kolb, M. *Synthesis* **1990**, *3*, 171. (c) Tominaga, Y. *J. Heterocycl. Chem.* **1989**, *26*, 1167.

(20) Wenli, M.; Peterson, B.; Kelson, A.; Laborde, E. *J. Comb. Chem.* **2009**, *11*, 697.

(21) (a) Adam, D. *Nature* **2003**, *421*, 571. (b) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250. (c) Ortiz, A. D.; Hoz, A.; Moreno, A. *Chem. Soc. Rev.* **2005**, *34*, 164. (d) Li, C. J. *Chem. Rev.* **1993**, *93*, 2023. (e) Li, C. J. *Chem. Rev.* **2005**, *105*, 3095.

(22) McKillop, A.; Sanderson, W. R. *Tetrahedron* **1995**, *51*, 6145.

(23) Bi, X.; Dong, D.; Liu, Q.; Pan, W.; Zhao, L.; Li, B. *J. Am. Chem. Soc.* **2005**, *127*, 4578.

(24) (a) Al-Ajlouni, A. M.; Daiafla, T. M.; El-Khateeb, M. *J. Mol. Catal. A: Chem.* **2007**, *275*, 139. (b) Egami, H.; Katsuki, T. *J. Am. Chem. Soc.* **2007**, *129*, 8940. (c) Shirini, F.; Zolfigol, M. A.; Abri, A. R. *Chin. Chem. Lett.* **2008**, *19*, 51. (d) Shaabani, A.; Rezayan, A. H. *Catal. Commun.* **2007**, *8*, 1112.

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Etidronic Acid: a New and Efficient Catalyst for the Synthesis of Novel 5-Nitro-3,4-Dihydropyrimidin-2(1*H*)-ones

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Abstract A simple, convenient and efficient one-pot cyclocondensation reaction of 1-(2-hydroxyphenyl)-2-nitroethanone, arylaldehydes and urea using etidronic acid to furnish nitro dihydropyrimidine derivatives is described. A new and efficient protocol is developed as a homogenous catalyst for the synthesis of dihydropyrimidines using substituted ω-nitro acetophenone. Various bisphosphonic acids were examined to synthesized pyrimidines via multicomponent cyclocondensation reaction. This methodology has the advantage of excellent yields with short reaction time.

Keywords Etidronic acid · Homogenous catalyst · Cyclocondensation · Nitro-dihydropyrimidine · Multicomponent

1 Introduction

The Biginelli reaction [1], one of the most useful multicomponent reactions, offers an efficient way to access multifunctionalized 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) and related heterocyclic compounds [2]. However, in biginelli reaction 1,3 diketone is used as a synthone. The ability of nitro group to enhance biological and therapeutic activities of certain organic compounds has led to widespread interest in the selective introduction of nitro groups into organic compounds [3] especially those heterocyclic molecules which possess potential biological activities. For example, DHPMs are important heterocycles in both natural and synthetic compounds, which exhibit various pharmacological properties such as calcium channel blockers, antihypertensive agents, antitumor, anti-inflammatory and neuropeptide antagonists [3, 4]. Dihydropyridines and dihydropyrimidinones contain an ester group in the position 5 of the heterocycle [2, 5]. However, Substitution of NO_2 for COOAlk in the dihydropyridines alters their biological action. Reports reveal that nitro group functionalized dihydropyrimidines which might have potential biological activities were less studied [6].

A major drawback to Biginelli's original reaction was poor to moderate yields [7]. Recently, many improved procedures have been reported using $InBr_3$ [8], $InCl_3$ [9], $LiClO_4$ [10], $FeCl_3.6H2O$ or $NiCl_2.6H2O$ [11], p-TsOH [12], $LaCl_3.7H_2O$ [13], IR radiation [14], $Bi(OTf)_3$ [15], $La(OTf)_3$ [16], BF_3 OEt_2 [17], ionic liquids (BMIm PF_6 and BMIm BF_4) [18], TEBA [19], natural HEU type zeolite [20], I_2 [21], *N*-bromosuccinimide (NBS) [22], polyaniline–bismoclite complex [23] and other Lewis acids [24] heteropoly acid [25], sulfated zirconia [26], $Sr(NO_3)_2$ [27], and covalently anchored sulfonic acid onto silica [28], PPE [29], Phosphoric acid [30]. However, some of the newer reported methods also suffer from drawbacks such as unsatisfactory yields, cumbersome product isolation procedures, and environmental pollution. Moreover, the main disadvantage of almost all existing methods is that the catalysts are destroyed in the workup procedure and cannot be recovered or reused. Therefore, still there is need for versatile, simple, and environmentally friendly processes whereby DHPMs may be formed under milder and practical conditions.

In continuation of our work on the development of useful synthetic methodologies by employing solid acid catalysts [31], we observed that bisphosphonic acid is an

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efficient catalyst for the synthesis of pyrimidines via biginelli condensation. To explore further, the utility of this catalyst in multicomponent cyclocondensation, herein we report some new nitro group containing dihydropyrimidine derivatives from substituted ω-nitro acetophenone instead of 1,3 diketone with excellent yield under various reaction conditions.

2 Experimental Section

2.1 Materials

Chemicals were supplied by E. Merck (Germany) and S. D. Fine Chemicals (India) and used without purification. The solvents were analytical grade. THF was distilled over sodium/benzophenone prior to use. Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F_{254} precoated plates. Silica gel (Loba, 100–200 mesh, 60 Å) for column chromatography was used as received.

2.2 Instrumentation

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO, and TMS was used as an internal reference on a Bruker AVANCE II spectrometer. Mass spectra were determined using direct inlet probe on a GCMS-QP2010 mass spectrometer. IR spectra were recorded on KBr discs, using FTIR-8400 spectrophotometer. The syntheses were carried out in a Questron Technologies Corporation QPro-M microwave synthesizer. Melting points were measured in open capillaries and are uncorrected.

2.3 General Procedure for the Synthesis of Nitro dihydropyrimidines **4a–n**

To a mixture of various aromatic aldehydes (10 mmol, **2a–n**) and urea (10 mmol, 0.60 g) in dry THF (5 mL) was added etidronic acid (0.1 mmol, 0.2 g) and stirred it for 5 min at r.t. to this add 1-(2-hydroxyphenyl)-2-nitroethanone (10 mmol, 0.18 g) and subjected to microwave irradiation at 360 W for appropriate time (Table 3). The reaction being monitored by TLC. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The separated solid was washed with water and followed by methanol, filtered, dried and crystallized from glacial acetic acid to furnish analytically pure products. The water layer is evaporated to recycle the catalyst.

2.3.1 Spectral Data for Selected Compounds

*2.3.1.1 3,4-Dihydro-6-(2-hydroxyphenyl)-5-nitro-4-phenyl-pyrimidin-2(1H)-one (**4a**)* Lemon yellow solid; mp 241–243 °C; IR (KBr): 3,624 (–OH), 3,076 (–NH), 1,674 (C=O) cm^{-1}; ^{1}H NMR: δ 5.74 (d, 1H, $J = 3.16$ Hz), 6.90–7.59 (m, 9H, Ar–H), 7.68 (s, 1H, NH), 8.97 (s, 1H, NH), 9.64 (s, 1H, OH); ^{13}C NMR: 55.30, 114.54, 116.08, 119.31, 120.38, 126.99, 128.73, 131.61, 142.12, 146.22, 151.53, 166.05; MS *m/z*: 311(M^+); Anal. calcd. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50%. Found: C, 61.58; H, 4.08; N, 13.33%.

*2.3.1.2 3,4-Dihydro-6-(2-hydroxyphenyl)-4-(3-chlorophenyl)-5-nitropyrimidin-2(1H)-one (**4b**)* Yellow solid; mp 255–257 °C; IR (KBr): 3,556 (–OH), 3,290(–NH), 1,672 (C=O) cm^{-1}; ^{1}H NMR: δ 5.71 (d, 1H, $J = 3.52$ Hz), 6.91–7.93 (m, 8H, Ar–H), 7.94 (s, 1H, NH), 9.17 (s, 1H, NH), 9.78 (s, 1H, OH); ^{13}C NMR: 54.40, 115.52, 117.18, 120.25, 122.31, 127.63, 129.63, 134.63, 145.22, 148.34, 153.53, 163.12; MS *m/z*: 345(M^+); Anal. calcd. for $C_{16}H_{12}ClN_3O_4$: C, 55.58; H, 3.50; N, 12.15. Found: C, 55.42; H, 3.38; N, 12.03%.

*2.3.1.3 3,4-Dihydro-6-(2-hydroxyphenyl)-4-(4-methoxyphenyl)-5-nitropyrimidin-2(1H)-one (**4c**)* Pale yello solid; mp 246–248 °C; IR (KBr): 3,649 (–OH), 3,292 (–NH), 1,678 (C=O) cm^{-1}; ^{1}H NMR: δ 3.79 (s, 1H, OCH_3), 5.65 (d, 1H, $J = 3.36$ Hz), 6.86–7.49 (m, 8H, Ar–H), 7.95 (s, 1H, NH), 9.42 (s, 1H, NH), 9.71 (s, 1H, OH); ^{13}C NMR: 54.65, 55.28, 113.91, 115.97, 119.24, 128.22, 128.61, 131.22, 134.45, 135.44, 136.30, 151.52, 159.28, 172.96, 181.34; MS *m/z*: 341(M^+); Anal. calcd. for $C_{17}H_{15}N_3O_5$: C, 59.83; H, 4.43; N, 12.31%. Found: C, 59.66; H, 4.32; N, 12.18%.

*2.3.1.4 3,4-Dihydro-6-(2-hydroxyphenyl)-4-(4-nitrophenyl)-5-nitropyrimidin-2(1H)-one (**4h**)* Lemon yellow solid; mp 268–270 °C; IR (KBr): 3,487 (–OH), 3,304 (–NH), 1688 (C=O) cm^{-1}; ^{1}H NMR: δ 5.82 (d, 1H, $J = 3.64$ Hz), 6.92–7.84 (m, 6H, Ar–H), 8.14 (s, 1H, NH), 8.20-8.22 (q, 2H, Ar–H), 9.31(s, 1H, NH), 9.76 (s, 1H, OH); MS *m/z*: 356 (M^+); Anal. calcd. for $C_{16}H_{12}N_4O_6$: C, 53.94; H, 3.39; N, 15.73%. Found: C, 53.75; H, 3.24; N, 15.26%.

3 Results and Discussions

Etidronic acid [(1-hydroxyethylidene) bisphosphonic acid] is one of the bisphosphonic acid derivative and also known

Fig. 1 Bisphophonic acid

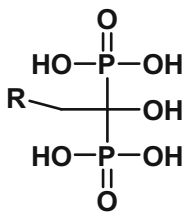

Table 1 Optimization of the reaction conditions for the synthesis of **4a**

Entry	Catalyst (equiv.)	Solvent	Yield (%)	Time
1	EDA (1.0)	THF[a]	89[c]	4.0 h
2	EDA (1.0)	THF[b]	95[d]	3.5 min
3	EDA (0.1)	THF[a]	86[c]	6.0 h
4	EDA (0.1)	THF[b]	93[d]	3.0 min
5	EDA (0.1)	MeOH[a]	73[e]	11.5 h
6	EDA (0.1))	MeOH[b]	82[e]	6.5 min
7	EDA (0.1)	EtOH[a]	78[e]	9.0 h
8	EDA (0.1)	EtOH[b]	86[e]	5.5 min

[a] All solution-phase reactions were conducted at reflux temperature of the solvent used All solution-phase reactions were conducted at reflux temperature of the solvent used

[b] The reaction was conducted under microwave irradiation (360 W)

[c] After 4–6 h reflux

[d] Isolated yield after purification

[e] After column chromatography

as bisphosphonate having molecular formula $C_2H_8O_7P_2$. The two PO_3 (phosphonate) groups covalently linked to carbon atom (Fig. 1).

It differs from Polyphosphate ester and polyphosphoric acid. Various bisphosphonic acids are known [32, 33]. Etidronic acid is mild enough as compare to another strong acid such as polyphosphoric acid etc. moreover, the catalyst did not affect acid sensitive aldehydes.

Indeed, condensation of the 1-(2-hydroxyphenyl)-2-nitroethanone **1** with benzaldehyde **2a** and urea **3** took place smoothly in the presence of EDA in THF resulted in the formation of dihydropyrimidine **4a** in 89% yield (entry 1, Table 1). We found that the final product obtained was dihydro biginelli product **4a** (Scheme 1). The condensation of **1** with **2a** and **3** to generate **4a** was investigated under a variety of conditions (Table 1), as a test case, to optimize the yield, and the results are gathered in Table 1. The condensation took place even with a catalytic amount of EDA (10%, entry 3). Though the condensation reaction with a catalytic amount of EDA was cleaned, it took a longer time (6 h). On the other hand, the reaction was relatively fast (4 h) when one equiv. of EDA was employed (entry 1). However, the reaction carried out under microwave irradiation gave excellent yields (entry 4, 2). The yield of desired product **4a** was moderate when methanol and ethanol was used as solvent (entry 5–8) and in this case product **4a** was separated by column chromatography over silica gel using hexane/EtOAc (7:3) as an eluent.

With the optimized conditions in hand, the reactions of **1** with benzaldehyde **2a** and urea **3** with various bisphosphonic acids were examined to explore the utility of these catalysts in multicomponent cyclocondensation reaction under microwave irradiation in THF. We found that bisphosphonic acid linked with alkyl amines (Table 2, entry 2–4) or with heterocyclic moieties (Table 2, entry 5–8) showed very poor catalytic activity compared to EDA (Table 2, entry 1). This can be explained by the fact that electron donating moieties attached with bisphosphonic acid at C_2 position may decreases the reactivity of these catalysts leading to moderate or poor yields of **4a**. Thus, it is clear from the aforementioned experiments that the best yield of compound **4a** could be obtained by employing catalytic amount of etidronic acid in THF under microwave irradiation.

When the reaction of the 1-(2-hydroxyphenyl)-2-nitroethanone **1** with various arylaldehydes **2a** and urea **3** was conducted it was observed that the electron deficiency and nature of the substituents on the aromatic ring aldehydes effect the conversion rate; aromatic aldehydes having electron-withdrawing groups on the aromatic ring (Table 3,

Table 2 Synthesis of nitro dihydropyrimidines **4a** using various catalyst and THF under microwave irradiation

Entry	*R*	Catalyst (equiv.)	Yield (%)	Time (min)
1	–H	Etidronic acid (0.1)	93	3.0
2	$-CH_2NH_2$	Pamidronic acid (0.1)	65	9.5
3	$-CH_2NH_2$	Pamidronic acid (1.0)	68	8.0
4	$-(CH_2)_2NH_2$	Alendronic acid (0.1)	66	8.0
5	$-(CH_2)_2NH_2$	Alendronic acid (1.0)	67	9.5
6	-3-Pyridyl	Risedronic acid (0.1)	55	11.0
7	-3-Pyridyl	Risedronic acid (1.0)	59	9.5
8	-1-Imidazolyl	Zoledronic acid (0.1)	45	11.5
9	-1-Imidazolyl	Zoledronic acid (1.0)	51	11.0

Scheme 1 Etidronic acid catalyzed one-pot synthesis of nitro functionalized dihydropyrimidines under microwave irradiation

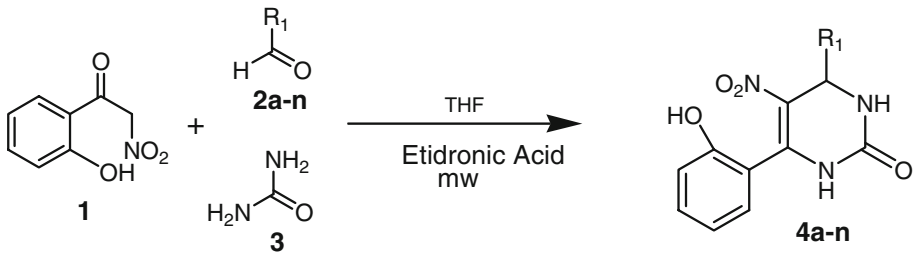

Table 3 Synthesis of nitro functionalized dihydropyrimidines using etidronic acid (catalyst) and THF under microwave irradiation

Entry	R^1	Products	Yields[a] (%)	Time (min)
1	Ph	**4a**	93	3.0
2	3-Cl Ph	**4b**	86	4.5
3	4-OCH_3 Ph	**4c**	91	4.0
4	4-Cl Ph	**4d**	89	4.0
5	4-F Ph	**4e**	90	4.5
6	3-OCH_3 Ph	**4f**	92	3.0
7	2-Cl Ph	**4g**	86	4.5
8	4-NO_2 Ph	**4h**	93	2.5
9	3-NO_2 Ph	**4i**	91	3.0
10	3,4-di-OCH_3 Ph	**4j**	93	3.5
11	4-OH Ph	**4k**	88	4.0
12	3-OH Ph	**4l**	86	4.5
13	3-Br Ph	**4m**	86	4.0
14	2,4-di-Cl Ph	**4n**	89	3.5

[a] Isolated yields after purification

entries 6, 8, 9) reacted faster than electron-donating groups (Table 3, entries 2, 11, 12). The synthesized compounds were characterized by spectroscopy analysis. In mass spectrum of **4a** molecular ion peak appears at 311 *m*/*z* which reveal the formation of dihydropyrimidine. The ^{1}H NMR spectrum of **4a** displayed one characteristic doublet for the methane proton at 5.74 δ ppm and one –OH proton at 9.64 δ ppm. The overall study indicates the catalyst is efficient to synthesize nitro dihydropyrimidines.

4 Conclusions

In summary, we have demonstrated a simple route for the synthesis of nitro group containg dihydropyrimidines via cyclocondensation reactions using etidronic acid as an efficient homogeneous catalyst. The use of etidronic acid was well tolerated with a range of aldehydes. This protocol is general and provides dihydropyrimidines in good to excellent yields depending on the reactivity of arylaldehydes. Thus, the present synthesis of pyrimidines will serve as an exclusive method of preparative importance for this class of compounds. We are currently engaged in the application of this catalyst for the electrophilic substitution.

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References

1. Biginelli P (1893) Gazz Chim Ital 23:360
2. Kappe CO (2000) Eur J Med Chem 35:1043
3. Arkadiy OB, Margarita PD, Irina VS, Tatiana GT, Valentina FS, Oleg Bioorg PS (2006) Med Chem Let 16:1418
4. Russowsky D, Canto RFS, Sanches SAA, Doca MGM, Fatima AD, Carvalho Bioorg JED (2006) Chem 34:173
5. Kappe CO (1993) Tetrahedron 49:6937
6. Remennikov GY (1997) Khim Geterotsikl Soedin 1587
7. Barluenga J, Thomas M, Ballesterus A (1989) A Lopez Tetrahedron Lett 30:4573
8. Fu NY, Yuan YF, Cao Z, Wang SW, Wang JT, Peppe C (2002) Tetrahedron 58:4801
9. Ranu B, Hajra A, Jana U (2000) J Org Chem 65:6270
10. Yadav JS, Reddy BVS, Srinivas R, Venugopal C, Ramalingam T Synthesis (2001) 1341
11. Sedova VF, Krivopalov VP, Shkurko OP (2007) Russian J Org Chem 43:90
12. Jin T, Zhang S, Li T (2002) Synth Commun 32:1847
13. Lu J, Bai Y, Wang Z, Yang B, Ma H (2000) Tetrahedron Lett 41:9075
14. Osnaya R, Arroyo GA, Parada L, Delgado F, Trujillo J, Salmon M, Miranda R (2003) Arkivoc 11:112
15. Varala R, Alam MM, Adapa SR (2003) Synlett 67
16. Ma Y, Qian C, Wang L, Yang M (2000) J Org Chem 65:3864
17. Hu EH, Slider DR, Dolling UH (1998) J Org Chem 63:3454
18. Eng J, Deng Y (2001) Tetrahedron Lett 42:5917
19. Bose DS, Sudharshan M, Chavhan SW (2005) Arkivoc 3:228
20. Ajbakhsh M, Mohajerani B, Heravi MM, Ahmadi AN (2005) J Mol Catal A Chemical 236:216
21. Srinvas KVN, Dash B (2004) Synthesis 2091
22. Hazarkhani H, Karimi B (2004) Synthesis 1239
23. Gangadasu B, Palaniappan S, Rao VJ (2004) Synlett 1285
24. Kappe CO, Kumar D, Varma RS (1999) Synthesis 1799
25. Rafiee E, Jafari Bioorg H (2006) Med Chem Lett 16:2463
26. Kumar D, Sundaree MS, Mishra BG (2006) Chem Lett 35:1074
27. Liu C, Wang J, Li Y (2006) J Mol Catal A Chem 258:367
28. Gupta R, Paul S, Gupta R (2007) J Mol Catal A Chem 266:50
29. Falsone FS, Kappe CO (2001) Arkivoc 2:122
30. Chen XH, Xu XY, Liu H, Cun LF, Gong LZ (2006) J Am Chem Soc 128:14802
31. Pansuriya AM, Savant MM, Bhuva CV, Singh J, Naliapara YT (2009) Arkivoc 7:79
32. Merck Index (2001) An encyclopedia of chemicals, drugs and biologicals, 13th ed, pp 683
33. Pandey SC, Haider H, Saxena S, Singh MK, Thaper RK, Dubey SK (2006) WO134603 A1

Use of cyclic aliphatic ketones for spiro 2-amino-3-cyano pyrano[3,2-*c*]chromene formation

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Abstract
The three component reaction between 4-hydroxycoumarin, malononitrile and carbonyl compounds in ethanol in the presence of morpholine as a catalyst was studied. Only cyclic aliphatic ketones afford spiro 2-amino-3-cyanopyrano[3,2-*c*]chromene derivatives.

Keywords: 4-Hydroxycoumarin, malononitrile, cyclic aliphatic ketone, spiro, pyrano[3,2-*c*]chromene

Introduction

A key intermediate in the synthesis of warfarin (rodenticide, a blood anticoagulant) is 2-amino-3-cyano-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene **3** prepared by heating 4-hydroxycoumarin **1** with benzylidenemalononitrile **2** in pyridine[1] or water.[2] Acid hydrolysis of the pyrano[3,2-*c*]chromene **3** affords compound **4**, which is subsequently transformed into warfarin **5**.[1-5]

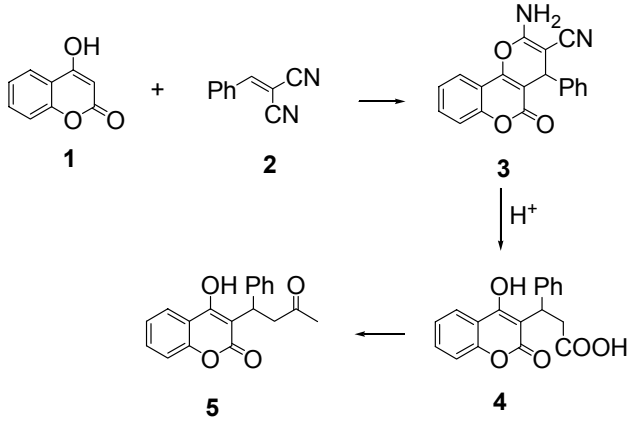


Figure 1

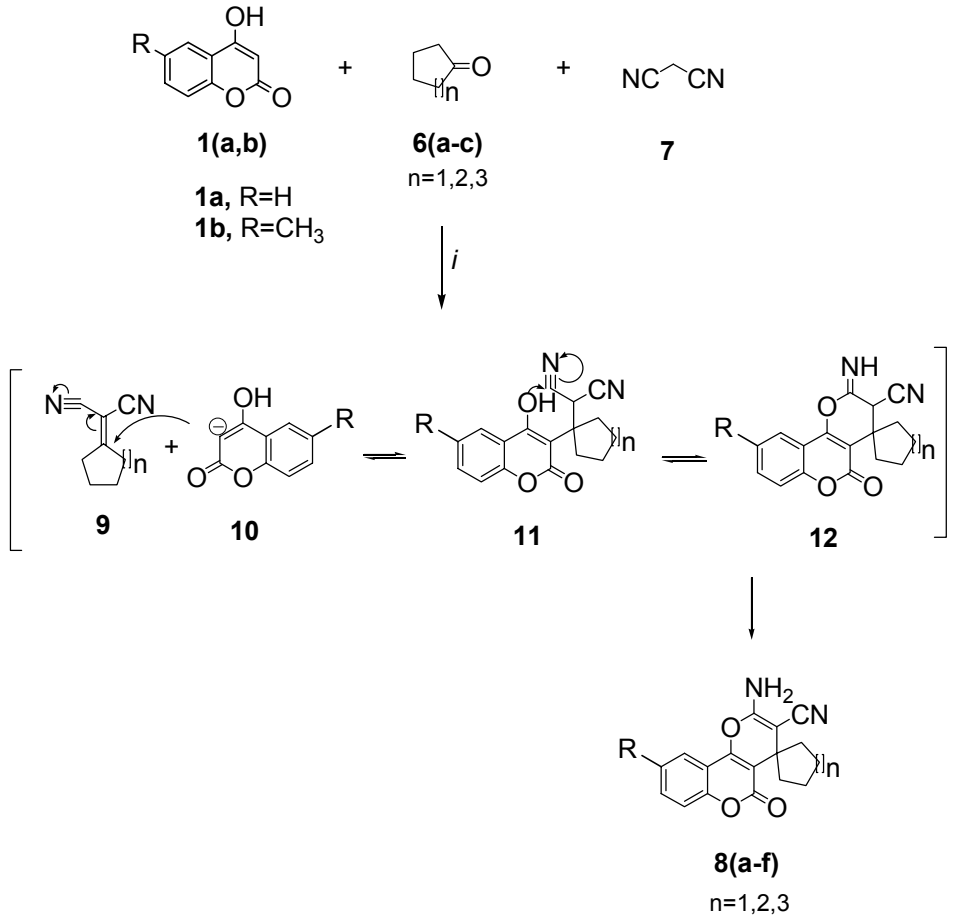
2-Amino-4-aryl-3-(thiocarbamoyl, alkoxycarbonyl, or cyano)-5-oxo-4,5-dihydropyrano[3,2-*c*]chromenes were also obtained[3,6] by morpholine catalyzed reaction between 4-hydroxycoumarin with arylidenecyanothioacetamide, alkyl arylidenecyanoacetates, or arylidenemalononitrile in hot benzene or ethanol. Despite modest achievements in recent years for the synthesis of compounds of type **3** having substituents in position 4, the preparation and isolation of unsaturated nitriles **2**, which are analogs of toxic agents (2-chlorobenzylidene)malononitrile (CS),[7-23] may substantially complicate the aforementioned synthesis (Figure1). In some cases, the condensation does not yield the target unsaturated nitrile at all. For instance reaction between pyridine-4-carbaldehyde and malononitrile yields 1-amino-2,4,4,6,6-pentacyano-3,5-di(4-pyridyl)cyclohex-1-ene.[24] This precludes the synthesis of pyrano[3,2-*c*]chromenes containing the 4-pyridyl substituent in position 4.

N-Substituted piperidine-4-one derivatives were failed to afford spiro[piperidine-4,4´-pyrano[3,2-*c*]chromenes],[25] while proficient to afford spiro[piperidine-4,4´-pyrano[3,2-*c*]quinoline][26] derivatives by one-pot multicomponent reaction of 4-hydroxyquinolone, cyanoacetic acid derivatives and substituted piperidine-4-one derivatives. However, isatin derivatives are proficient as cyclic ketone to afford spiro[2–amino–5–oxo–4,5–dihydropyrano[3,2–*c*]chromenes][25] by cross coupling reaction of 4-hydroxycoumarin, cyanoacetic acid derivatives and isatin derivatives.

In further investigations of cross coupling between cyanoacetic acid derivatives and carbonyl compounds with the aim of developing one step syntheses of functionalized heterocycles, we studied three component system reactions of 4-hydroxycoumarin, malononitrile, and carbonyl compounds (cyclic and non-cyclic aliphatic ketones).

Results and Discussion

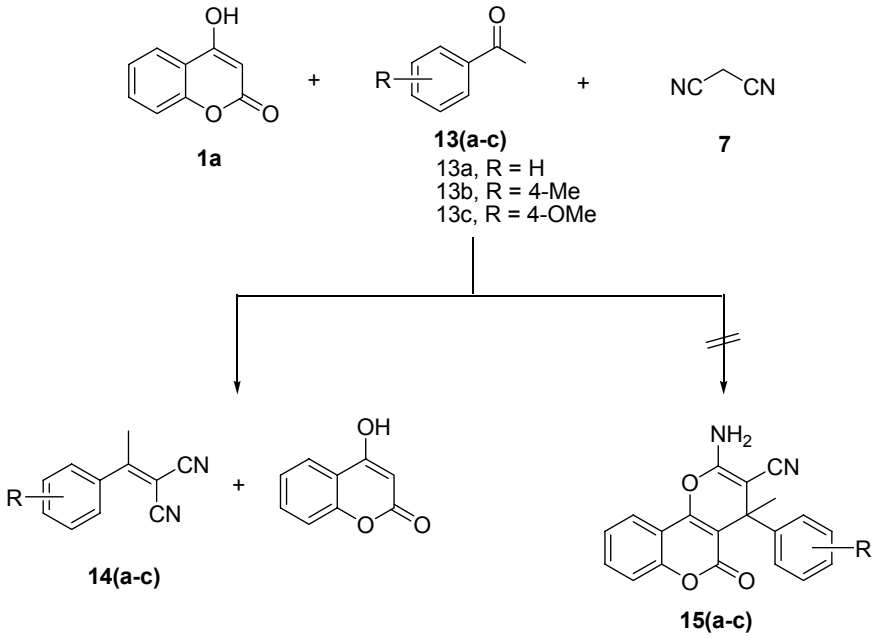
Brief heating of 4-hydroxycoumarin **1(a,b)** with cyclic aliphatic ketones (cyclopentanone, cyclohexanone, and cycloheptanone) **6(a-c)** and malononitrile **7** in boiling ethanol in the presence of morpholine as a catalyst gave 2-amino-3-cyanopyrano[3,2-*c*]chromene derivatives **8(a-f)** in high yields (65–85%). The observed high regioselectivity is most probably associated with the reaction sequence outlined in scheme **1.** Initial Knoevenagel reaction between cyclic ketones **6** and malononitrile **7** produces the unsaturated nitrile **9**, which, undergoes a Michael reaction with the base derived coumarin anion **10**. The resulting Michael adduct **11** then undergoes intramolecular cyclization producing the annelated iminopyran **12**. Subsequent tautomeric [1,3]sigmatropic shift gives compound **8** (Scheme 1).



Scheme 1. Mechanistic pathway towards **8(a-f)**.

Under these conditions, the reaction proceeds sufficiently rapidly and smoothly to afford the target chromenes **8(a-f)** in high yields without Michael adducts **11** being detected. However, the proposed mechanism is supported to some degree by isolation of analogous Michael adducts in the previously studied reaction of 4-hydroxycoumarin with arylidenecyanoacetamides.[3]

On the other hand the three component reaction system of 4-hydroxycoumarin **1a**, malononitrile **7** and substituted acetophenones **13(a-c)** as the carbonyl compound under analogous conditions failed to produce any chromenes **15(a-c)**. It was found that the acetophenones **13(a-c)** did react with malononitrile **7** to give unsaturated nitriles **14(a-c)** but these did not undergo Michael reaction with the coumarin anion. The plausible reason for the formation of spiro molecules with cyclic ketones is, in cyclic ketone the electrons are not localized in C−C bond but are actually spread out over the whole system, moreover the C in cyclic saturated ketones has sp^3 hybridization. While in aromatic ketones C in benzene ring has sp^2 hybridization due to this effect it may be cannot take part in cyclization.



Scheme 2. Synthetic approach towards spiropyrano[3,2-c]chromene using acetophenones as carbonyl compounds.

The pyranochromenes **8(a-f)** obtained are air stable and colorless solid powders, which are well soluble in acetone, DMF and DMSO. The structures of these compounds were confirmed by IR, Mass spectrometry, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. The IR spectra of pyranochromene **8(a-f)** exhibit characteristic absorption bands of the amino, nitrile, and methylene fragments: ν (NH_2) 3300-3500 cm^{-1}, ν (CN) 2200-2350 cm^{-1}, and ν (CH_2) 2800-2900 cm^{-1}. The IR spectra of pyranochromenes show a particular absorption band of the lactone group at 1680-1720 cm^{-1}. All the mass spectra show a molecular ion peak in agreement with the molecular weight of the respective compound. The ^{1}H NMR spectra show signals for the proton of amino group and methylene group at 5.6 to 5.8 δ ppm and 1.5 to 2.8 δ ppm, respectively. The signals for the benzenoid protons of coumarin are observed at in the interval 7.3 to 7.8 δ ppm. The ^{13}C NMR spectral data for compound 8a are consistent with the assigned structure.

Conclusions

In the three component system between 4-hydroxycoumarin, malonitrile and ketones for the formation of spiro 2-amino-3-cyanopyrano[3,2-*c*]chromene derivatives the ketones have to be cyclic in nature.

Experimental Section

General. Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was performed on 0.2-mm precoated plates of silica gel G60 F_{254} (Merck). Visualization was made with UV light (254 and 365nm) or with iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS (diffusion reflectant spectroscopy) probe. ^{1}H NMR spectra were recorded on a Bruker AVANCE II (400 MHz) spectrometer in DMSO. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using a direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). All reagents were purchased from Fluka, Sigma Aldrich, Merck and Rankem and used without further purification.

Preparation of spiro pyrano[3,2-*c*]chromene derivatives: General procedure
A stirred mixture of 4-hydroxycoumarin **1(a,b)** (10 mmol), carbonyl compound **6(a-c)** (cyclopentanone, cyclohexanone, and cycloheptanone) (10 mmol), malononitrile **7** (10 mmol), and morpholine (0.5 mmol) in anhydrous EtOH (50 mL) was heated under reflux for 20 min and allowed to crystallize at 4 °C for 12 h. The precipitate that formed was filtered off, washed with ethanol and hexane, and recrystallized from 1,4-dioxane to give compounds **8(a-f)** as white powders.

Spiro(2–amino–3–cyano pyrano[3,2–*c*]chromene–4,1'–cyclopentane) (8a). White solid; mp 230-232°C; Yield – 83%. IR (KBr): 3450, 2928, 2904, 2865, 2845, 2360, 1724, 1602, 1558, 1313 cm^{-1}. ^{1}H NMR: δ = 7.82 (d, 1H, Ar), 7.60–7.55 (m, 1H, Ar), 7.34–7.30 (m, 2H, Ar), 5.78 (s, 2H, NH_2), 2.39–2.33 (m, 2H, CH_2), 2.06–1.90 (m, 4H, CH_2), 1.89–1.64 (m, 2H, CH_2). ^{13}C NMR: δ = 159.7, 154.6, 152.4, 152.2, 132.3, 124.3, 122.3, 119.2, 116.5, 113.0, 109.3, 70.9, 43.1, 41.0, 27.6. Mass: *m/z* = 295 [M^++1], 294 [M^+]. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.19; H, 4.51; N, 9.43.

Spiro(2–amino–3–cyano pyrano[3,2–*c*]chromene–4,1'–cyclohexane) (8b). White solid; mp 230-232 °C; Yield – 78 %. IR (KBr): 3595, 2928, 2368, 1718, 1678, 1649, 1539, 1321, 1084 cm^{-1}. ^{1}H NMR: δ = 7.78 (m, 1H, Ar), 7.60–7.54 (m, 1H, Ar), 7.29–7.28 (m, 2H, Ar), 5.78 (s, 2H, NH_2), 2.41–2.35 (m, 4H, CH_2), 2.12–2.01 (m, 4H, CH_2), 1.89–1.61 (m, 2H, CH_2). Mass: *m/z* = 308 [M^+]. Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.06; H, 4.56; N, 8.82.

Spiro(2–amino–3–cyano pyrano[3,2–*c*]chromene–4,1'–cycloheptane) (8c). White solid; mp 225-226 °C; Yield – 66 %. IR (KBr): 3458, 2930, 2868, 2344, 1720, 1649, 1545, 1080 cm^{-1}. ^{1}H NMR: δ = 7.78 (m, 1H, Ar), 7.68–7.63 (m, 1H, Ar), 7.36–7.29 (m, 2H, Ar), 5.79 (s, 2H, NH_2), 2.41–1.69 (m, 12H, CH_2). Mass: *m/z* = 322 [M^+]. Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.23; H, 5.34; N, 8.51.

Spiro(2–amino–3–cyano–9–methyl pyrano[3,2–*c*]chromene–4,1'–cyclopentane (8d). White solid; mp 228-230 °C; Yield–85 %. IR (KBr): 3464, 2983, 2928, 2856, 2846, 2364, 1690, 1645,

1554, 1371, 1317, 1024 cm^{-1}. ^1H NMR: δ = 7.83–7.65 (m, 1H, Ar), 7.34–7.21 (m, 2H, Ar), 5.77 (s, 2H, NH_2), 2.37–2.33 (m, 4H, CH_2), 2.31 (s, 3H, CH_3), 2.12–1.88 (m, 4H, CH_2). Mass: *m/z* = 308 [M^+]. Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 69.94; H, 5.08; N, 8.98.

Spiro(2–amino–3–cyano–9–methyl pyrano[3,2–*c*]chromene–4,1'–cyclohexane) (8e). White solid; mp 231-232 °C; Yield–72 %. IR (KBr): 3502, 2960, 2956, 2845, 2304, 1678, 1649, 1545, 1368, 1321, 1045 cm^{-1}. ^1H NMR: δ = 7.68–7.54 (m, 1H, Ar), 7.41–7.28 (m, 2H, Ar), 5.78 (s, 2H, NH_2), 2.40–2.31 (m, 4H, CH_2), 2.31 (s, 3H, CH_3), 2.12–1.75 (m, 6H, CH_2). Mass: *m/z* = 322 [M^+]. Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.55; H, 5.48; N, 8.51.

Spiro(2–amino–3–cyano–9–methyl pyrano[3,2–*c*]chromene–4,1'–cycloheptane) (8f). White solid; mp 220-222 °C; Yield–70 %. IR (KBr): 3478, 2928, 2910, 2300, 1692, 1664, 1539, 1380, 1308, 1023 cm^{-1}. ^1H NMR: δ = 7.65–7.52 (m, 1H, Ar), 7.40–7.29 (m, 2H, Ar), 5.78 (s, 2H, NH_2), 2.32 (s, 3H, CH_3), 2.34–1.60 (m, 12H, CH_2). Mass: *m/z* = 336 [M^+]. Anal. Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.30; H, 5.76; N, 8.24.

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References

1. US Pat. 3 097 213; *Ref. Zh., Khim.* **1965**, 12N257P.
2. Jpn Pat. 12 901; *Ref. Zh., Khim.* **1968**, 12N568P.
3. Sharanin, Y. A.; Goncharenko, M. P.; Litvinov, V. P. *Usp. Khim.* **1998**, *67*, 442 [*Russ. Chem. Rev.* **1998**, *67* (Engl. Transl.)].
4. Shestopalov, A. M.; Emeliyanova, Y. M. In *Selected Method for Synthesis and Modification of Heterocycle,s* Kartsev, V. G., Ed.; IBS Moscow, 2003; Vol. 2, p 363.
5. *The Merck Index*, Ed. O´Neil, V. J.; Merck and Co., Inc.: Whitehouse Station: New York, 2001, 1818.
6. Mousawi, S. M. A.; Elkholy, Y. M.; Mohammad, M. A.; Elnagdi, M. H. *Org. Prep. Proc. Int.* **1999**, *31*, 305.
7. Freeman, F. *Chem. Rev.***1980**, *80*, 329.
8. Shmunes, E.; Taylor, J. S. *Archiv. Dermatology* **1973**, *107(2)*, 212.
9. Bandman, A. L.; Mil'shein, G. I. *Voenno-Meditsinskii Zh.* **1974**, *11*, 85.

10. Brimblecombe, R. W.; Green, D. M.; Muir, A. W. *British J. Pharmacology* **1972**, *44(3)*, 561.
11. Ballantyne, B.; Gazzard, M. F.; Swanston, D. W.; Williams, P. *Archiv fuer Toxikologie* **1974**, *32(3)*, 149.
12. Alarie, Y.; Wakisaka, I.; Oka, S. *Environmental Physiology & Biochemistry* **1973**, *3(2)*, 53.
13. Holland, P.; White, R. G. *British J. Dermatology* **1972**, *86(2)*, 150.
14. Chung, C. W.; Giles, A. L., Jr. *J. Immunology* **1972**, *109(2)*, 284.
15. Foster, R. W.; Ramage, A. G. *British J. Pharmacology* **1976**, *57(3)*, 436.
16. Ballantyne, B.; Swanston, D. W. *Acta Pharmacologica et Toxicologica* **1974**, *35(5)*, 412.
17. Cotes, J. E.; Dabbs, J. M.; Evans, M. R.; Holland, P. *Quarterly Journal of Expl.l Physiology* **1972**, *57(2)*, 199.
18. Upshall, D. G. *Toxicology and Applied Pharmacology* **1973**, *24*, 45.
19. Ballantyne, B.; Beswick, F. W. *Medicine, Science and the Law* **1972**, *12*, 121.
20. Ulrich, C. E.; Haddock, M. P.; Alarie, Y. *Arch. Environmental Health* **1972**, *24(1)*, 37.
21. Ballantyne, B.; Swanston, D. W. *Acta Pharmacologica et Toxicologica* **1973**, *32(3-4)*, 266.
22. Beswick, F. W.; Holland, P.; Kemp, K. H. *British J. Indust. Medicine* **1972**, *29*, 298.
23. Frankenberg, L.; Sorbo, B. *Arch. Toxikologie* **1973**, *31(2)*, 99.
24. Shestopalov, A. M.; Niazimbetova, Z. I.; Evans, D. H.; Niyazymbetov, M. E. *Heterocycles* **1999**, *51*, 1101.
25. Shestopalov, A. A.; Rodinovskaya, L. A.; Shestopalov, A. M.; Litvinov, V. P. *Russian Chemical Bulletin, International Ed.* **2005**, *54(4)*, 992.
26. Dandia, A.; Gautam, S.; Jain, A. *J. Fluorine Chem.* **2007**, *128*, 1454.