STUDIES ON PHARMACOLOGICALLY ACTIVE HETEROCYCLES

A

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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.

Date:

Place:Rajkot **Piyush V. Pipaliya**

Certificate

This is to certify that the present work "**Studies on Pharmacologically active Heterocycles**" submitted for the Ph. D. Degree Chemistry of Saurashtra University, Rajkot, Gujarat, India by Mr. Piyush V. Pipaliya 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.

Date:
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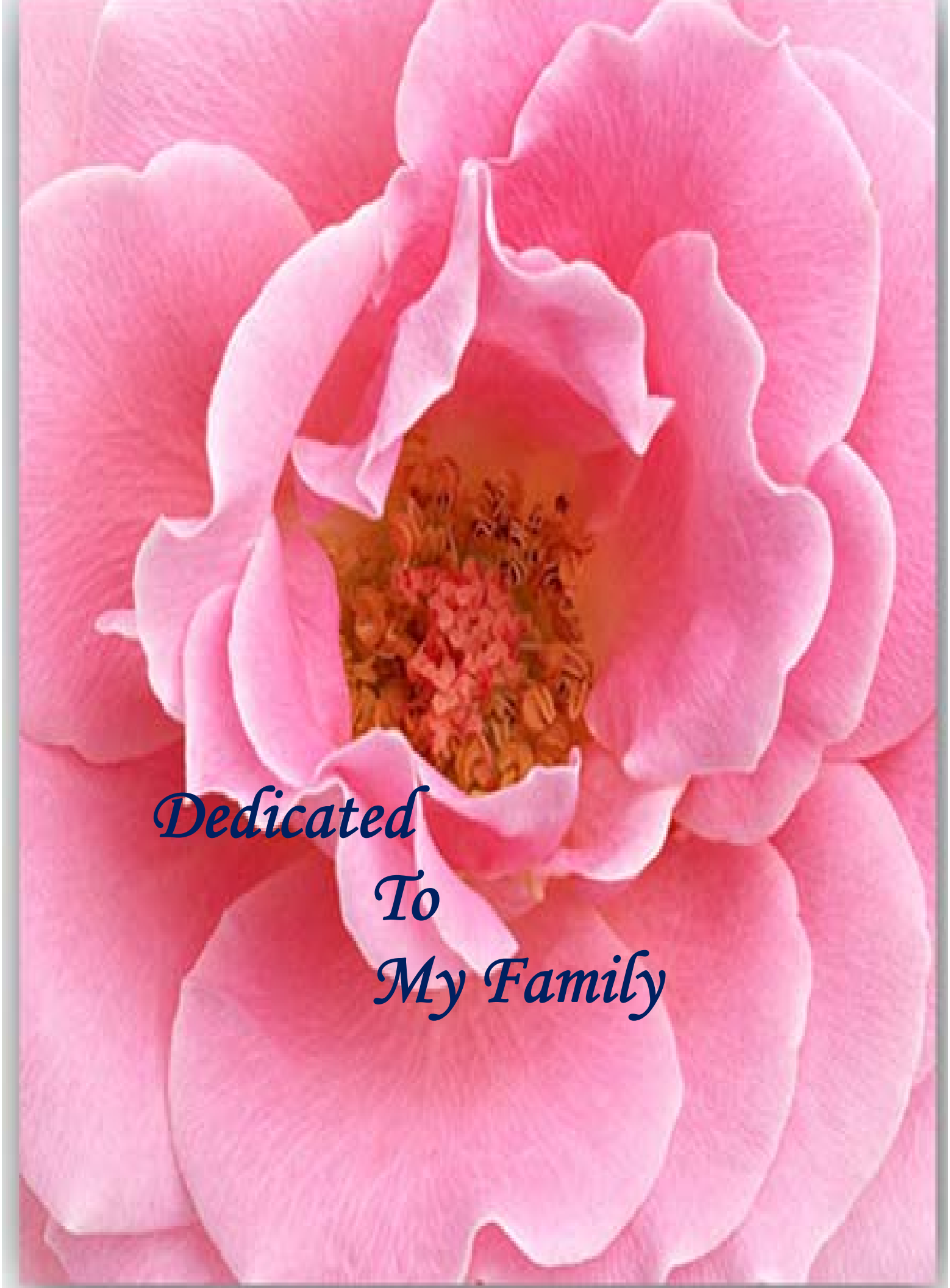
Dedicated
To
My Family

Acknowledgement

Firstly, I bow my head humbly before the Almighty God for making me capable of completing my Ph.D. Thesis; with his blessings only I have accomplished this huge task.

I express deep sense of gratitude and thankfulness to my supervisor Dr. Yogesh T. Naliapara who has helped me at each and every point of my research work with patience and enthusiasm. I am much indebted to him for his inspiring guidance, affection, generosity and everlasting enthusiasm throughout the tenure of my research work.

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At Last, I have no words to state anything about my parents but at this occasion, I would like to thanks my father and mother, without their support, motivation and understanding it would have been impossible for me to finish my research work. I sincerely thank to my whole family for encouraging me and providing every help to fulfill my task. My special gratitude is due to my sister for their loving support during tenure of my work. To them I dedicate this thesis.

Piyush Pipaliya

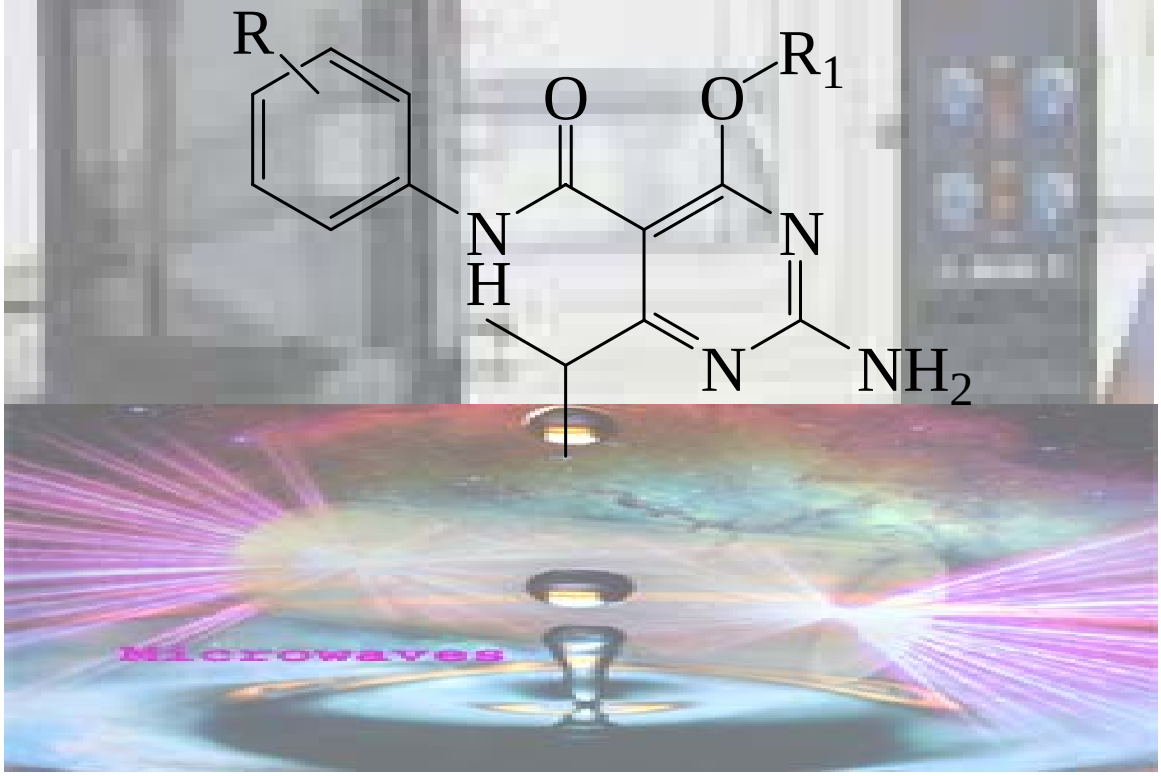
Content

Abbreviations

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
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

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NOVEL HIGHLY FUNCTIONALIZED PYRIMIDINE DERIVATIVES.



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]

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS.

Alloxan **(1)** is known for its diabetogenic action in a number of animals.[15] Uracil **(2)**, thymine **(3)** and cytosine **(4)** are the three important constituents of nucleic acids (**Figure 1**).

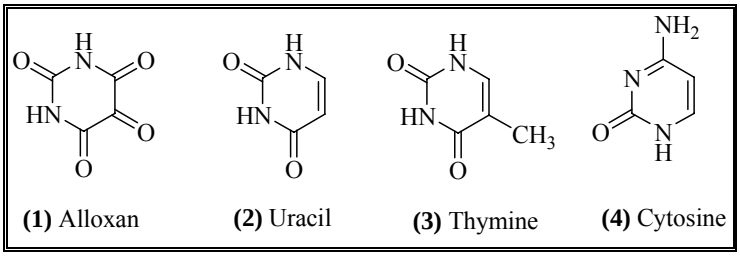


Figure 1

The pyrimidine ring is found in vitamins like thiamine **(5)**, riboflavin **(6)** (**Figure 2**) and folic acid **(7)**[16] Barbitone **(8)**, (**Figure 3**) the first barbiturate hypnotic, sedative and anticonvulsant are pyrimidine derivatives.

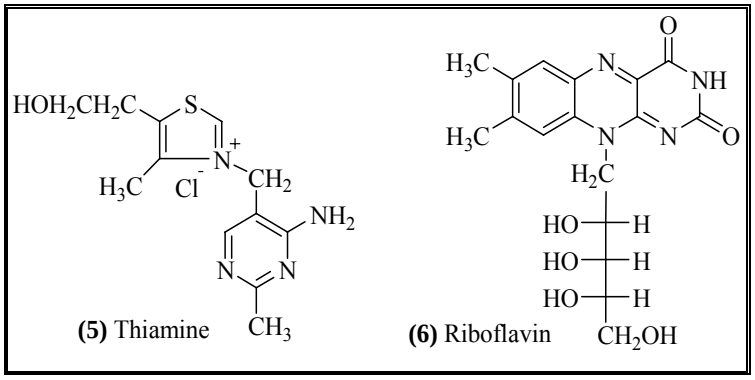


Figure 2

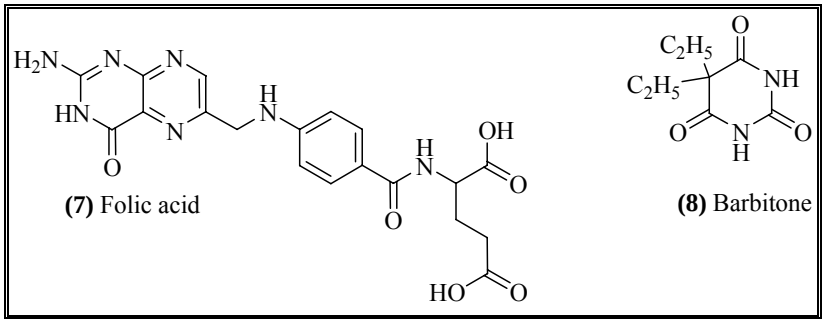


Figure 3

1.2 Pharmacological Profile

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.[17,18]

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.[5-7,19-20] 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 4**).

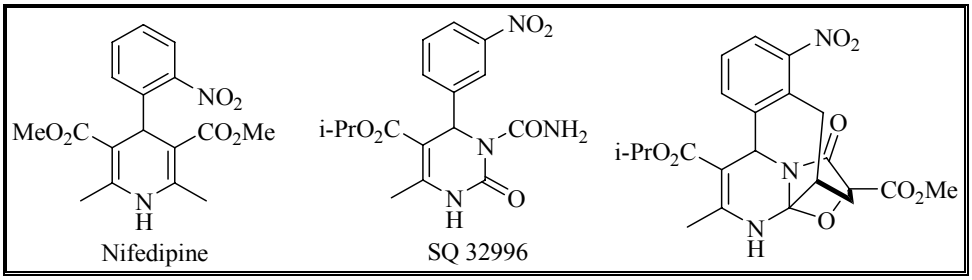


Figure 4

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 5**). 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.[21,22]

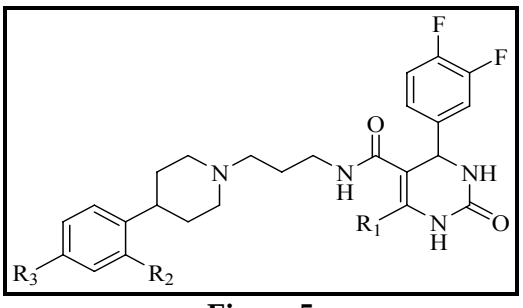


Figure 5

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 6**). 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.[23,24]

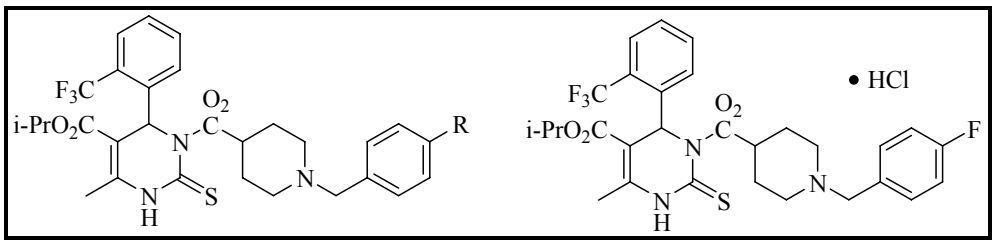


Figure 6

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 7**).[6]

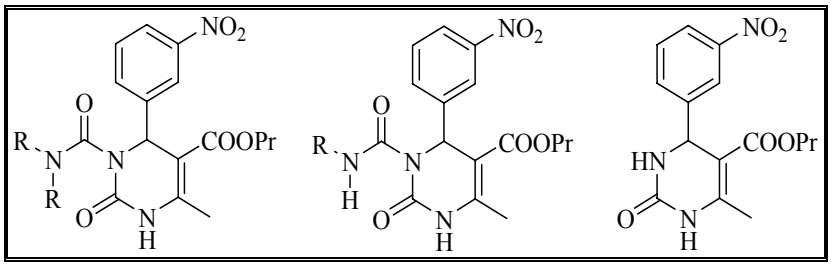


Figure 7

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 8**). 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

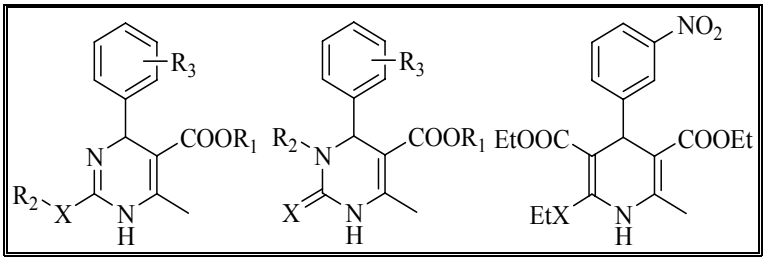


Figure 8

2-Heterosubstituted-4-aryl-l,4-dihydro-6-methyl-5-pyrimidinecarboxylicesters **(Figure 9**), 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.[25]

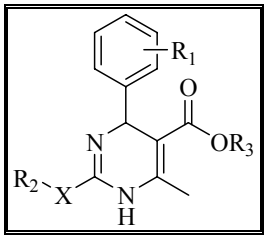


Figure 9

Bryzgalov A. O. et al has studied the antiarrhythmic activity of 4,6-di(het)aryl-5-nitro-3,4-dihydropyrimidin-(1*H*)-2-ones (**Figure 10**) 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.[26]

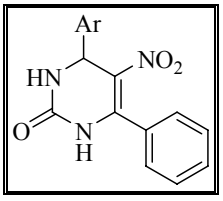


Figure 10

Remennikov G. Y. et al[27] has synthesized some novel 4-aryl-5-nitro substituted DHPMs (**Figure 11**) using nitro acetone 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 was analogous to that of nifedipine. This suggests that they share a common mechanism of action - blockade of calcium ion influx

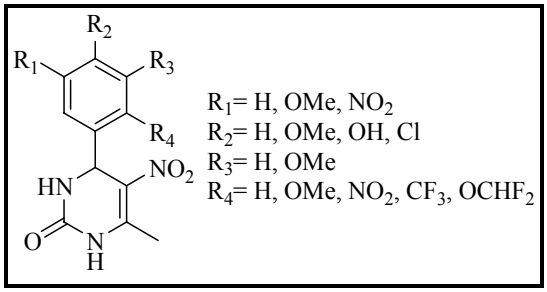


Figure 11

Brain C. Shook et al[28] has synthesized a novel series of arylindenopyrimidines **(Figure 12)** were identified as A_{2A} and A_1 receptor antagonists. The series was optimized for vitroactivit by substituting the 8-and-9-positions with methylene amine substituents. The compounds show excellent activity in mouse models of perkinson's disease when dosed orally.

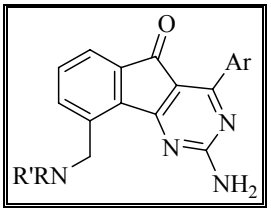


Figure 12

1.3. Alternative synthetic routes for better yield, shorter reaction time and 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[29] have synthesized the different DHPMs by using different inorganic salts as a catalyst (**Figure 13**). 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.

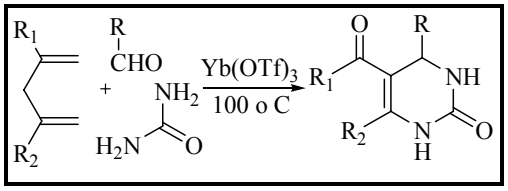


Figure 13

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[30] reported indium (III) chloride ($InCl_3$) as an efficient catalyst for the

synthesis of 3,4-dihydropyrimidn-2(1*H*)-ones (**Figure 14**). 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.

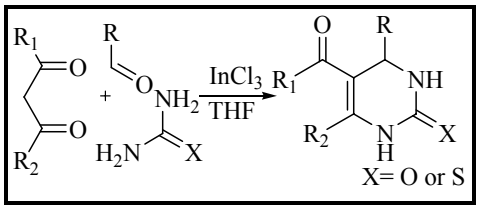


Figure 14

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[31] and 12-molybdophosphoric acid[32] as recyclable catalyst (**Figure 15**).

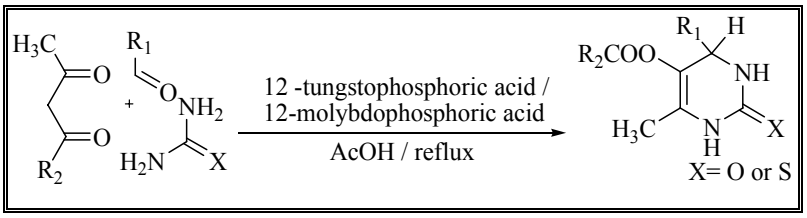


Figure 15

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.[33]

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 h).[34] 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.[35]

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.[36]

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.[37]

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.[38]

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(1*H*)-ones in excellent yields (**Figure 16**). The catalyst can be recovered and reused, making this method friendly and environmentally acceptable.[39]

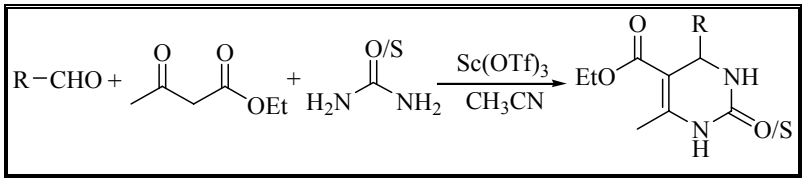


Figure 16

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%. 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 17**).[40]

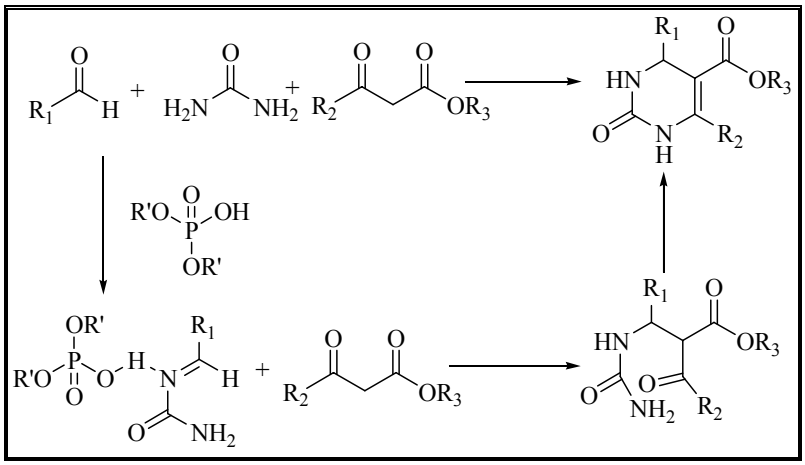


Figure 17

Shkurko, O. P. et al have synthesized 4,6-diaryl-5- nitro-3,4-dihydropyrimidin-2(1*H*)-ones and *N*-benzoyl-*N*′-(1-aryl-2-nitroethyl)urea (**Figure 18**) 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.[41]

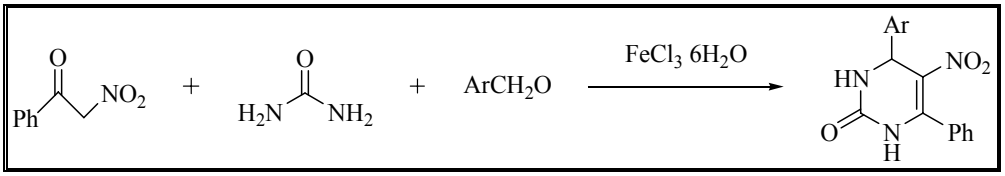


Figure 18

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.[42] Many researchers[43-44] 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)[45,46-47] 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[48] in 1995 (**Figure 19**). 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.

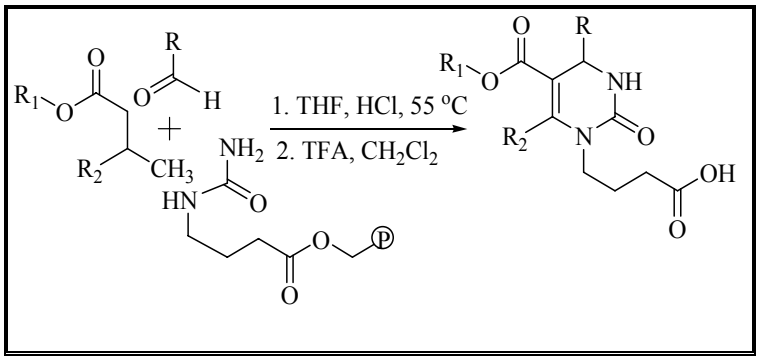


Figure 19

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 20**). 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.

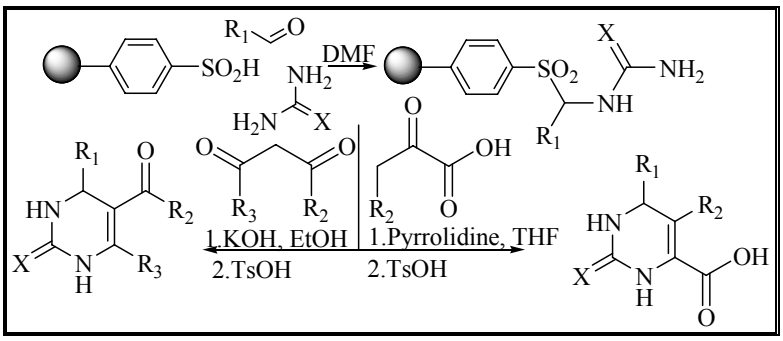


Figure 20

Gross et al[78] developed a protocol to increase the diversity of DHPM which based on immobilized α-ketoamides (**Figure 21**). 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.

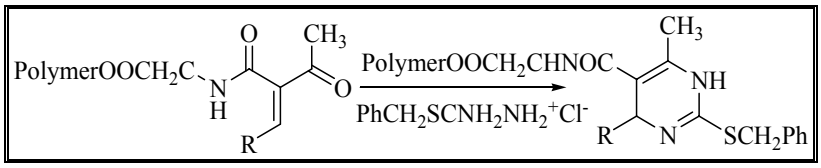


Figure 21

❖ **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.[51] Recently, organic synthesis of small molecular compounds on soluble polymers, i.e. liquid phase chemistry has increasingly become attractive field.[52] 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[53] 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[54] (**Figure 22**). 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).[55]

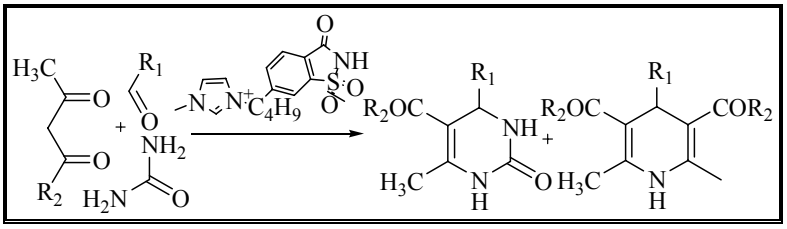


Figure 22

❖ **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.[56] The publication by Dandia A. et al[57] described microwave-enhanced solution-phase Biginelli reactions employing ethyl acetoacetate, thiourea and a wide variety of aromatic aldehydes as building blocks (**Figure 23**). 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.

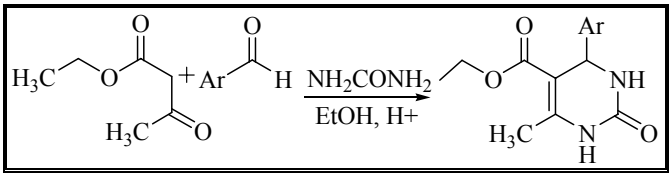


Figure 23

In recent years, solvent free reactions using either organic or inorganic solid supports have received more attention.[58] 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.[59]

❖ **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.[60] 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[61] using aldehydes, *β*-ketoester and urea.

Liu C. et al[62] 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 24**), 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.

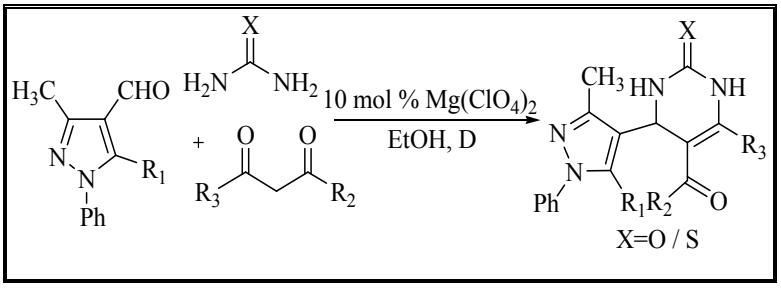


Figure 24

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 25**).[63]

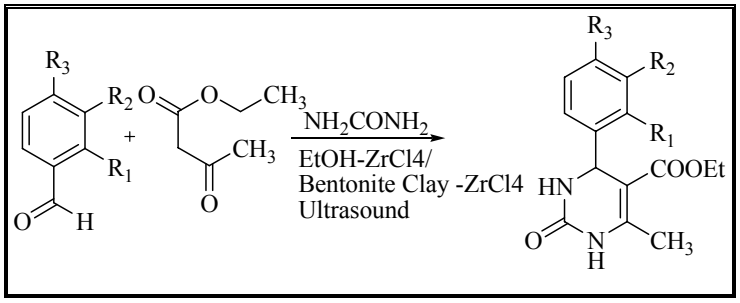


Figure 25

❖ **Synthesis of substituted pyrimidines using various ketene dithio acetals.**

Junjappa and coworkers[64] extended the generality of this strategy by using guandine and thiourea s the nucleophilic reagents. Reaction of *α*-unsubstituted *β,β'*-bis(alkylthio)-*α,β*-enones with guanidinium nitrate in refluxing methanolic sodium methoxide affords the 2-amino-4-methoxy pyrimidines **(Figure 26,27).** While utilization of thiourea affords the corresponding 2-mercapto-4-alkoxy analogs. This procedure is particularly useful for the synthesis of the latter compounds, which

would be difficult to prepare by alkylation of a free hydroxyl group in the presence of thiol functionality. These procedures have been extended to α-aryl substituted β,β'-bis(alkylthio)-α,β-enones and to α-oxo ketene dithioacetals derived from cyclic ketones

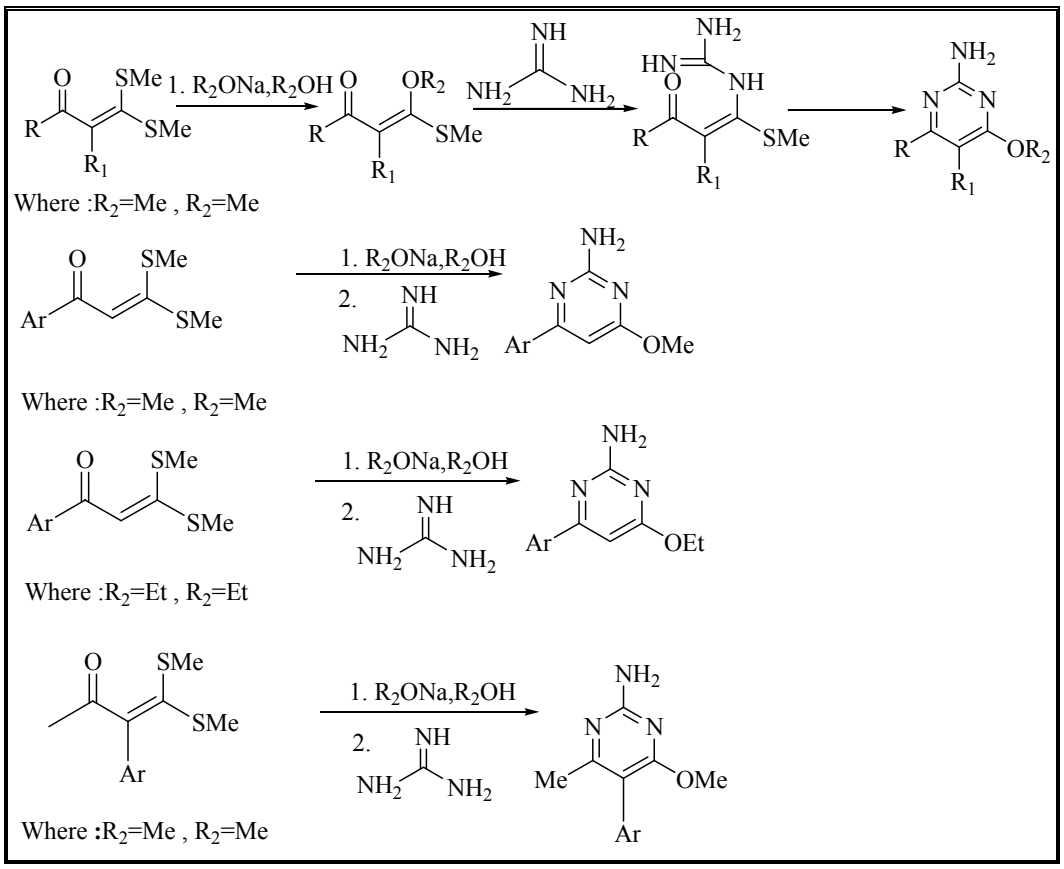


Figure 26

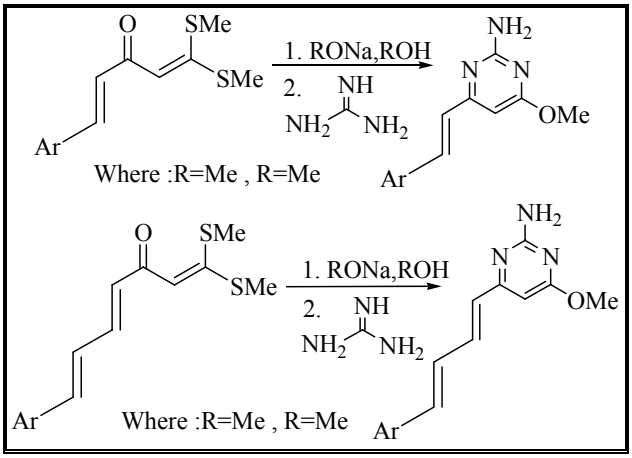


Figure 27

A. Kumar and V. Agrawal et al[65] have synthesized a novel series of 2-amino-4-(*N*-alkyl-*N*-arylamino)-pyrimidines using polarized ketene *S,S*-and *S,N*-acetals (**Figure 28**.) by reaction of ketene *S,S*-acetal **1** with aniline **2** in boiling ethanol the corresponding *S,N*-acetal **3** was obtained in good yield. Treatment of **3** with guanidine nitrarte in the presence of sodium ethoxide followed by refluxing in ethanol, yielded 2-amino-4-anilino-5-cyno-1,6-dihydro-6-oxopyrimidine **4** in 57% yield.

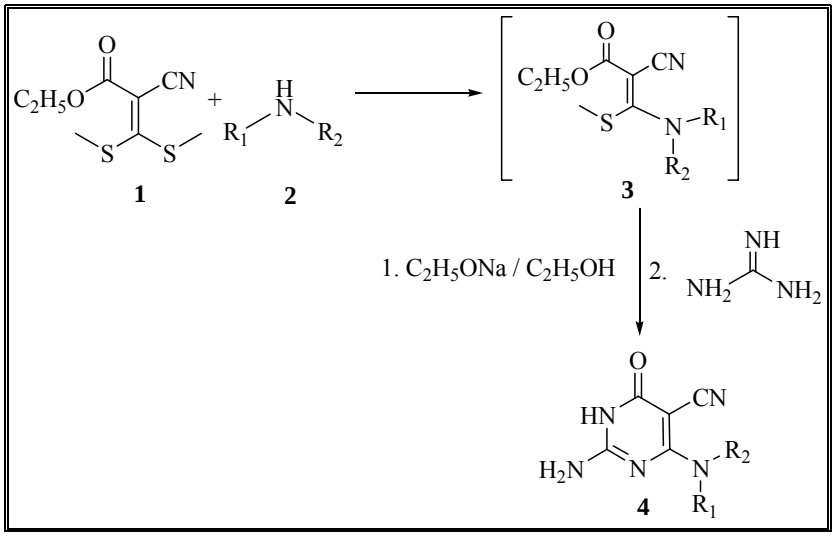


Figure 28

H. Ila and H. Junjappa et al[66] have synthesized a novel series of 2-amino-4-ethoxy dibenz[b,f]oxepino-[4,5-*d*]pyrimidine **(Figure 29).** Guanidine nitrate was added to a stirred solution of sodium ethoxide then after 5 min ketene dithioacetal was added and reflux the reaction mix for 5-12 h which yielded pyrimidine.

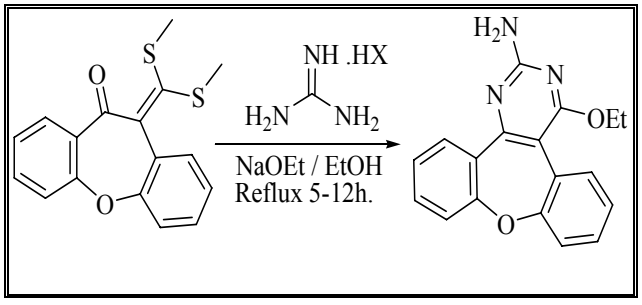


Figure 29

U. K. Syamkumar et al[67] have reported a heteroannulation of 2-[Bis (methylthio) methylene]-1-methyl-3-oxoindole with guanidine nitrate and sodium methoxide or ethoxide in 50b ml of the respective alcohol and the reaction mix was refluxed for 12-15 h which yielded 2-amino-5-*N*-methyl-4-(alkoxy)pyrimido[5,4-b]indole **(Figure 30)** in good yield.

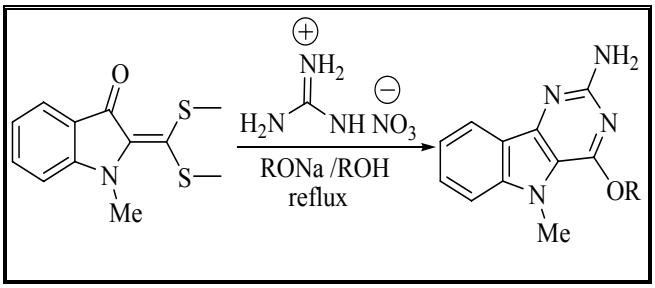


Figure 30

M. A. Ebraheem et al[68] have reported a novel synthesis of polysubstituted pyrimidines **(2)** via the reaction of α,α -oxoketene dithioacetals **(1)** with urea and thiourea in EtOH reflux 3 h which yielded pyrimidine **(Figure 31)** in good yield.

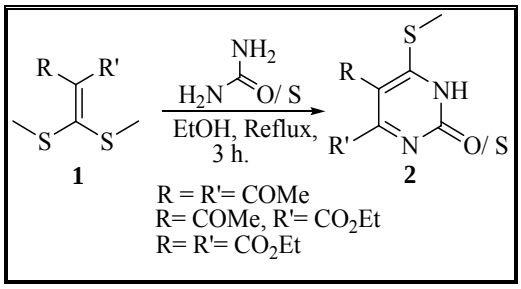


Figure 31

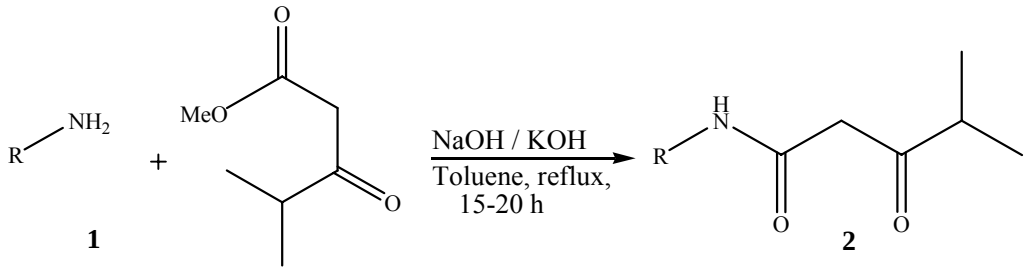
1.4. CURRENT RESEARCH WORK

Nitrogen containing heterocyclic compounds has received considerable attention due to their wide range of pharmacological activity. In this context, the pyrimidine derivatives have been reported to possess a variety of potent biological activity, among which are the analgesic, antihypertensive, antipyretic, antiviral and anti-inflammatory activity. These are also associated with nucleic acid, antibiotic, antimalarial and anti cancer drugs. Many of the pyrimidine derivatives are reported to possess potential CNS depressant properties. The tremendous biological potential of pyrimidine derivatives encouraged us to synthesize some novel carboxamide functionalized pyrimidine derivatives. Various methodologies have been described for the synthesis of pyrimidine derivatives. However, the existing methods have suffered with some drawbacks, such as yield, time and product isolation.

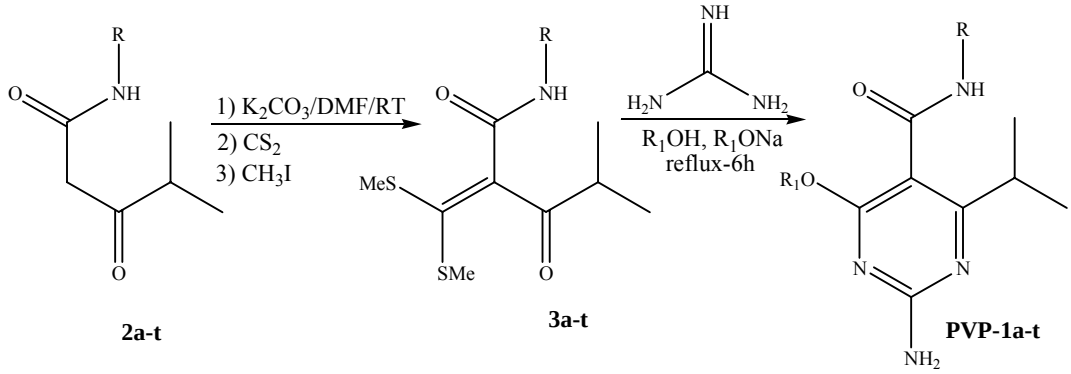
During the course of our ongoing interest on the synthesis various heterocycles we observed that functionalized ketene dithioacetals are versatile intermediates in organic synthesis for the construction of substituted heterocycles. Thus, we have synthesized some novel ketene dithioacetals starting from, 4-methyl-3-oxo-*N*-aryl pentanamide for the construction of small molecule library of 2-amino-4-isopropyl-6-alkoxy-*N*-arylpyrimidine-5-carboxamide. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. All synthesized compounds were evaluated for their antimicrobial activity.

1.5. RESULTS AND DISCUSSION

Scheme:-1 Synthesis of substituted pyrimidines using ketene dithioacetals.



Scheme:-2



Various substituted 4-methyl-3-oxo-*N*-arylpentanamide **2a-t** were prepared by reacting substituted amines **(1)** and methyl-4-methyl-3-oxopentanoate in toluene with a catalytic amount of NaOH or KOH (**Scheme 1**). The reaction mixture was reflux for 15-20 h. Various acetoacetanilide were synthesized bearing different electron donating and electron withdrawing groups like 2,3-diCH_3; 3,4-diCH_3; 4-CH_3; H; 2,5-diCH_3; 2,4-diCH_3; 3-Cl-4-F; 4-F; 4-Cl; 2-Cl; 2-F; 4-OCH_3; 2,5-diCl and 3-NO_2 on the phenyl ring.

Thus, it has been found that reaction of substituted acetoacetanilide **2a-t** derivatives **(Scheme 1)** with carbon disulfide in the presence of potassium carbonate followed by the alkylation with methyl iodide gives the novel ketene dithioacetals **3a-t**, when **3a-t** was reacted with guanidine nitrate in refluxing methanolic sodium methoxide or ethanolic sodium ethoxide **(scheme 2)** affords the 2-amino-4-isopropyl-6-alkoxy-*N*-arylpyrimidine-5-carboxamide derivatives **PVP-1a-t** was obtained in excellent yield.

The structures of **PVP-1a-t** were established on the basis of their elemental analysis and spectral data (MS, IR, and ^{1}H NMR). The analytical data for **3m** revealed a molecular formula $C_{16}H_{21}NO_2S_2$ (*m/z* 323). The ^{1}H NMR spectrum revealed a two singlet at δ = 1.18-1.20 ppm assigned to isopropyl-CH_3, a singlet at δ = 1.57 ppm assigned to the –CH_3 protons, a singlet at δ = 2.44 ppm assigned to (2 × SCH_3), a multiplet at δ = 3.17-3.24 ppm assigned to the isopropyl-CH protons, a multiplet at δ = 6.99–7.54 ppm assigned to the aromatic protons, and one broad singlets at δ = 8.38 ppm assigned to -CONH groups.

Table 1: Synthesis of substituted pyrimidines using ketene dithioacetals.

Entry	R	R_1	Yield %	Time h.
PVP-1a	4-BrC_6H_4	C_2H_5	92	6.0
PVP-1b	4-O$CH_3C_6H_4$	CH_3	91	6.0
PVP-1c	C_6H_5	CH_3	84	5.7
PVP-1d	C_6H_{11}	C_2H_5	90	5.0
PVP-1e	2,5-di-$CH_3C_6H_3$	CH_3	86	5.5
PVP-1f	C_6H_{11}	CH_3	92	5.6
PVP-1g	4-FC_6H_4	CH_3	90	6.0
PVP-1h	4-ClC_6H_4	C_2H_5	86	5.8
PVP-1i	3-Cl,4-FC_6H_3	CH_3	93	5.5
PVP-1j	3,4-di-FC_6H_3	CH_3	91	6.0
PVP-1k	3-ClC_6H_4	CH_3	88	5.4
PVP-1l	3,4-di-ClC_6H_3	CH_3	92	5.7
PVP-1m	4-$CH_3C_6H_4$	CH_3	90	5.6
PVP-1n	2-$CH_3C_6H_4$	CH_3	87	5.8
PVP-1o	2-O$CH_3C_6H_4$	CH_3	85	5.5
PVP-1p	2-FC_6H_4	CH_3	93	6.0
PVP-1q	2-BrC_6H_4	CH_3	90	5.6
PVP-1r	C_6H_5	C_2H_5	89	6.0
PVP-1s	4-ClC_6H_4	CH_3	85	5.7
PVP-1t	3-ClC_6H_4	C_2H_5	92	5.4

The reaction of compounds **3a-t** with guanidine nitrate in refluxing methanolic sodium methoxide or ethanolic sodium ethoxide afforded the 2-amino-4-isopropyl-6-alkoxy-*N*-arylpyrimidine-5-carboxamide derivatives **PVP-1a-t.**

The structures of **PVP-1a** were established on the basis of their elemental analysis and spectral data (MS, IR, ^{1}H NMR, and ^{13}C NMR). Structure **PVP-1a** was supported by its mass (*m/z* 379), which agrees with its molecular formula $C_{16}H_{19}BrN_4O_2$, its ^{1}H NMR spectrum had signals at δ= 1.18-1.20 (d, 6H 2 x ^{i}prCH$_3$), 1.29-1.33 (t, 3H, CH_3), 3.07-3.13 (m, 1H, CH), assigned to the isopropyl-CH protons, 4.32-4.37 (q, 2H, CH_2), 5.86 (s, 2H, NH_2), 7.38-7.40 (d, 2H, Ar-H, *j*=8.8*Hz*), 7.63-7.65 (d, 2H, Ar-H, *j*=8.8*Hz*), 9.97 (br, s, 1H, -CONH).

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 guanidine in the presence of base attacks on carbonyl carbon of systems and formed heterocyclic product by removal of water molecule followed by methylthio as good leaving group.

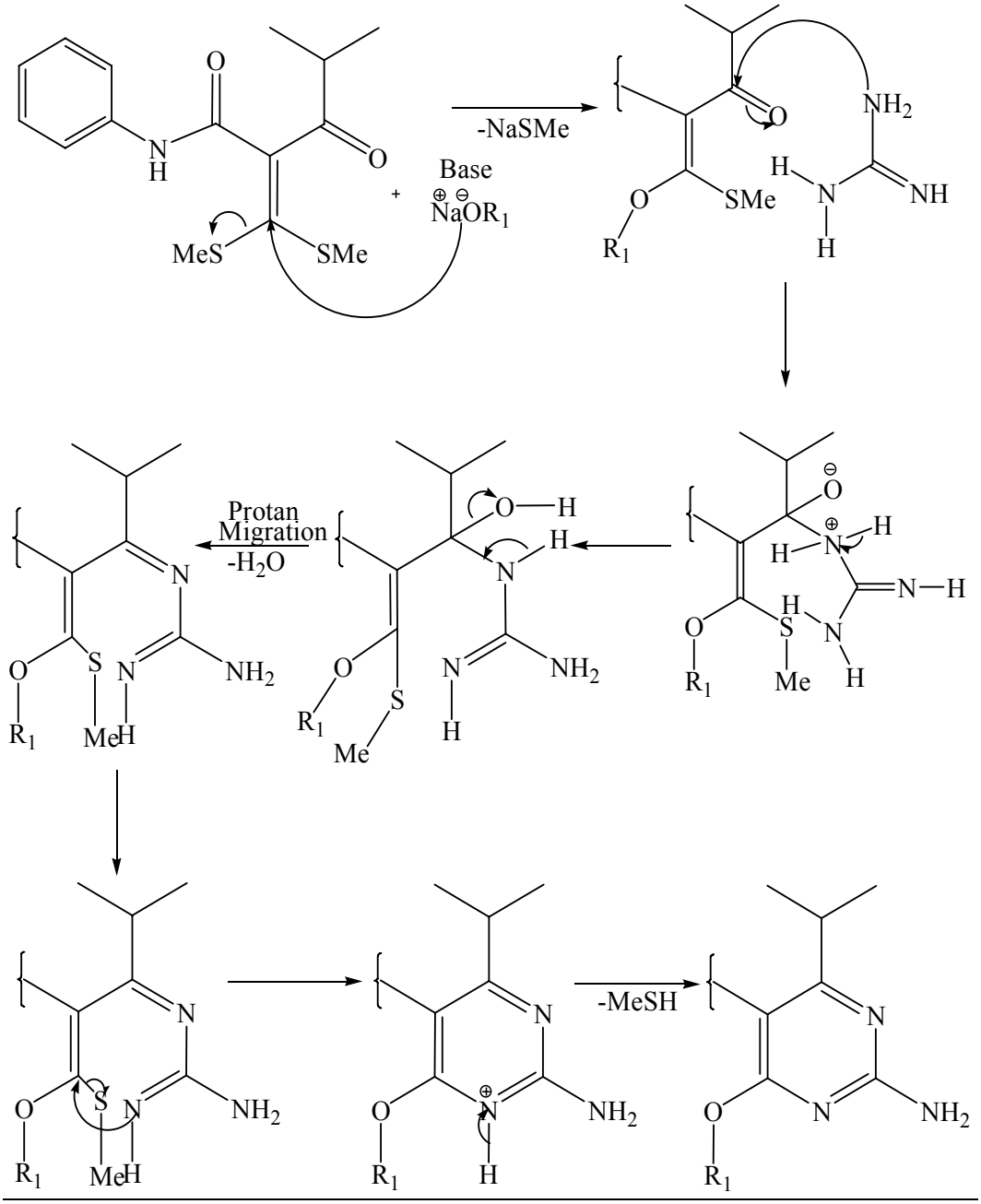


Figure 35: Proposed mechanism for the formation of pyrimidine.

1.6. ANTIMICROBIAL SENSITIVITY TESTING

WELL DIFFUSION / AGAR CUP METHOD (Lt. General Raghunath D. 1998, Ashok Rattan, 1998; Patel R., Patel K. 2004,)

In vitro effectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective then many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive then using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Requirements

1. Young broth culture of a standard test organism
2. Sterile Mueller Hinton Agar plate
3. Solution of antimicrobial substance
4. Cup borer
5. Alcohol etc.

Inoculum preparation

Inoculum was prepared by selecting 4-5 colonies from slope of stock culture of the indicator organism and emulsifying them in a suitable broth. The inoculated broth was incubated at 37ºC till it equals turbidity of a 0.5 McFarland standard. This happens in 2-8 h.

Procedure

1. Inoculate test organism on the top of Mueller Hinton Agar plate with help of sterile swab. (it can be inoculated in melted agar also)
2. The swab was dipped in the inoculum and surface of plate was streaked with swab.
3. Streaking was repeated for 3 times and each time the plate was rotated at angle of 60º.
4. Sterilize the cup-borer make four cups of the diameter of 8-10 mm. at equal distance in the plate previously inoculated with seed culture.
5. The depth of well was 2.5-5.0 mm.
6. The wells have been clearly punched so the surrounding medium is not lifted when the plug was removed out.
7. The plates were incubated at 37ºC for 24 h. Then the zone of inhibition measured and the size of zone cited in table.

❖ *Antibiotic Sensitivity Assay*

(Concentration250/500/ 1000 µG/ml)

Sr. No.	CODE No.	*Pseudomonas aeruginosa*			*Proteus vulgaris*			*Escherichia coli*			*Staphylococcus aureus*			*Candida albicans*		
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
1.	**1a**	R	1.1	1.2	R	1.1	1.3	R	R	R	R	R	R	R	1	1.2
2.	**1b**	1.2	1.4	2	1.1	1.3	1.6	R	R	R	R	1	1.2	R	1.2	1.5
3.	**1c**	1.2	1.3	1.7	1.1	1.4	1.6	R	R	R	1.2	1.3	1.6	1	1.3	1.8
4.	**1d**	1.1	1.3	1.5	R	1.1	1.4	1.1	1.2	1.3	R	1	1.2	1.1	1.5	2
5.	**1e**	1.1	1.2	1.4	1	1.3	1.6	R	R	R	1.3	1.4	1.6	1.1	1.4	1.8
6.	**1f**	1.2	1.3	1.6	R	1.2	1.4	R	R	R	1.2	1.4	1.6	1	1.3	1.7
7.	**1g**	1.1	1.2	1.3	R	1	1.2	R	R	R	1.2	1.3	1.5	1	1.1	1.3
8.	**1h**	1	1.3	1.5	1.1	1.4	1.7	1.2	1.4	1.8	1.1	1.3	1.4	R	1.1	1.4
9.	**1i**	1.1	1.3	1.6	1.2	1.6	2	1.3	1.5	1.9	1.1	1.5	2.2	1.2	1.6	2.3
10.	**1j**	1.3	1.5	1.9	1	1.2	1.3	1.3	1.4	1.7	1.1	1.4	1.5	1.1	1.4	1.8
11.	**1k**	1.3	1.5	1.8	1.1	1.4	1.7	1.2	1.4	1.8	1.4	1.5	2	1.2	1.4	1.7
12.	**1l**	1.4	1.7	2	1.1	1.3	1.5	1.1	1.1	1.3	1.4	1.6	2	1.1	1.3	1.5
13.	**1m**	1.1	1.3	1.5	R	R	R	R	R	R	1.3	1.4	1.7	R	1.3	1.7
14.	**1n**	1.3	1.5	1.5	1.9	R	R	1.5	1.5	1.7	R	1.3	R	1.3	R	1
15.	**1o**	1.5	1.6	1.3	1.1	1.4	1.3	1.4	1.7	1	R	1.2	1.7	1.1	1.5	1.3
16.	**1p**	1.7	1.8	1.5	1	1.6	1.2	1.3	1.9	1.1	1.7	1.5	1.5	R	1.1	1.4
17.	**1q**	1.6	1	1.2	1.5	1.4	1.2	1.2	1.5	1.4	1.6	1.8	1.3	1.5	1.3	1.8
18.	**1r**	2	1.8	1.3	1.1	1.3	1.5	1	1.2	1.5	1.1	1	1.4	1.8	1.1	1.6
19.	**1s**	1.2	1.1	1.1	1.7	1.8	1.4	1.1	1	1.3	1.5	1.6	1.9	1.6	1	2
20.	**1t**	R	1	2	1.3	1.3	1.2	1.5	1.7	1.2	1.3	1.2	1	1.2	1.8	1.7
21.	**A**	1.8			1.8			1.9			1.9			-		
22.	**CPD**	2.2			2.1			2.1			2.2			-		
23.	**GF**	1.8			1.9			2.0			2.0			-		
24.	**GRF**	-			-			-			-			2.6		
25.	**FLC**	-			-			-			-			2.8		

Note: Zone of inhibition interpretation is as follows.

1. ZONE SIZE <1.0 C.M.- RESISTENT(R)
2. ZONE SIZE 1.0 To 1.5 – INTERMEDIATE
3. ZONE SIZE >1.5 – SENSITIVE

STD Antibiotic Sensitivity Assay Concentration 40 µG/ml

A: AMPICILLIN
CPD: CEFPODOXIME
GF: GATIFLOXACIN
GRF: GRESIOFULVIN
FLC: FLUCONAZOLE

1.7 CONCLUSION

In summary, we have described the synthesis of substituted pyrimidine derivatives in excellent yields. The reaction of various ketene dithioacetals with guanidine nitrate in refluxing methanolic sodium methoxide or ethanolic sodium ethoxide affords the 2-amino-4-isopropyl-6-alkoxy-*N*-arylpyrimidine-5-carboxamide derivatives with good yields. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-1a-t** showed moderate to potent activity. The compounds **PVP -1i, 1k** and **1l** showed comparatively good activity against all the bacterial strains.

1.8 EXPERIMENTAL SECTION

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. Melting points were measured in open capillaries and are uncorrected.

❖ **General synthesis of 4-methyl-3-oxo-*N*-arylpentanamide 2a-t.**

A mixture containing the primary amine (10 mmol), methyl isobutyrylacetate (10 mmol), and catalytic amount of sodium or potassium hydroxide lie (10 %) was reflux at 110^{o}C for the approximately 15-20 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under *vaccuo* when the reaction was completed. The solid or oil was crystallized from methanol to give pure product **2a-t**.

❖ **General synthesis of ketene dithioacetals 3a-t.**

A 100mL conical flask equipped with magnetic stirrer and septum was charged with a solution of 4-methyl-3-oxo-*N*-arylpentanamide **2a-t**, (10 mmol) in DMF (10 mL). Dried K_2CO_3 (10 mmol) was added and the mixture was stirred for 2 h at room temperature. CS_2 (30 mmol) was added and the mixture was stirred for an additional 2 h at room temperature. Methyl iodide (20 mmol) was added at 0-5 ^{o}C and the mixture was stirred for 4 h before being poured onto water (40 mL). The precipitated crude product was purified by filtration followed by crystallization from EtOH. When the product was oil, the organic phase was extracted with Et_2O (3 × 10 mL). The combined organic extracts were washed with H_2O (2 × 10 mL), dried ($MgSO_4$), and concentrated in *vaccuo* to afford ketene dithioacetals directly used for the next step.

❖ **General procedure for the synthesis of substituted pyrimidines PVP-1a-t.**

To a well stirred mixture of guanidine nitrate (10 mmol) and sodium methoxide or sodium ethoxide (20 mmol) in methanol or ethanol was added the solution of ketene dithioacetals **3a-t** (10 mmol) in methanol or ethanol within 10-15 min. The resulting reaction mixture was further stirred at rt for 15 min. then, reflux the reaction mixtures for 6h on water bath. After completion of the reaction, the mixture was poured onto ice cold water. Thus, the obtained solid was filtered, wash with water and dried it and crystallization from EtOH to afford analytically pure products **PVP-1a-t.**

❖ **Spectral data of the synthesized compounds**

2-(bis(methylthio)methylene)-4-methyl-3-oxo-*N*-*p*-tolylpentanamide 3m. yellow solid, mp 155-157°C; IR (KBr): 3373, 3072, 2895, 2828, 1694, 1635, 1482, 1343, 1298 cm^{-1}; ^1H NMR (400 MHz, DMSO): δ ppm 1.18-1.20 (d, 6H, 2 × $^iprCH_3$), 1.57 (s, 3H, CH_3), 2.44 (s, 6H, 2 × SCH_3), 3.17-3.24 (m, 1H, ^iprCH), 6.99–7.54 (m, 4H, Ar-H), 8.38 (br, s, 1H, -CONH); MS (*m/z*): 323 (M^+); Anal. Calcd for $C_{16}H_{21}NO_2S_2$: C, 59.41; H, 6.54; N, 4.33; Found: C, 59.33; H, 6.45; N, 4.23.

2-amino-*N*-(4-bromophenyl)-4ethoxy-6-isopropylpyrimidine-5-carboxamide (PVP-1a): White solid; R_f 0.45 (6:4 hexane-EtOAc); mp 185-187°C; IR (KBr): 3459, 3327, 3193, 2999, 1648, 1586, 1261, 1061 cm^{-1}; ^1H NMR: δ 1.18-1.20 (d, 6H, 2 x $^iprCH_3$), 1.29-1.33 (t, 3H, CH_3), 3.07-3.13 (m, 1H, ^iprCH), 4.32-4.37 (q, 2H, CH_2), 5.86 (s, 2H, NH_2), 7.38-7.40 (d, 2H, Ar-H, *j*=8.8*Hz*), 7.63-7.65 (d, 2H, Ar-H, *j*=8.8*Hz*), 9.97 (br, s, 1H, -CONH); MS (*m/z*): 379 (M^+); Anal. Calcd for $C_{16}H_{19}BrN_4O_2$: C, 50.67; H, 5.05; N, 14.77; Found: C, 50.48; H, 5.15; N, 14.52.

2-amino-4isopropyl-6-methoxy-*N*-(4-methoxyphenyl)pyrimidine-5-carboxamide (PVP-1b): yellow solid; R_f 0.24 (6:4 hexane-EtOAc); mp 180-184°C; IR (KBr): 3452, 3307, 3223, 2980, 1653, 1509, 1461, 1051 cm^{-1}; ^1H NMR: δ 1.19-1.25 (d, 6H, 2 x $^iprCH_3$), 3.07-3.13 (m, 4H, ^iprCH,), 3.78 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.69 (s, 2H, NH_2), 6.82-6.85 (d, 2H, Ar-H, *j*=8.8*Hz*), 7.60-7.62 (d, 2H, Ar-H, *j*=8.8*Hz*), 9.58 (s, 1H, CONH); MS (*m/z*): 316 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71; Found: C, 60.48; H, 6.15; N, 17.52.

2-amino-4isopropyl-6-methoxy-*N*-phenylpyrimidine-5-carboxamide (PVP-1c): yellow solid; R_f 0.26 (6:4 hexane-EtOAc); mp 210-212°C; IR (KBr): 3412, 3317, 3253, 2950, 1613, 15039, 1431, 1041 cm^{-1}; MS (*m/z*): 286 (M^+); Anal. Calcd for $C_{15}H_{18}N_4O_2$: C, 60.92; H, 6.34; N, 19.57; Found: C, 60.48; H, 6.15; N, 19.32.

2-amino-*N*-cyclohexyl-4ethoxy-6-isopropylpyrimidine-5-carboxamide (PVP-1d): White solid; R_f 0.47 (6:4 hexane-EtOAc); mp 196-198°C; IR (KBr): 3429, 3307, 3123, 2959, 1658, 1546, 1265, 1041 cm^{-1}; ^1H NMR: δ 1.19-1.20 (d, 6H, 2 x $^iprCH_3$), 1.22-1.25 (t, 3H, CH_3), 1.28-1.93 (m, 10H, 5 x CH_2), 3.07-3.14 (m, 1H, ^iprCH), 3.79-3.86 (m, 1H, CH), 4.26-4.31 (q, 2H, CH_2), 5.73 (s, 2H, NH_2), 7.21 (s, 1H, CONH);

MS (*m/z*): 306 (M^+); Anal. Calcd for $C_{16}H_{26}N_4O_2$: C, 62.72; H, 8.55; N, 18.29; Found: C, 62.38; H, 8.10; N, 18.

2-amino-4-isopropyl-6-methoxy-*N*-(2,5-dimethylphenyl)pyrimidine-5-carboxamide (PVP-1e): yellow solid; R_f 0.24 (6:4 hexane-EtOAc); mp 186-188°C; IR (KBr): 3420, 3341, 32631, 2930, 1610, 1553, 1411, 1060 cm^{-1}; MS (*m/z*): 314 (M^+); Anal. Calcd for $C_{17}H_{22}N_4O_2$: C, 64.58; H, 7.05; N, 17.82; Found: C, 64.48; H, 7.15; N, 17.62.

2-amino-*N*-cyclohexyl-4isopropyl-6-methoxypyrimidine-5-carboxamide(PVP-1f): yellow solid; R_f 0.22 (6:4 hexane-EtOAc); mp 190-192°C; IR (KBr): 3462, 3307, 3223, 2990, 1653, 1509, 1461, 1061 cm^{-1}; ^{13}C NMR: δ 21.20, 24.42, 25.02, 31.60, 32.16, 38.90-40.16, 47.97, 53.01, 106.73, 161.89, 164.94, 166.47, 173.41. MS (*m/z*): 292 (M^+); Anal. Calcd for $C_{15}H_{24}N_4O_2$: C, 61.62; H, 8.27; N, 19.16; Found: C, 61.58; H, 8.15; N, 19.12.

2-amino-*N*-(4-fluorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1g): yellow solid; R_f 0.25 (6:4 hexane-EtOAc); mp 180-182°C; IR (KBr): 3459, 3327, 3173, 2989, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 304 (M^+); Anal. Calcd for $C_{15}H_{17}FN_4O_2$: C, 59.20; H, 5.63; N, 18.41; Found: C, 59.13; H, 5.45; N, 18.52.

2-amino-*N*-(4-chlorophenyl)-4ethoxy-6-isopropylpyrimidine-5-carboxamide (PVP-1h): White solid; R_f 0.42 (6:4 hexane-EtOAc); mp 194-196°C; IR (KBr): 3449, 3331, 3182, 3055, 2952, 1651, 1568, 1491, 1247, 1049 cm^{-1}; ^1H NMR: δ 1.17-1.94 (d, 6H, 2 x ^{i}prCH$_3$), 1.28-1.31 (t, 3H, CH_3), 3.05-3.09 (m, 1H, ^{i}prCH), 4.31-4.36 (q, 2H, CH_2), 6.09 (s, 2H, NH_2), 7.24-7.26 (d, 2H, Ar-H, *j*=8.8*Hz*), 7.68-7.71 (d, 2H, Ar-H, *j*=8.8*Hz*), 10.07 (s, 1H, CONH); MS (*m/z*): 334 (M^+); Anal. Calcd for $C_{16}H_{19}ClN_4O_2$: C, 57.40; H, 5.72; N, 16.73; Found: C, 57.41; H, 5.55; N, 16.63.

2-amino-*N*-(3-chloro-4-fluorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1i): yellow solid; R_f 0.21 (6:4 hexane-EtOAc); mp 185-187°C; IR (KBr): 3443, 3325, 3153, 2989, 1648, 1506, 1251, 1064 cm^{-1}; MS (*m/z*): 338 (M^+); Anal. Calcd for $C_{15}H_{16}ClFN_4O_2$: C, 53.18; H, 4.76; N, 16.54; Found: C, 53.13; H, 4.65; N, 16.52.

2-amino-*N*-(3,4-di-fluorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1j): yellow solid; R_f 0.26 (6:4 hexane-EtOAc); mp 245-247°C; IR (KBr): 3442, 3226, 3143, 2986, 1642, 1566, 1241, 1061 cm^{-1}; MS (*m/z*): 322 (M^+); Anal. Calcd for $C_{15}H_{16}F_2N_4O_2$: C, 55.90; H, 5.00; N, 17.38; Found: C, 55.73; H, 4.94; N, 17.32.

2-amino-*N*-(3-chlorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1k): yellow solid; R_f 0.25 (6:4 hexane-EtOAc); mp 256-258°C; IR (KBr): 3459, 3252, 3143, 2919, 1648, 1586, 1241, 1051 cm^{-1}; MS (*m/z*): 320 (M^+); Anal. Calcd for $C_{15}H_{17}ClN_4O_2$: C, 56.16; H, 5.34; N, 17.47; Found: C, 56.23; H, 5.25; N, 17.52.

2-amino-*N*-(3,4-di-chlorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1l): yellow solid; R_f 0.23 (6:4 hexane-EtOAc); mp 240-242°C; IR (KBr): 3420, 3226, 3143, 2988, 1632, 1546, 1231, 1061 cm^{-1}; MS (*m/z*): 354 (M^+); Anal. Calcd for $C_{15}H_{16}Cl_2N_4O_2$: C, 50.72; H, 4.54; N, 15.77; Found: C, 55.73; H, 4.64; N, 15.62.

2-amino-4isopropyl-6-methoxy-*N*-*p*-tolylpyrimidine-5-carboxamide (PVP-1m): yellow solid; R_f 0.27 (6:4 hexane-EtOAc); mp 194-196°C; IR (KBr): 3412, 3317, 3253, 2950, 1613, 1539, 1431, 1061 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65; Found: C, 63.88; H, 6.65; N, 18.52.

2-amino-4isopropyl-6-methoxy-*N*-*o*-tolylpyrimidine-5-carboxamide (PVP-1n): yellow solid; R_f 0.21 (6:4 hexane-EtOAc); mp 185-187°C; IR (KBr): 3442, 3327, 3253, 2980, 1623, 1569, 1431, 1051 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65; Found: C, 63.88; H, 6.65; N, 18.52.

2-amino-4isopropyl-6-methoxy-*N*-(2-methoxyphenyl)pyrimidine-5-carboxamide (PVP-1o): yellow solid; R_f 0.22 (6:4 hexane-EtOAc); mp 165-167°C; IR (KBr): 3462, 3327, 3220, 2980, 1623, 1509, 1461, 1051 cm^{-1}; MS (*m/z*): 316 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71; Found: C, 60.68; H, 6.55; N, 17.62.

2-amino-*N*-(2-fluorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1p): yellow solid; R_f 0.25 (6:4 hexane-EtOAc); mp 178-180°C; IR (KBr): 3442,

3327, 3173, 2989, 1653, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 304 (M^+); Anal. Calcd for $C_{15}H_{17}FN_4O_2$: C, 59.20; H, 5.63; N, 18.41; Found: C, 59.16; H, 5.55; N, 18.32.

2-amino-*N*-(2-bromophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1q): yellow solid; R_f 0.24 (6:4 hexane-EtOAc); mp 190-192°C; IR (KBr): 3459, 3327, 3173, 2989, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 364 (M^+); Anal. Calcd for $C_{15}H_{17}BrN_4O_2$: C, 49.33; H, 4.69; N, 15.34; Found: C, 49.13; H, 4.45; N, 15.22.

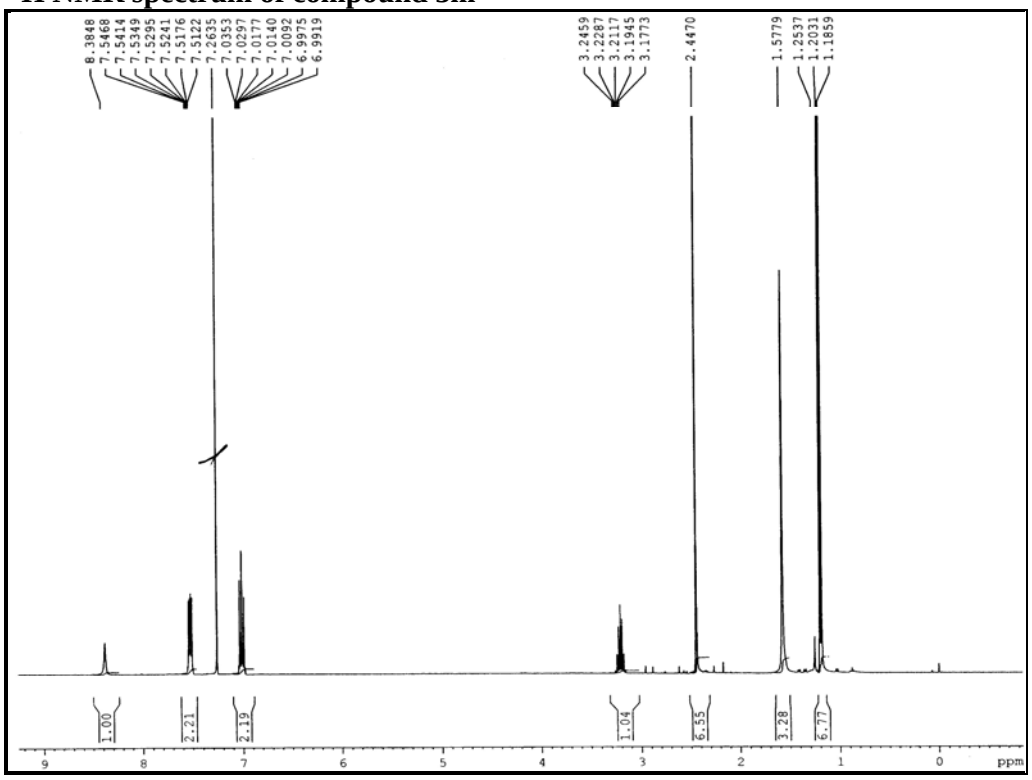
2-amino-4-ethoxy-6-isopropyl-*N*-phenyl pyrimidine-5-carboxamide (PVP-1r): White solid; R_f 0.43 (6:4 hexane-EtOAc); mp 198-200°C; IR (KBr): 3459, 3327, 3193, 2999, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65; Found: C, 63.92; H, 6.65; N, 18.56.

2-amino-*N*-(4-chlorophenyl)-4isopropyl-6-methoxypyrimidine-5-carboxamide (PVP-1s): yellow solid; R_f 0.23 (6:4 hexane-EtOAc); mp 185-187°C; IR (KBr): 3459, 3252, 3143, 2919, 1648, 1586, 1241, 1051 cm^{-1}; MS (*m/z*): 320 (M^+); Anal. Calcd for $C_{15}H_{17}ClN_4O_2$: C, 56.16; H, 5.34; N, 17.47; Found: C, 56.20; H, 5.25; N, 17.42.

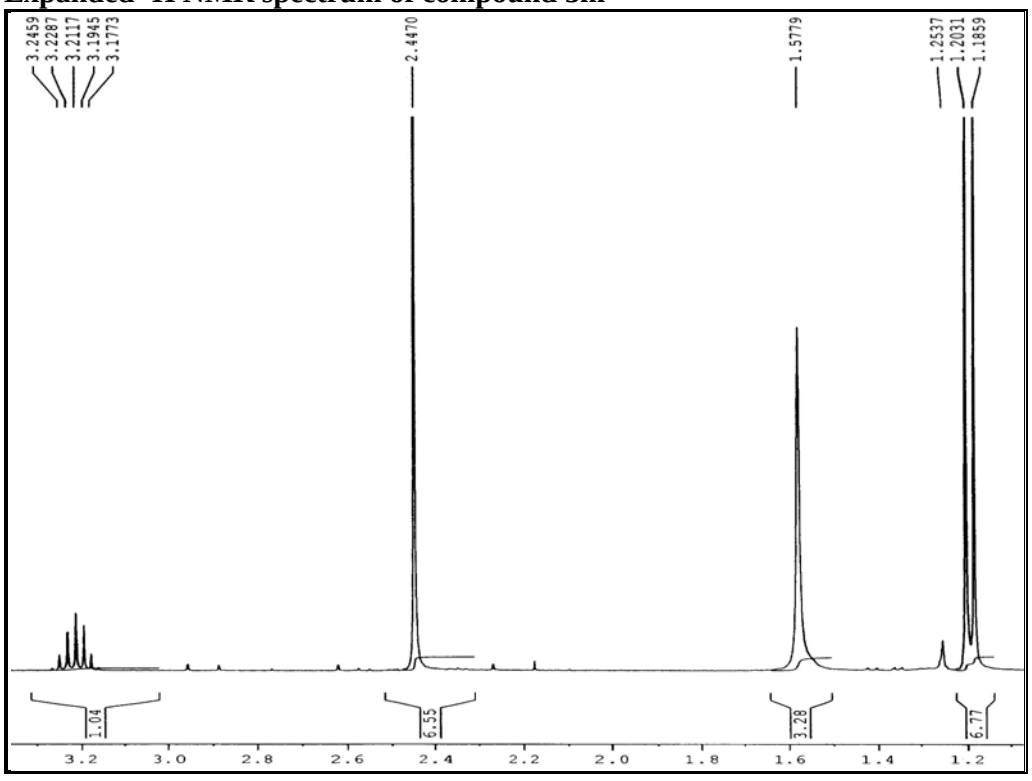
2-amino-*N*-(3-chlorophenyl)-4ethoxy-6-isopropylpyrimidine-5-carboxamide (PVP-1t): White solid; R_f 0.42 (6:4 hexane-EtOAc); mp 210-212°C; IR (KBr): 3449, 3227, 3193, 2966, 1628, 1522, 1217, 1041 cm^{-1}; MS (*m/z*): 334 (M^+); Anal. Calcd for $C_{16}H_{19}ClN_4O_2$: C, 57.40; H, 5.72; N, 16.73; Found: C, 57.44; H, 5.65; N, 16.67.

❖ **Spectral representation of synthesized compounds**

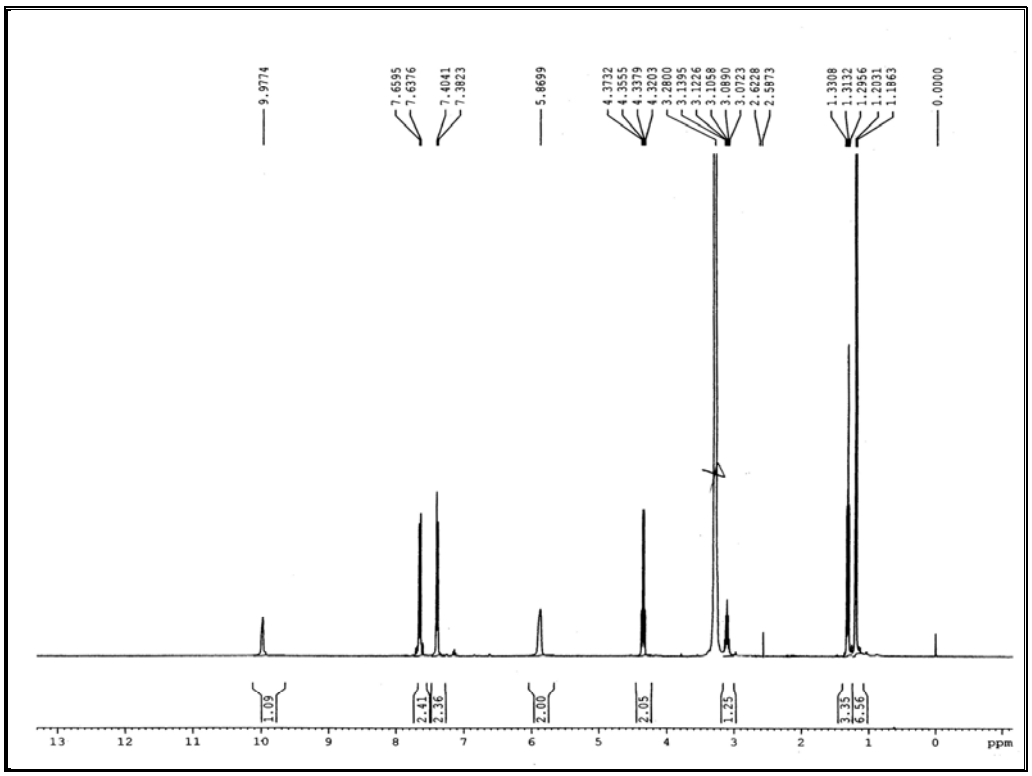
^{1}H NMR spectrum of compound 3m



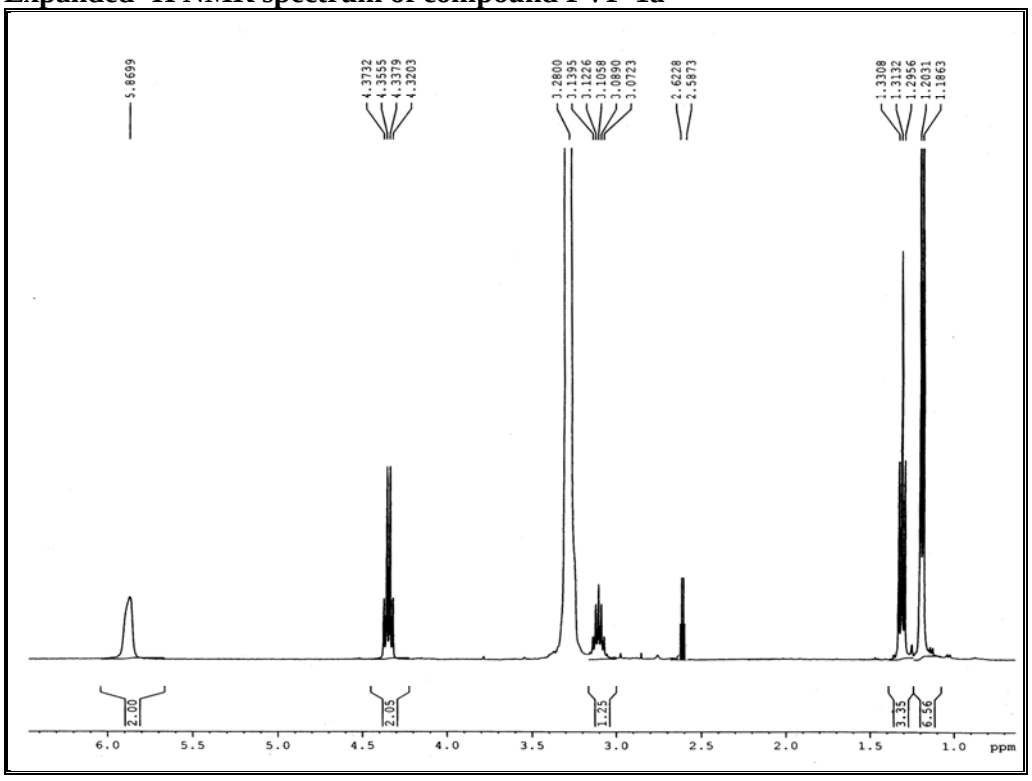
Expanded ^{1}H NMR spectrum of compound 3m



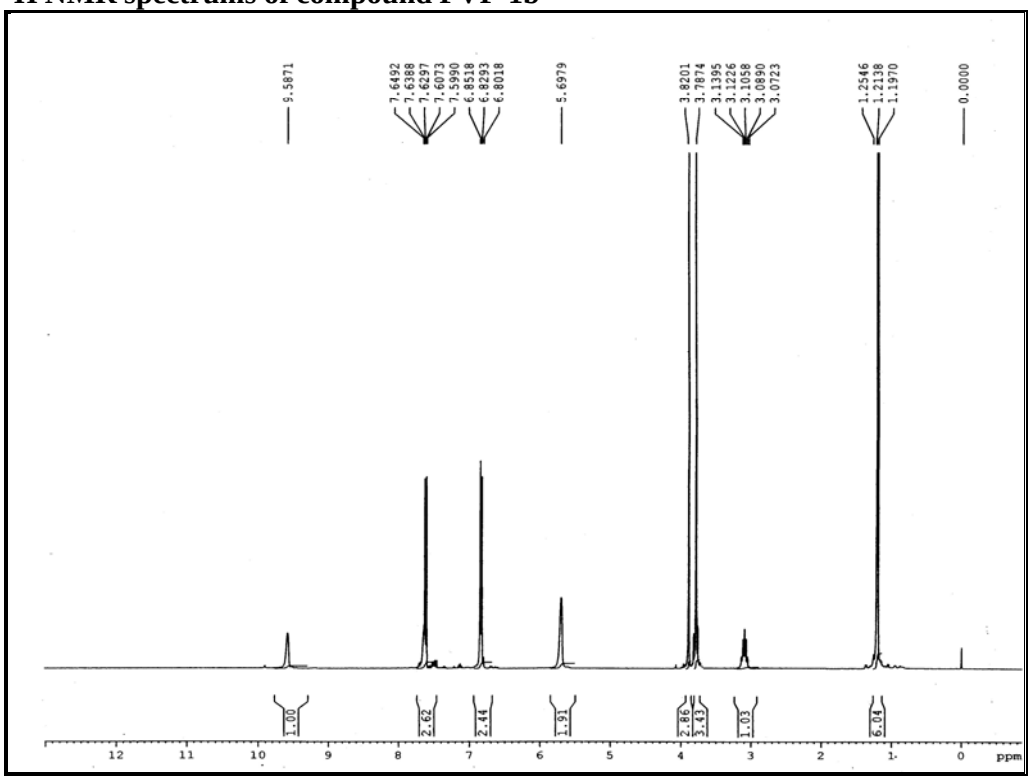
^{1}H NMR spectrums of compound PVP-1a



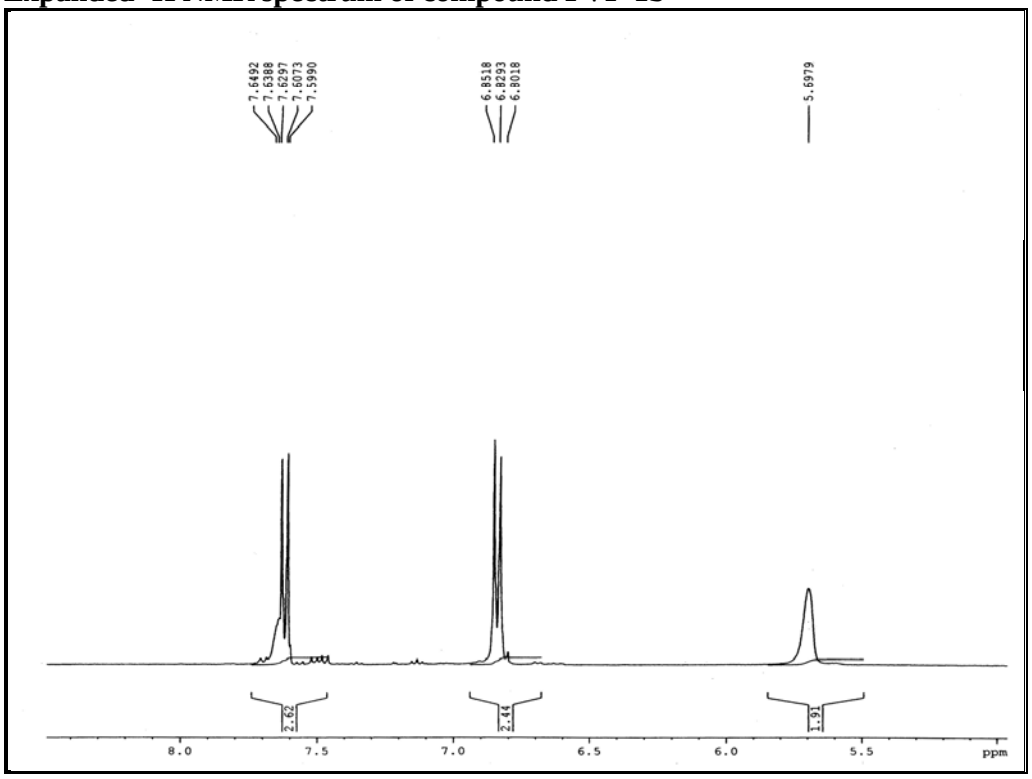
Expanded ^{1}H NMR spectrum of compound PVP-1a



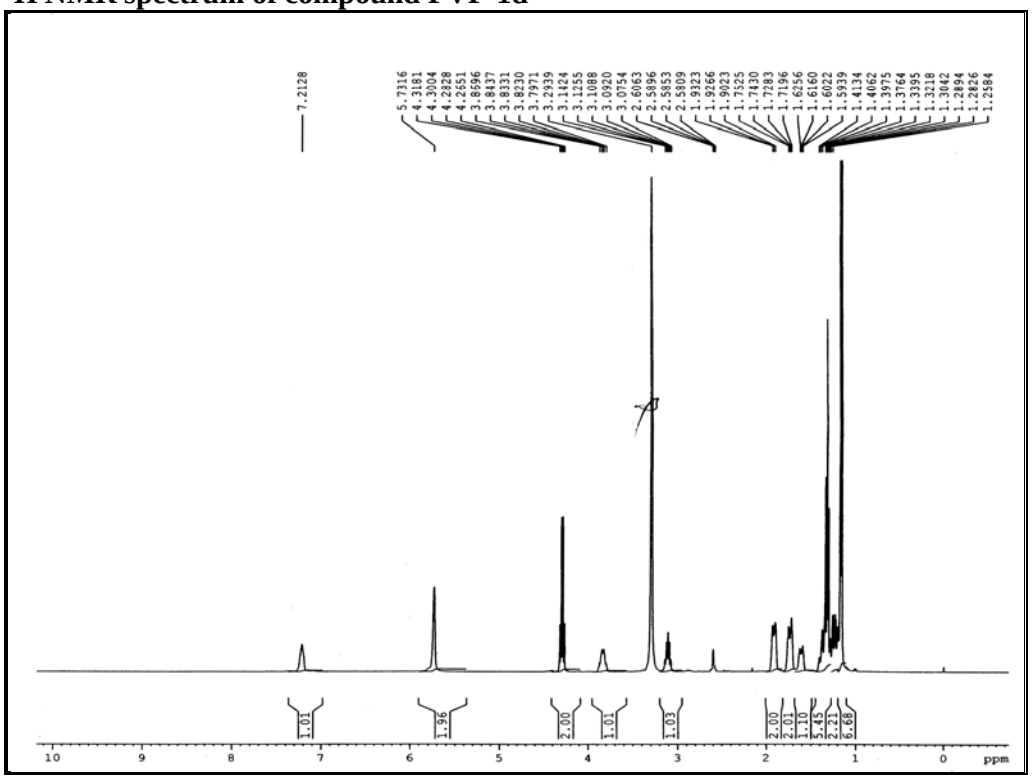
^{1}H NMR spectrums of compound PVP-1b



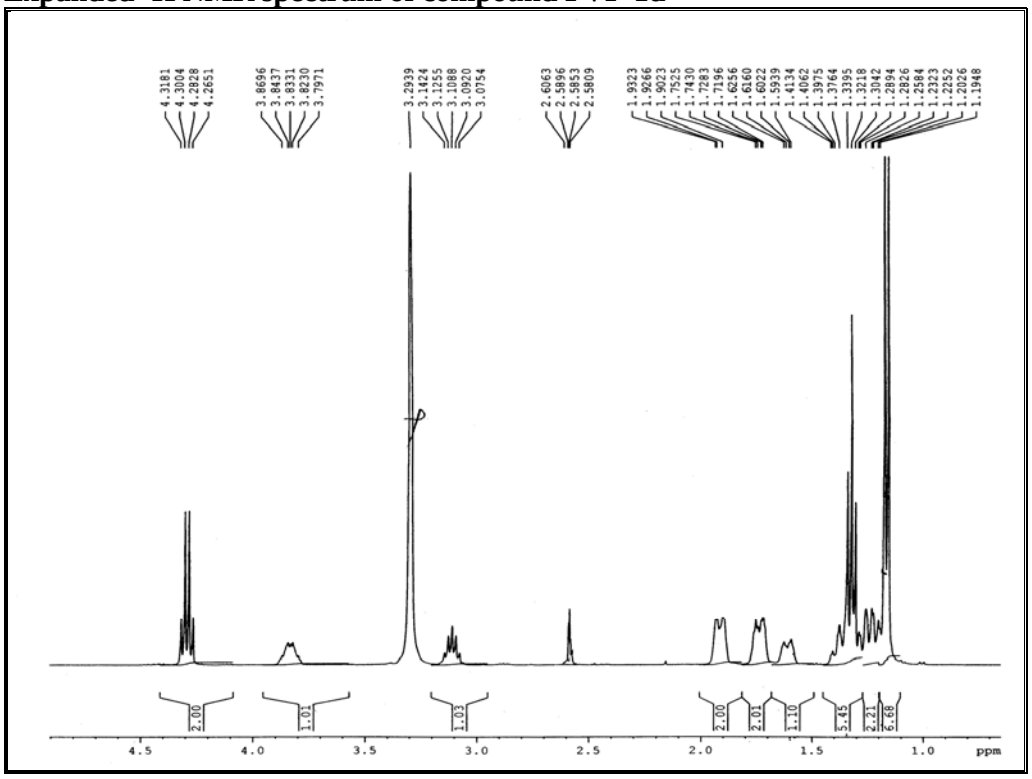
Expanded ^{1}H NMR spectrum of compound PVP-1b



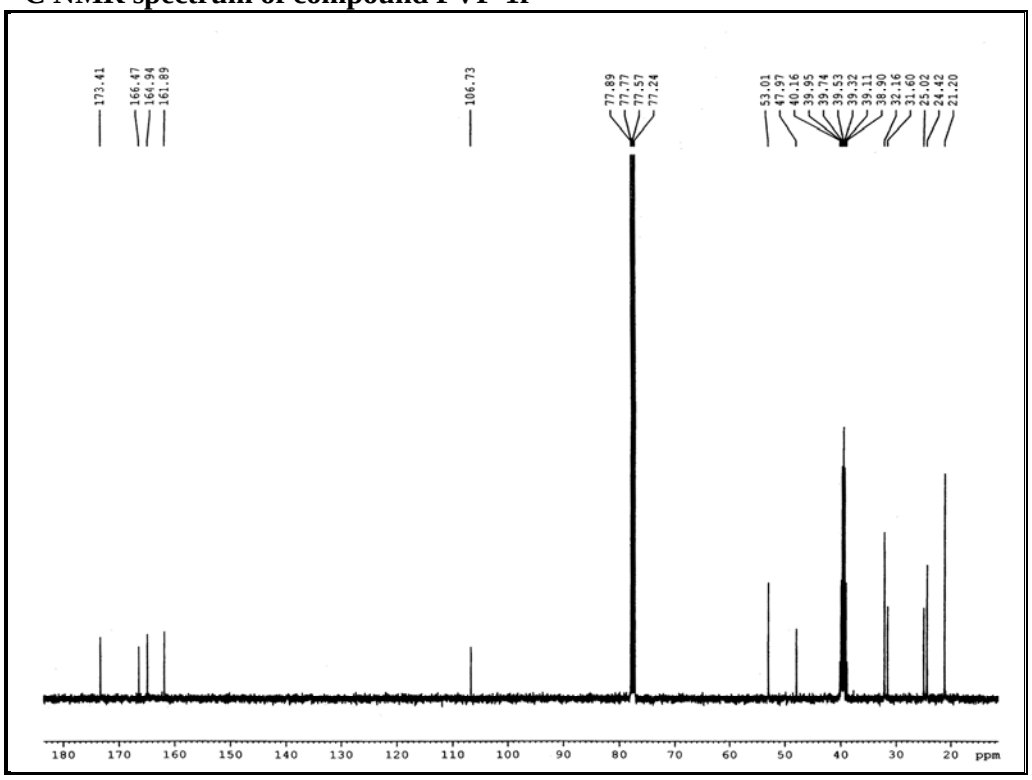
^{1}H NMR spectrum of compound PVP-1d



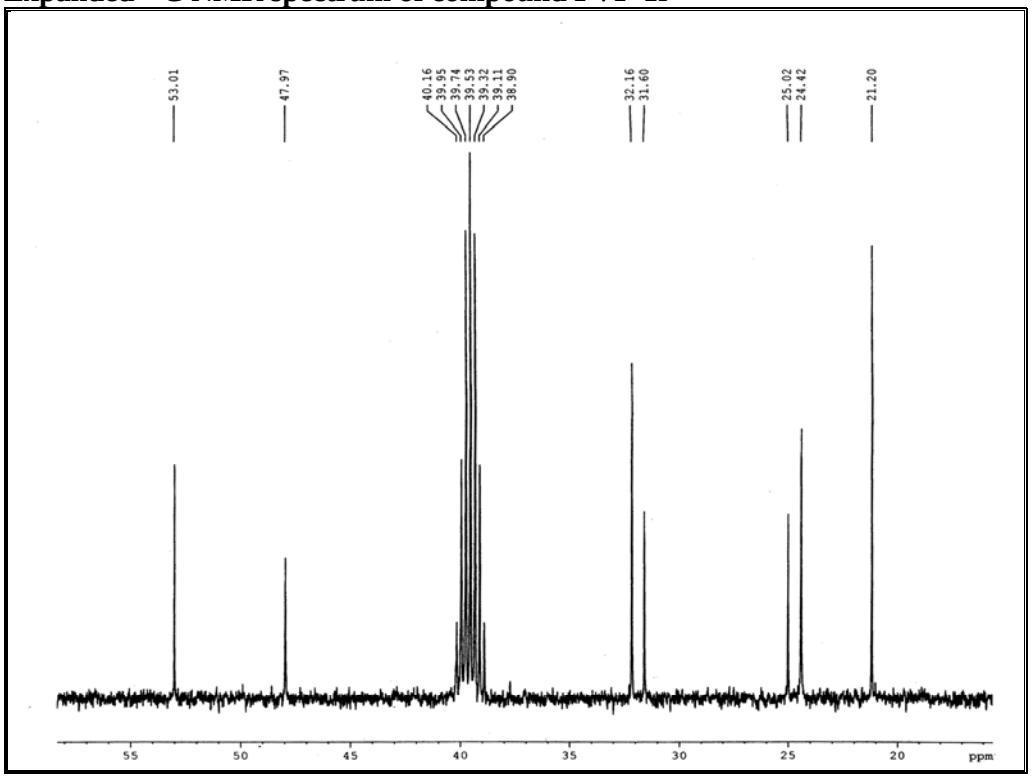
Expanded ^{1}H NMR spectrum of compound PVP-1d



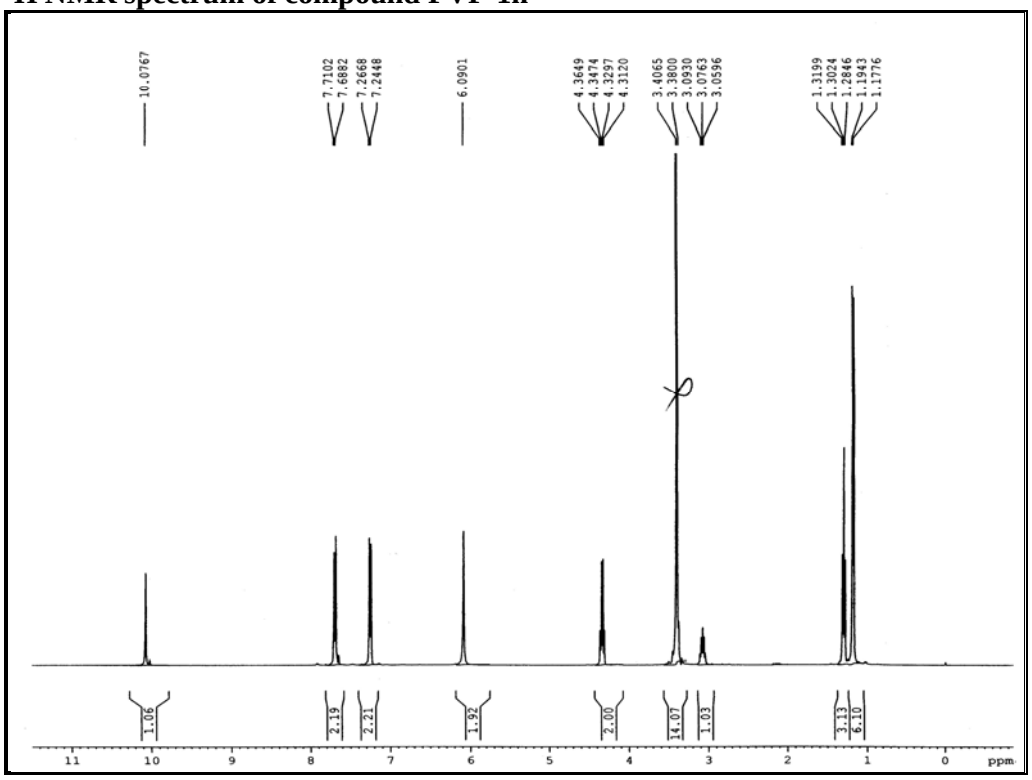
^{13}C NMR spectrum of compound PVP-1f



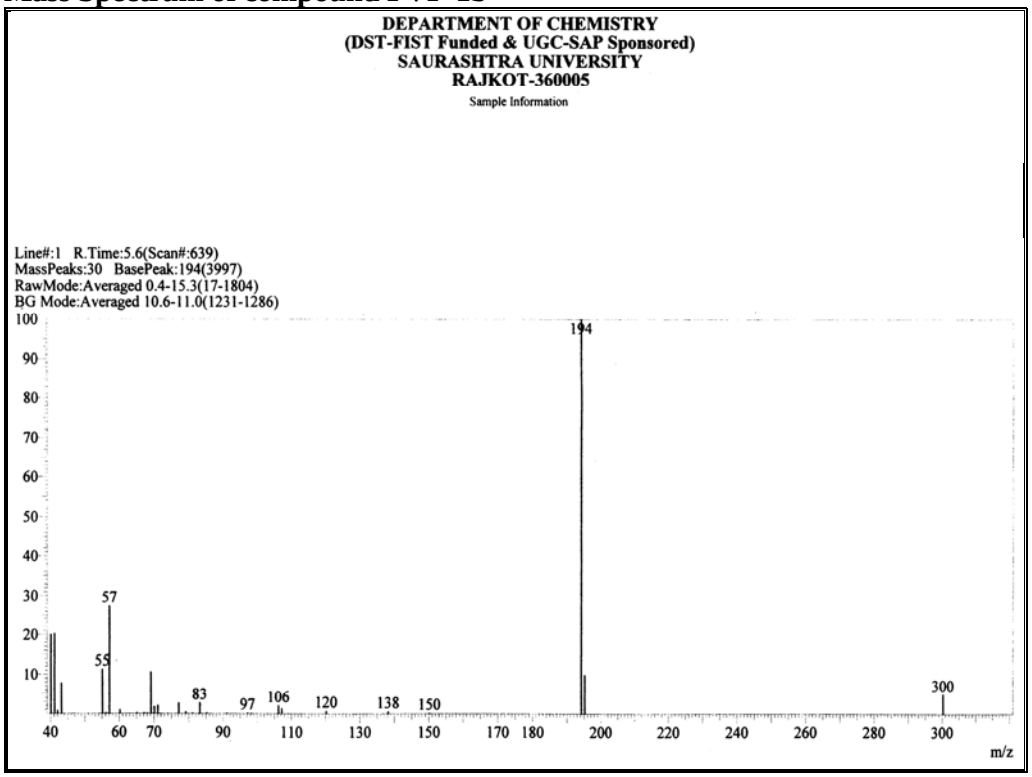
Expanded ^{13}C NMR spectrum of compound PVP-1f



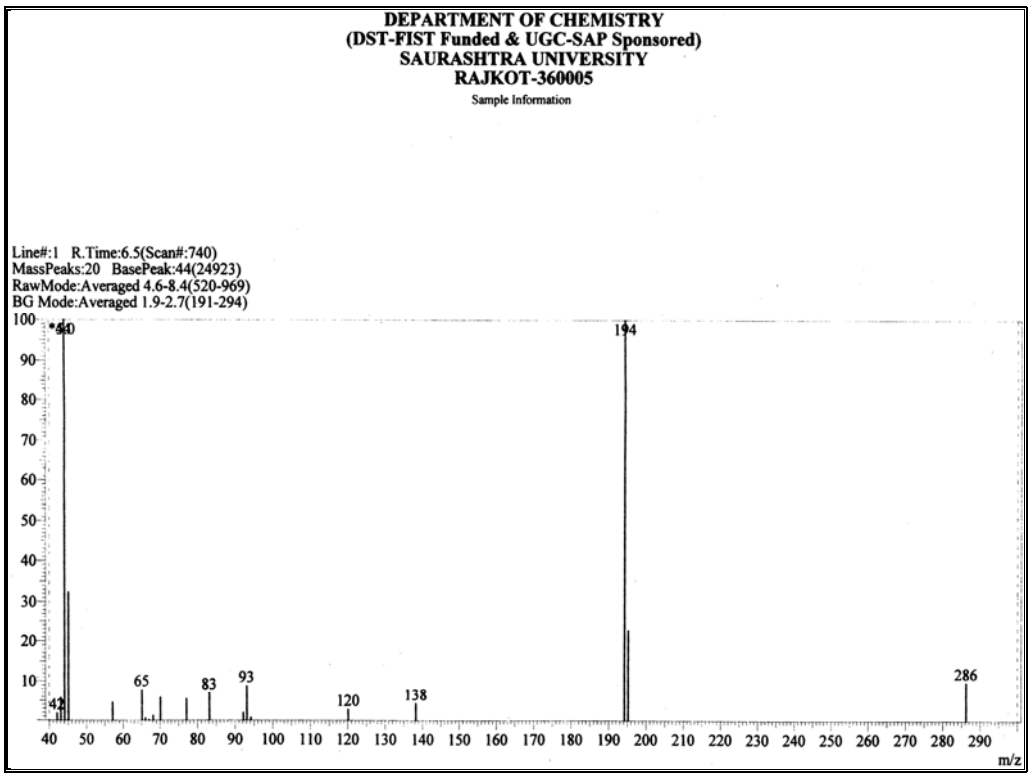
^{1}H NMR spectrum of compound PVP-1h



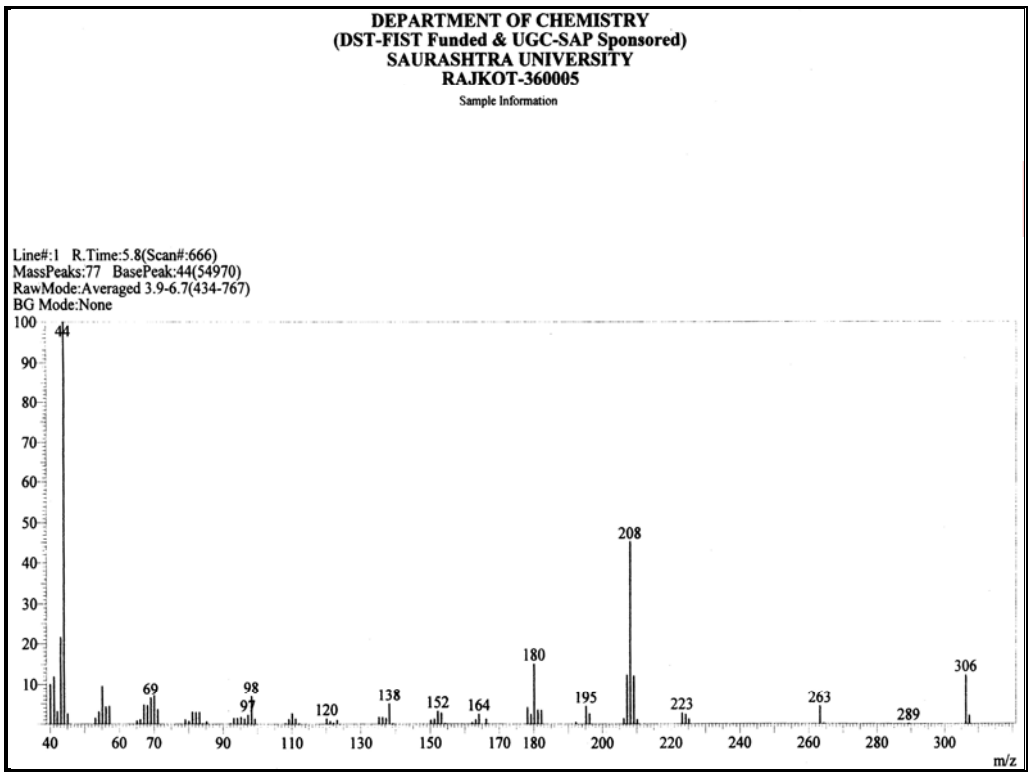
Mass Spectrum of compound PVP-1b



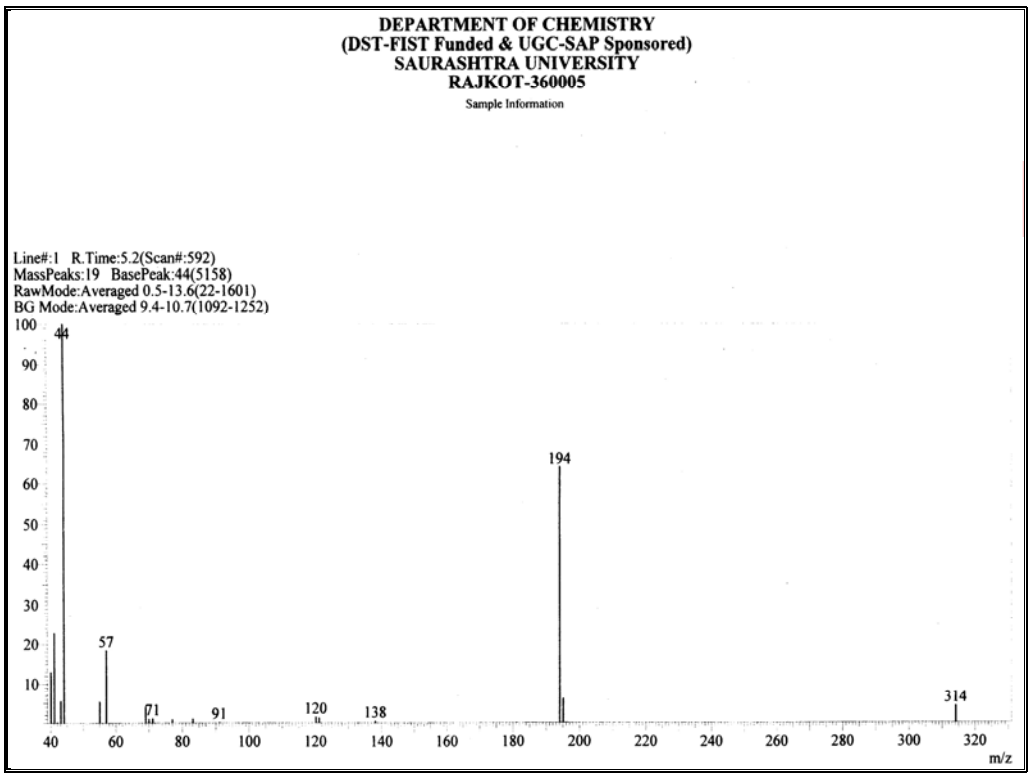
Mass Spectrum of compound PVP-1c



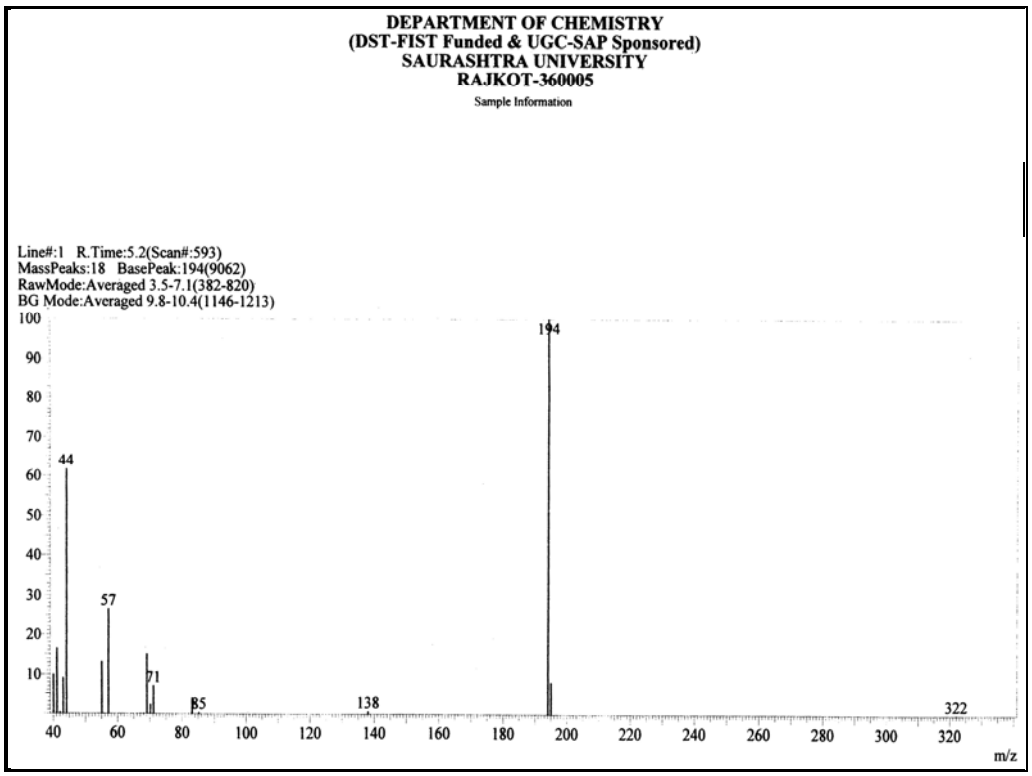
Mass Spectrum of compound PVP-1d



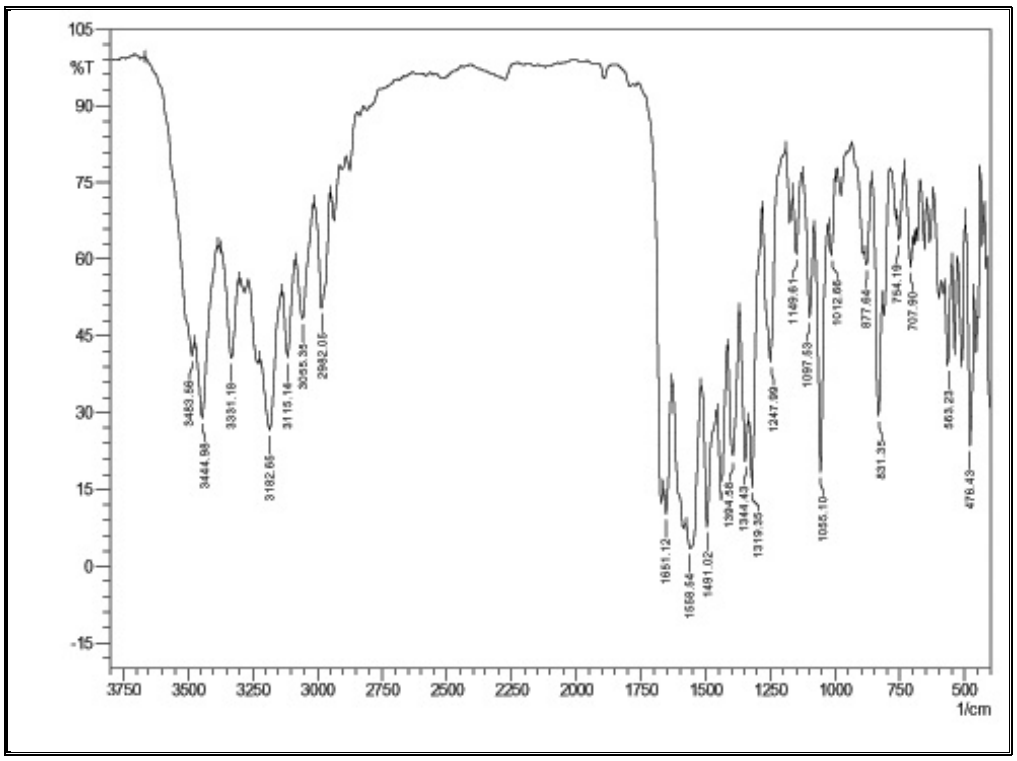
Mass Spectrum of compound PVP-1e



Mass Spectrum of compound PVP-1j



IR Spectrum of compound PVP-1h



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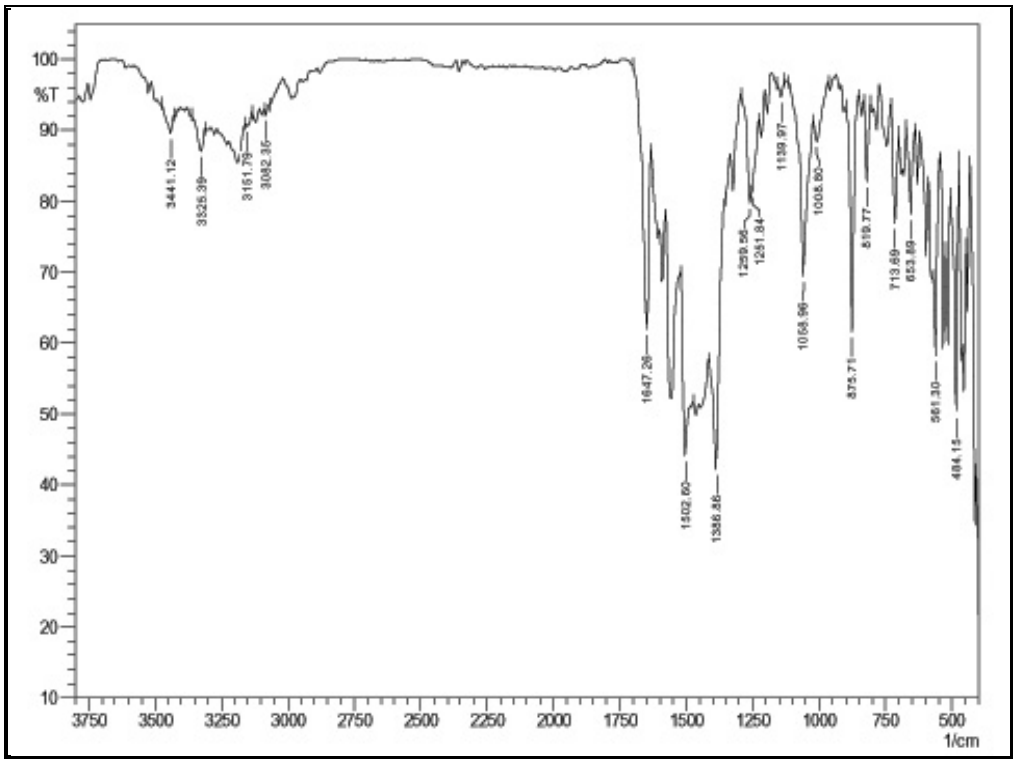

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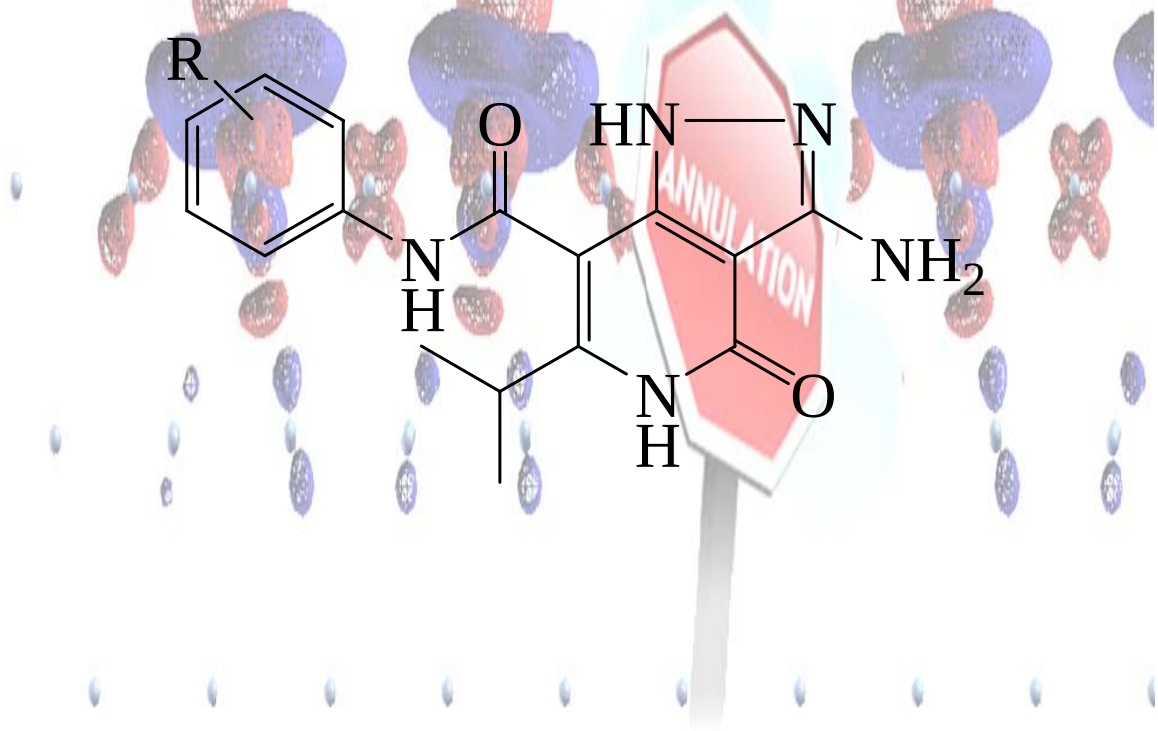
Chapter 2

SYNTHESIS OF SOME NOVEL PYRAZOLOPYRIDONE DERIVATIVES USING KETENEDITHIOACETALS AND THEIR ANTIMICROBIAL ACTIVITY.



2.1 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.

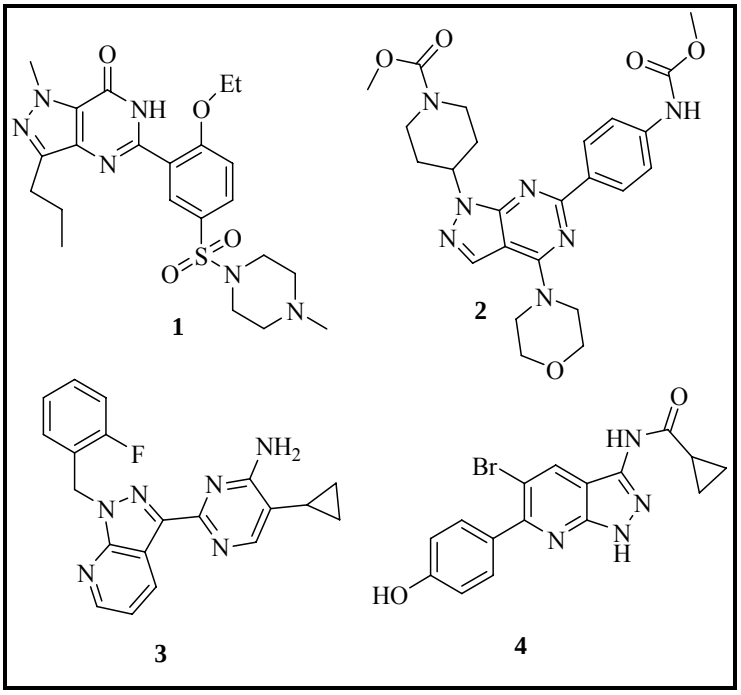


Figure 1

2.2 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]

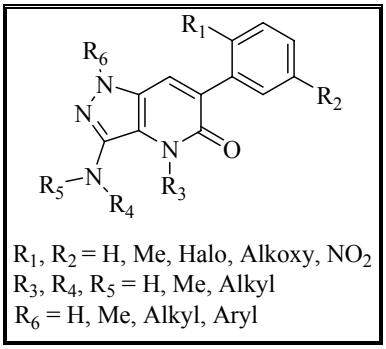


Figure 2

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

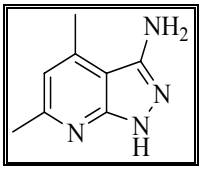


Figure 3

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 *i*M.

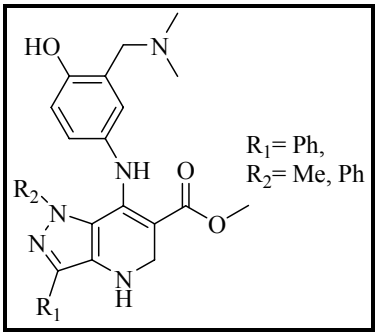


Figure 4

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.

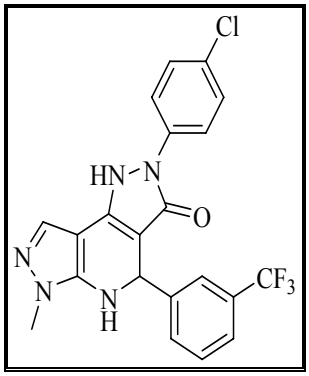


Figure 5

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 messenger's 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]

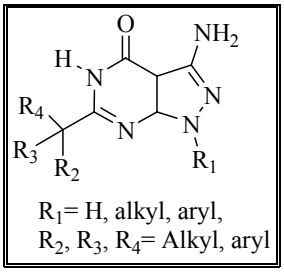


Figure 6

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.

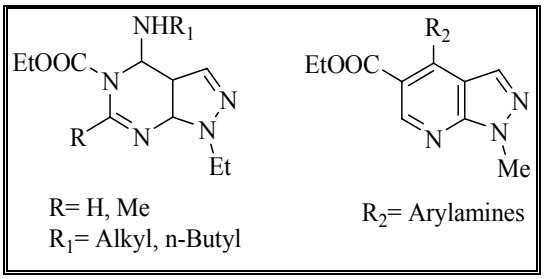


Figure 7

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]

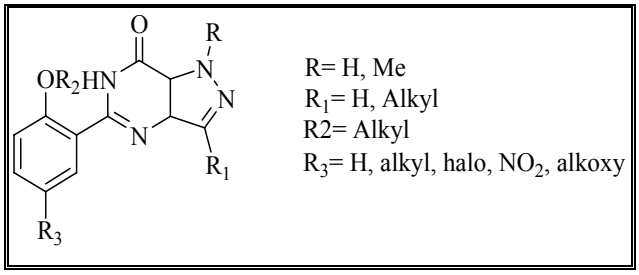


Figure 8

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.

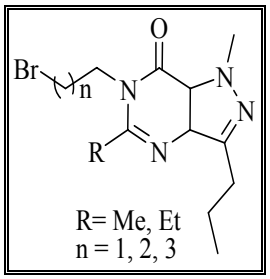


Figure 9

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.

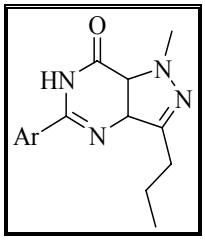


Figure 10

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 and1-ethyl-3-methyl-6-(2-propoxy-5-(4-methylthiazol-2-yl)phenyl)-1,5dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**Figure 11**) displayed outstanding *in vivo* activities at 5 mg/kg/os and good metabolic stabilities.

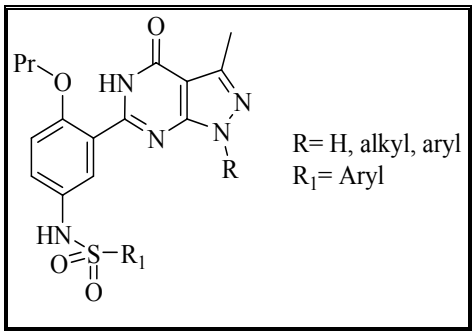


Figure 11

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.

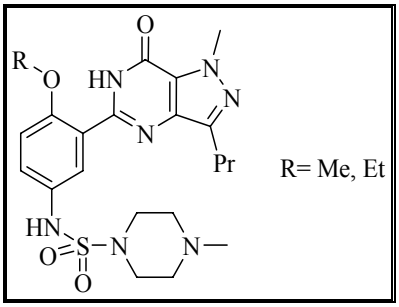


Figure 12

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.

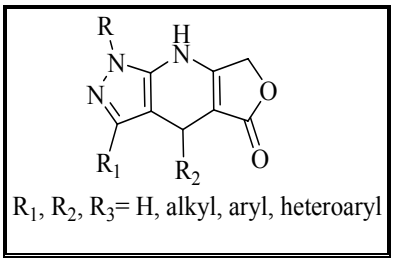


Figure 13

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.

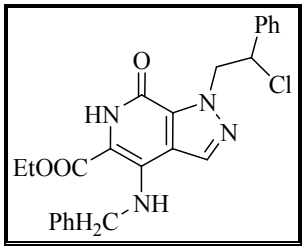


Figure 14

Thierry sifferlen et al[20] have been reported the synthesis and structure-activity relationship of a novel series of dual orexin reseptoar antagonists was prepared by heteroaromatic five-membered ring system replacement of dimethoxyphenl moiety contained in the tetrahydroquinoline core skeleton of almorexant.(**Figure 15**) Thus, replacement of the dimethoxyphenyl by a substituted pyrazole and additional optimization of the substitution pattern of the phenethyl motit allowed the identification of potent antagonists with nanomolar affinity for hOX_1R and hOX_2R.

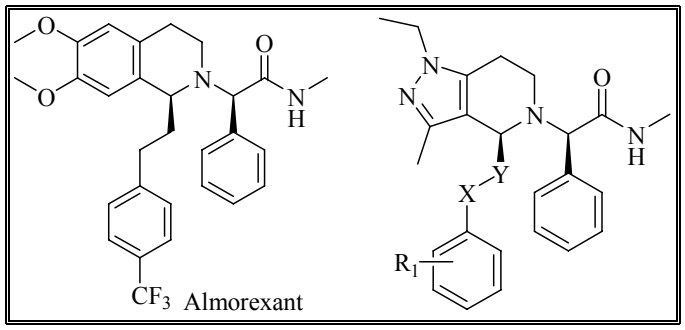


Figure 15

Raymond V. F. et al[21] have synthesized a series of 2-amino-pyrazolopyridines (**Figure 16**) was designed as polo-like kinase (plk) inhibitors based on a low micro molar hit.The SAR was developed to provide compounds exhibiting low nano molar inhibitory activity of plk 1; the phenotype of treated cells is consistent with plk1 inhibition.

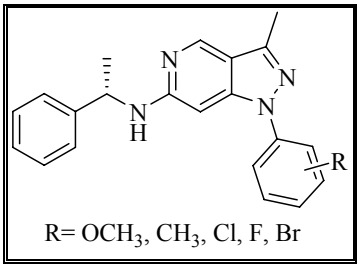


Figure 16

Xiacong M. et al[22] have discovered a series of pyrazolopiperidine sulfonamide based **(Figure 17)** γ-secretes Inhibitors and its SAR evolution is described. Significantly increases in APP potency on the pyrazolopipridine scaffold over the original *N*-bicyclic sulfonamide scaffold were achieved and this potency increase translated in an improved in vivo efficacy.

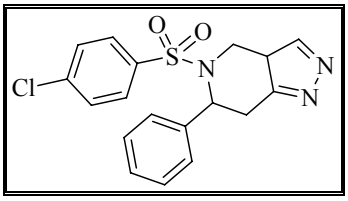


Figure 17

2.3 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[23] have described the preparation of two novel heterocyclic nuclei isoxazolopyridone and pyrazolopyridone (**Figure 18**) 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.

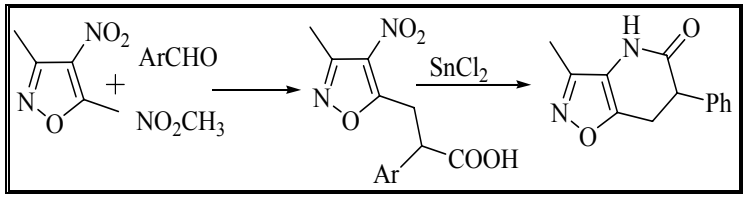


Figure 18

Sayed G. L. et al[24] 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 19**). 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.

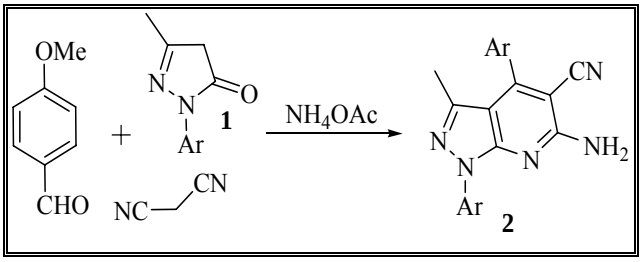


Figure 19

Attaby F. A. et al[25] have synthesized some pyrazolopyridone derivatives (**Figure 20**). 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**.

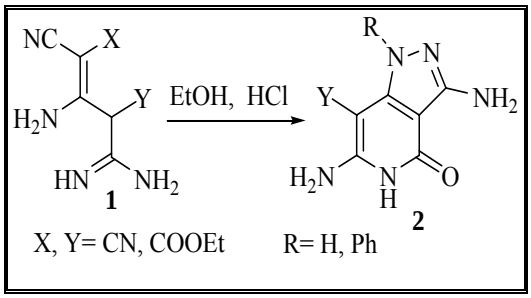


Figure 20

Junjappa H. et al[26] have developed a novel process for the synthesis of substituted *N*-methylpyrazolopyridones (**Figure 21**). 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.

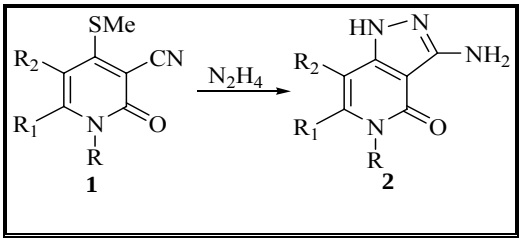


Figure 21

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 22**).[27]

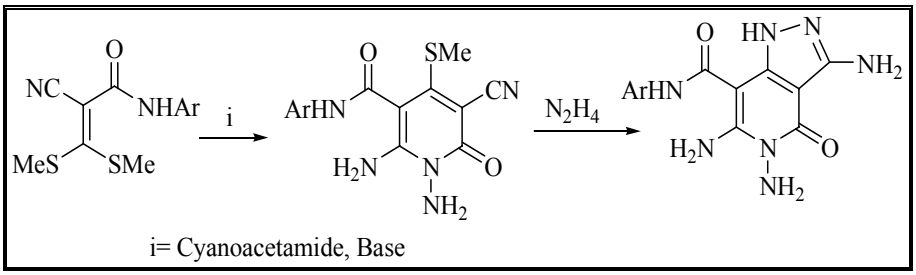


Figure 22

Additionally, a novel and efficient method for the synthesis of substituted 4-alkylthio-*N*-arylsulphonylamino-2-pyridones *via* the reaction of ketene-*S,S*-acetals with *N*-cyanoacetoarylsulfonylhydrazides has been developed by Elgemeie G. H. and coworkers.[28] 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 23**).

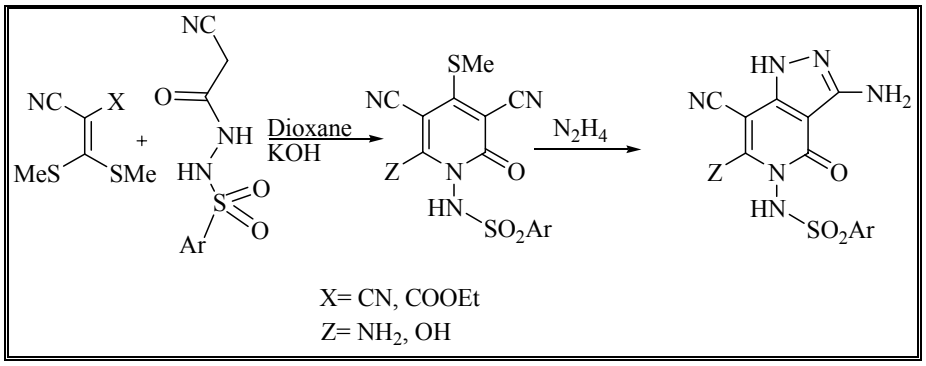


Figure 23

Lacova M. et al[29] have developed one-pot and facile preparations of 6-(2-hydroxy-5-*R*-benzoyl)-4-methyl-2-aryl-pyrazolo[3,4-*b*]pyridines (**Figure 24**), 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.

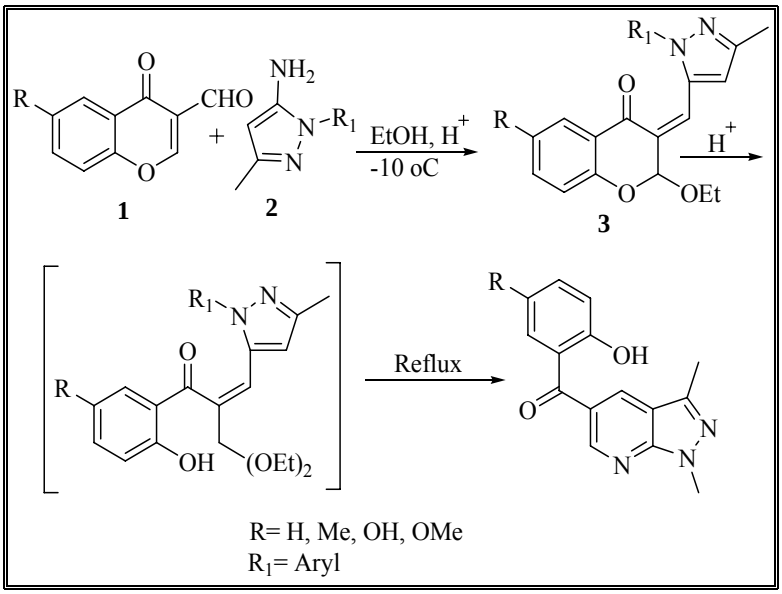


Figure 24

Abass M. has synthesized several fused pyrazolopyrimidones (**Figure 25**) with quinolone scaffold. He has described the synthesis of amino-ester, its hydrolysis and chloroacetylatio, which were utilized for the synthesis of pyrazoloyridones.[30]

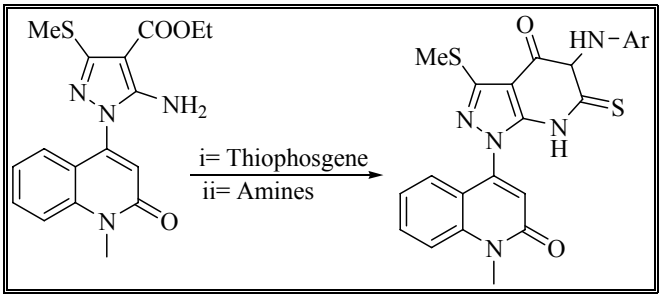


Figure 25

Hassanein E. M. et al[31] have synthesized some pyrazolopyridone derivatives *via* the reaction of compound **1** with ketene dithioacetal **2,** yielded compound **3** in good yields (**Figure 26**). Further, the reaction of **3** with hydrazine afforded pyrazolopyridones **4** in high yield.

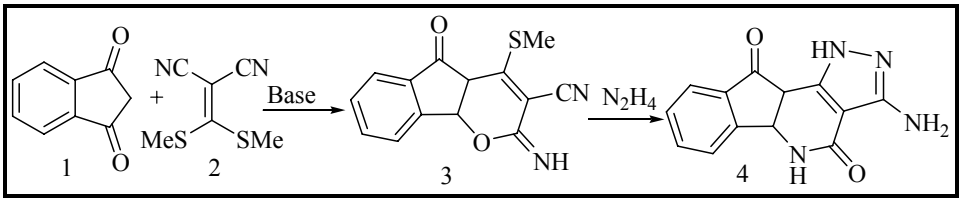


Figure 26

A mild one-step synthetic method to access privileged pyrazolearylpyrazole[3,4-*b*]pyridines (**Figure 27**) from indole-3-carboxaldehyde derivatives and a variety of aminopyrazoles has been developed by Park S. B. and coworker.[32] This novel method constructs heterobiaryls with the wide scope of substrate generality and excellent regioselectivity *via* indole ring opening.

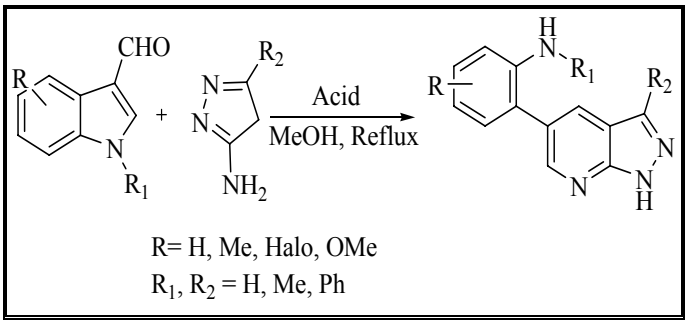


Figure 27

Rodrigues L. M. et al[33] have synthesized some pyrazolopyridine derivatives (**Figure 28**). The reaction of *N*-substituted-5-amino-4-cyanopyrazoles with malononitrile occurs with formation of 6-substituted pyrazole[3,4-*b*]pyridines respectively.

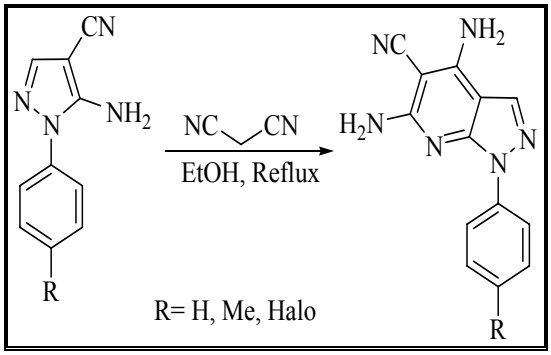


Figure 28

Recently, Yao H. et al[34] 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 29**).

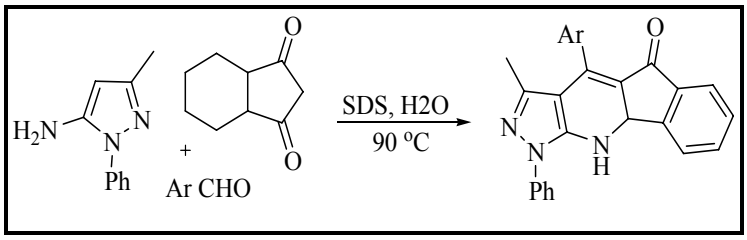


Figure 29

Li J. R. et al[35] have developed synthesis of pyrazolopyrimidinones under microwave irradiation (**Figure 30**). They have demonstrated that the direct reaction of *o*-aminopyrazocarbonitriles and carbonyl compounds afforded pyrazolopyrimidinones under microwave irradiation with high yields.

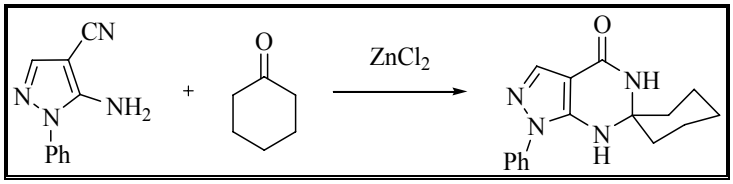


Figure 30

Mekheimer R. et al[36] have synthesized some benzoannulated pyrazolopyridones **2** by the reaction of **1** with hydrazine hydrate (**Figure 31**).

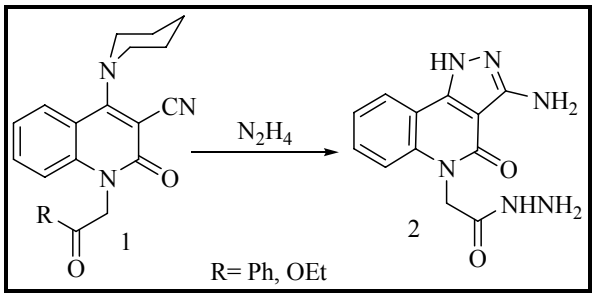


Figure 31

Swett L. R. et al[37] have synthesized two isomeric pyrazolopyridones (**Figure 32**) which were identified as their tetrahydropyrazolopyridine derivatives by the reaction of 5-amino-1,3-dimethylpyrazole with ethyl acetoacetate.

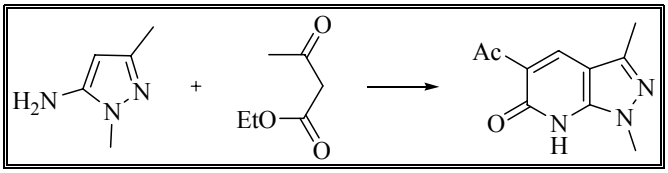


Figure 32

Lynette A. Smyth et al,[38] have synthesized 3-amino-1*H*-pyrazolo[4, 3-c]pyridine-4-(5*H*)ones represent apotentially attractive heteroaromatic scaffold. (**Figure 33)** The reaction of bis(methylthio)but-3-en-2one **1** with cyanoacetamide to get 6-arylpyridones **2** Further, the pyridones on reaction with hydrazine hydrated in isopropyl alcohol furnished the novel pyrazolopyridone **3** derivatives in excellent yields.

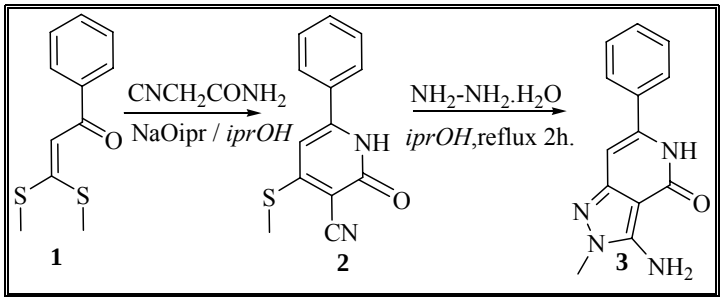


Figure 33

2.4 CURRENT RESEARCH WORK

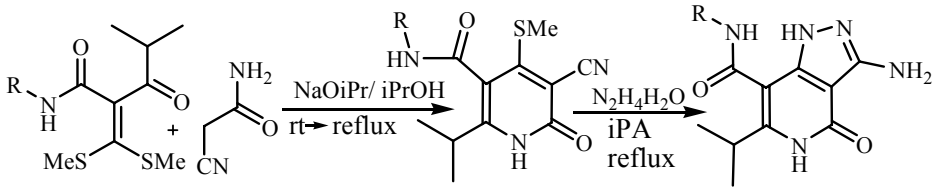
The pyrazolopyridine and pyrazolopyrimidine derivatives have considerable chemical and pharmacological importance because of a broad range of biological activities displayed by these classes of molecules. As we demonstrated, the tremendous biological potential of pyrazolopyridine derivatives encouraged us to synthesize some pyrazolopyridone 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 their anti microbial activity.

2.5 RESULTS AND DISCUSSION

Initially, the reaction of 2-(bis(methylthio)methylene)-3-oxo-*N*-arylbutanamide (**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-t **2** **3a-t** **PVP -2a-t**

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-t** were further reacted with hydrazine hydrate in isopropyl alcohol to afford the pyrazolopyridone **PVP-2a-t** 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 anti microbial activity.

Table 2: Synthesis of various pyrazolopyridones PVP-2a-t.

Entry	R	Time min	Yield %	mp ^{o}C
PVP-2a	4-$OCH_3C_6H_4$	50	89	280-282
PVP-2b	C_6H_5	65	86	270-272
PVP-2c	C_6H_{11}	40	89	283-285
PVP-2d	2,5-di-$CH_3C_6H_3$	45	87	265-277
PVP-2e	4-BrC_6H_4	55	88	285-287
PVP-2f	4-FC_6H_4	65	89	288-290
PVP-2g	4-ClC_6H_4	60	85	270-272
PVP-2h	3-Cl,4-FC_6H_3	45	84	290-292
PVP-2i	3,4-di-FC_6H_3	50	84	280-285
PVP-2j	3-ClC_6H_4	60	85	270-272
PVP-2k	3,4-di-ClC_6H_3	65	87	275-287
PVP-2l	4-$CH_3C_6H_4$	70	88	288-290
PVP-2m	2-$CH_3C_6H_4$	60	89	290-292
PVP-2n	2-$OCH_3C_6H_4$	70	83	280-282
PVP-2o	2-FC_6H_4	75	92	268-270
PVP-2p	2-BrC_6H_4	75	84	268-270
PVP-2q	4-$NO_2C_6H_4$	60	85	284-286
PVP-2r	3,4-di-C_6H_3	55	89	278-280
PVP-2s	2-ClC_6H_4	60	85	275-277
PVP-2t	3-$CH_3C_6H_4$	55	84	290-292

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.

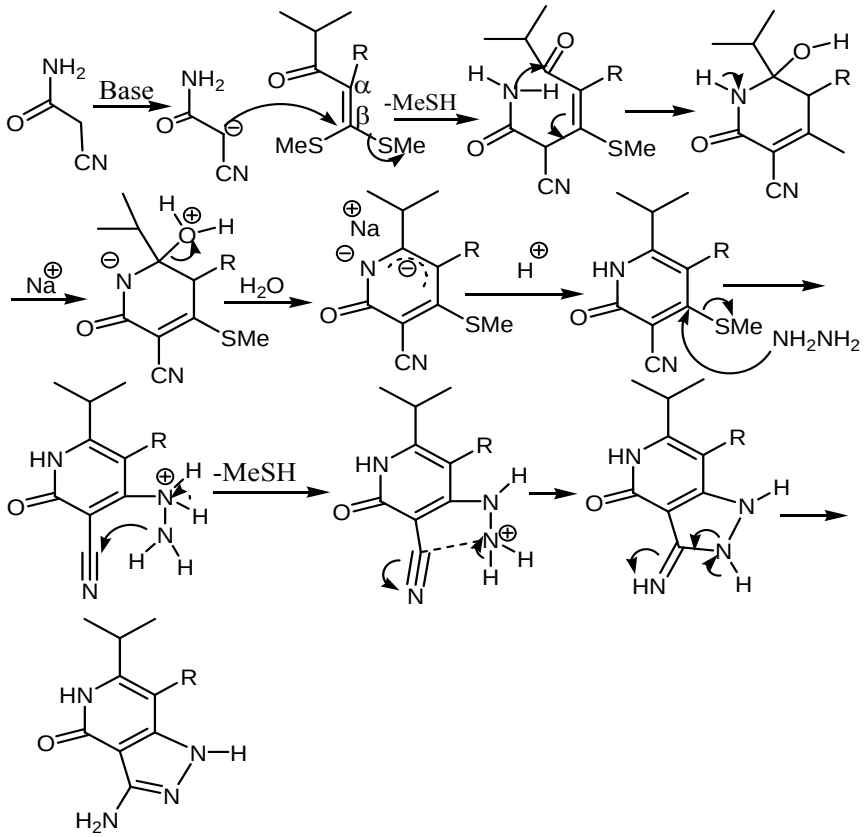


Figure 32: Proposed mechanism for the formation of pyrazolopyridone.

2.6 ANTIMICROBIAL SENSITIVITY TESTING

WELL DIFFUSION / AGAR CUP METHOD (Lt. General Raghunath D. 1998, Ashok Rattan, 1998; Patel R., Patel K. 2004,)

In vitro effectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective then many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive then using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Requirements

1. Young broth culture of a standard test organism
2. Sterile Mueller Hinton Agar plate
3. Solution of antimicrobial substance
4. Cup borer
5. Alcohol etc.

Inoculum preparation

Inoculum was prepared by selecting 4-5 colonies from slope of stock culture of the indicator organism and emulsifying them in a suitable broth. The inoculated broth was incubated at 37ºC till it equals turbidity of a 0.5 McFarland standard. This happens in 2-8 h.

Procedure

1. Inoculate test organism on the top of Mueller Hinton Agar plate with help of sterile swab. (it can be inoculated in melted agar also)
2. The swab was dipped in the inoculum and surface of plate was streaked with swab.
3. Streaking was repeated for 3 times and each time the plate was rotated at angle of 60º.
4. Sterilize the cup-borer make four cups of the diameter of 8-10 mm. at equal distance in the plate previously inoculated with seed culture.
5. The depth of well was 2.5-5.0 mm.
6. The wells have been clearly punched so the surrounding medium is not lifted when the plug was removed out.
7. The plates were incubated at 37ºC for 24 h. Then the zone of inhibition measured and the size of zone cited in table.

❖ *Antibiotic Sensitivity Assay*

(Concentration250/500/ 1000 µG/ml)

Sr. No.	COD E No.	*Pseudomonas aeruginosa*			*Proteus vulgaris*			*Escherichia coli*			*Staphylococcus aureus*			*Candida albicans*		
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
1.	**2a**	1.1	1.2	1.3	R	1.1	1.2	1.1	1.2	1.3	R	1.1	1.2	1.2	1.5	2
2.	**2b**	R	R	R	R	R	R	1.2	1.5	1.7	R	R	R	R	R	R
3.	**2c**	1.1	1.2	1.4	1	1.3	1.7	1.4	1.5	2	R	R	R	R	1.2	1.5
4.	**2d**	1.3	1.4	1.8	1.2	1.5	1.9	1.2	1.7	2.1	R	R	R	R	1	1.3
5.	**2e**	1.1	1.5	1.7	1.1	1.3	1.5	1.3	1.4	1.7	R	R	R	R	R	R
6.	**2f**	1.1	1.4	1.6	1	1.2	1.3	1.2	1.3	1.6	1.2	1.4	1.6	1.1	1.2	1.7
7.	**2g**	1.4	1.7	2	1.2	1.5	1.8	1.2	1.2	1.5	1.4	1.7	2.1	1	1.2	1.8
8.	**2h**	1.3	1.6	2	1.3	1.6	2	R	R	R	1.1	1.8	2.3	1.1	1.4	1.8
9.	**2i**	1.2	1.6	1.8	1.1	1.3	1.4	R	R	R	1.2	1.5	1.7	1.2	1.6	2.1
10.	**2j**	R	1	1.1	1	1.2	1.8	1.1	1.1	1.3	R	R	R	1	1.4	2
11.	**2k**	R	1.1	1.3	1.1	1.3	1.7	R	1.6	2	R	R	R	R	1.1	1.5
12.	**2l**	1.1	1.4	1.7	1	1.1	1.3	1.1	1.1	1.3	R	R	R	1	1.3	1.5
13.	**2m**	1.1	1.1	1.3	R	1.1	1.4	1.2	1.4	1.7	R	R	1.2	1.1	1.5	2
14.	**2n**	1.1	1.1	R	1.1	1.1	R	1.2	1.1	1.2	2	1.6	1.2	1.5	R	1.9
15.	**2o**	1.2	1.3	1.6	1.2	1.3	1.1	1.2	1.2	1.6	1.1	1.1	1.2	1.1	R	1.7
16.	**2p**	1.1	1.2	1.2	1.2	1.4	2	1.3	1.1	1.4	1.3	R	1.3	1.4	1.1	1.6
17.	**2q**	1.3	1.1	1.8	1.6	1.1	1.2	1.2	1.3	1.1	1.6	1.1	1.6	1.6	1.6	1.2
18.	**2r**	1.2	1.5	1.1	1.4	2	1.1	1.1	1.3	1.5	R	1.5	1.5	1.6	1.6	1.5
19.	**2s**	1.4	R	2	1.1	1.1	1.5	1.2	1.8	1.3	1.1	R	R	1.1	1.5	1.7
20.	**2t**	1.5	2	1.3	1.1	1.1	1.9	1.1	1.7	1.1	1.1	1.2	1.7	1.8	1.3	1.9
21.	**A**	1.8			1.8			1.9			1.9			-		
22.	**CPD**	2.2			2.1			2.1			2.2			-		
23.	**GF**	1.8			1.9			2.0			2.0			-		
24.	**GRF**	-			-			-			-			2.6		
25.	**FLC**	-			-			-			-			2.8		

Note: Zone of inhibition interpretation is as follows.

1. ZONE SIZE <1.0 C.M.- RESISTENT(R)
2. ZONE SIZE 1.0 To 1.5 – INTERMEDIATE
3. ZONE SIZE >1.5 - SENSITIVE

STD Antibiotic Sensitivity Assay Concentration 40 µG/ml

A: AMPICILLIN
CPD: CEFPODOXIME
GF: GATIFLOXACIN
GRF: GRESIOFULVIN
FLC: FLUCONAZOLE

2.7 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. All the synthesized compounds were evaluated for their anti microbial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-2a-t** showed moderate to potent activity. The compounds **PVP- 2h** and **2g** showed comparatively good activity against all the bacterial strains.

2.8 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-t.**

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-t** (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 room temperature to afford analytically pure products. The solid products were used for next step without further purification.

❖ **General procedure for the synthesis of pyrazolopyridones PVP-2a-t.**

The mixture of substituted pyridones **3a-t** (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 room temperature and filter the separated product washed with iPA and dried at room temperature to furnished analytically pure products.

❖ **Spectral data of the synthesized compounds**

2-(bis(methylthio)methylene)-*N*-(4-methoxyphenyl)-4-methyl-3-oxopentanamide 3a: yellow solid; R_f 0.42 (9:1Chloroform: Methanol);IR (KBr): 3439, 3007, 2928, 2808, 1599, 1462, 1327, 1255cm^{-1}; ^{1}H NMR: δ 1.18-1.20 (d, 6H, 2 × ^{i}prCH$_3$), 2.44 (s, 6H, 2 × SCH$_3$), 3.17-3.24 (m, 1H, ^{i}prCH), 3.75 (s, 3H, OCH$_3$), 6.99–7.54 (m, 4H, Ar-H), 8.38 (br, s, 1H, -CONH); MS (*m/z*): 339 (M$^+$); Anal. Calcd for $C_{16}H_{21}NO_3S_2$: C, 56.61; H, 6.24; N, 4.13; Found: C, 56.53; H, 6.14; N, 4.06.

3-amino-4,5-dihydro-6-isopropyl-*N*-(4-methoxyphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2a): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol); IR (KBr): 3379, 3024, 2902, 1651, 1516,1483, 1332, 1166, 954 cm^{-1}; ^{1}H NMR: δ 1.29-1.31 (d, 6H, 2 × ^{i}prCH$_3$), 3.79 (s, 3H, OCH$_3$), 4.21 (m, 1H, ^{i}prCH), 5.64 (s, 2H, NH$_2$), 6.84-6.86 (d, 2H, Ar-H, *J* = 8.00 *Hz*), 7.59-7.61 (d, 2H, Ar-H, *J* = 8.00 *Hz*), 9.50 (s, 1H, NH), 10.62 (s, 1H, CONH), 11.93 (s, 1H, NH) ; MS (*m/z*): 341 (M$^+$); Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52; Found: C, 59.61; H, 5.54; N, 20.29.

3-amino-4,5-dihydro-6-isopropyl-4-oxo-*N*-phenyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2b): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol); IR (KBr): 3347, 3104, 2842, 1681, 1496, 1331, 1152, 857 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, 61.65; H, 5.40; N, 22.42.

3-amino-*N*-cyclohexyl-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7- carboxamide (PVP-2c): Creamish solid; R_f 0.42 (9:1Chloroform: Methanol); IR (KBr): 3309, 3014, 2908, 1671, 1563, 1473,1287, 1246, 927 cm^{-1}; ^{1}H NMR: δ 1.29-1.31 (d, 6H, 2 × ^{i}prCH$_3$), 2.31-2.56 (m, 10H, 5 x CH$_2$), 3.44 (m, 1H, CH), 4.65 (m, 1H, ^{i}prCH), 5.92 (s, 2H, NH$_2$), 9.90 (s, 1H, NH), 10.62 (s, 1H, CONH), 12.13 (s, 1H, NH) MS (*m/z*): 317 (M$^+$); Anal. Calcd for $C_{16}H_{23}N_5O_2$: C, 60.55; H, 7.30; N, 22.07; Found: C, 60.50; H, 7.23; N, 22.02.

3-amino-4,5-dihydro-6-isopropyl-*N*-(2,5-dimethylphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2d): Creamish solid; R_f 0.41 (9:1Chloroform: Methanol); IR (KBr): 3406, 3115, 2902, 1660, 1514, 1483,1280, 1178, 952 cm^{-1}; MS

(*m/z*): 339(M^+); Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64; Found: C, 63.61; H, 6.20; N, 20.58.

3-amino-*N*-(4-bromophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2e): Creamish solid; R_f 0.44 (9:1Chloroform: Methanol); IR (KBr): 3330, 3142, 2830, 1688, 1489, 1241, 1118, 827 cm^{-1}; MS (*m/z*): 389 (M^+); Anal. Calcd for $C_{16}H_{16}BrN_5O_2$: C,49.25; H, 4.13; N, 17.95; Found: C, 49.14; H, 4.04; N, 17.90.

3-amino-*N*-(4-fluorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2f): Creamish solid; R_f 0.39 (9:1Chloroform: Methanol);IR (KBr): 3324, 3143, 2812, 1675, 1532,1482, 1298, 1114, 897 cm^{-1}; ^{13}C NMR: δ 20.32, 38.89-40.14, 114.58, 114.80, 121.48, 121.55, 134.94, 160.50, 163.25, 179.74, 187.97. MS (*m/z*): 329 (M^+); Anal. Calcd for $C_{16}H_{16}FN_5O_2$: C, 58.35; H, 4.90; N, 21.27; Found: C, 58.24; H, 4.84; N, 21.18.

3-amino-*N*-(4-chlorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2g): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol); IR (KBr): 3369, 3020, 2897, 1685, 1573, 1494, 1157, 854 cm^{-1}; MS (*m/z*): 345 (M^+); Anal. Calcd for $C_{16}H_{16}ClN_5O_2$: C, 55.58; H, 4.66; N, 20.25.; Found: C, 55.49; H, 4.61; N, 20.23.

3-amino-*N*-(3-chloro-4-fluorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2h): Creamish solid; R_f 0.42 (9:1Chloroform: Methanol); IR (KBr): 3348, 323, 3126, 2993, 1670, 1471, 1267, 1206, 1085, 956 cm^{-1}; MS (*m/z*): 363 (M^+); Anal. Calcd for $C_{16}H_{15}ClFN_5O_2$: C, 52.83; H, 4.16; N, 19.25; Found: C, 52.71; H, 4.08; N, 19.10.

3-amino-*N*-(3,4-difluorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2i): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol);IR (KBr): 3348, 3250, 3003, 2984, 1620, 1428, 1286, 1180, 897 cm^{-1}; MS (*m/z*): 347 (M^+); Anal. Calcd for $C_{15}H_{14}F_2N_5O_2$: C, 55.33; H, 4.35; N, 20.16; Found: C, 55.26; H, 4.24; N, 20.09.

3-amino-*N*-(3-chlorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2j): Creamish solid; R_f 0.44 (9:1Chloroform: Methanol);IR (KBr): 3347, 3041, 2820, 1681, 1546, 1473,1322, 1183, 974 cm^{-1}; MS (*m/z*): 345 (M^+); Anal. Calcd for $C_{16}H_{16}ClN_5O_2$: C, 55.58; H, 4.66; N, 20.25.; Found: C, 55.45; H, 4.61; N, 20.18.

3-amino-*N*-(3,4-dichlorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2k): Creamish solid; R_f 0.41 (9:1Chloroform: Methanol);IR (KBr): 3358, 3108, 2920, 1698, 1543, 1481,1248, 1198, 854 cm^{-1}; MS (*m/z*): 380 (M^+); Anal. Calcd for $C_{16}H_{15}Cl_2N_5O_2$: C, 50.54; H, 4.66; N,18.42; Found: C, 50.42; H, 4.55; N, 18.40.

3-amino-4,5-dihydro-6-isopropyl-4-oxo-*N*-*p*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2l): Creamish solid; R_f 0.42 (9:1Chloroform: Methanol);IR (KBr): 3364, 2927, 1674, 1533, 1462, 1281, 1112, 873 cm^{-1}; ^1H NMR: δ 1.29-1.37 (d, 6H, 2 × ^{i}prCH**3**), 2.30 (s, 3H, CH_3), 4.21 (m, 1H, ^{i}prCH), 5.61 (s, 2H, NH_2), 7.09-7.11 (d, 2H, Ar-H, *J* = 8.00 *Hz*), 7.56-7.58 (d, 2H, Ar-H, *J* = 8.00 *Hz*), 10.00 (s, 1H, NH), 10.66 (s, 1H, CONH), 12.02 (s, 1H, NH); MS (*m/z*): 325 (M^+); Anal. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.75; H, 5.89; N, 21.52; Found: C, 62.65; H, 5.85; N, 21.49.

3-amino-4,5-dihydro-6-isopropyl-4-oxo-*N*-*o*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2m): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol);IR (KBr): 3379, 3124, 2857, 1685, 1426, 1212, 1106, 854 cm^{-1}; MS (*m/z*): 325 (M^+); Anal. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.75; H, 5.89; N, 21.52; Found: C, 62.69; H, 5.71; N, 21.48.

3-amino-4,5-dihydro-6-isopropyl-*N*-(2-methoxyphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2n): Creamish solid; R_f 0.38 (9:1Chloroform: Methanol); IR (KBr): 3369, 3024, 2952, 1651, 1516,1463, 1332, 1166, 954 cm^{-1}; MS (*m/z*): 341 (M^+); Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52; Found: C, 59.71; H, 5.52; N, 20.43.

3-amino-*N*-(2-fluorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2o): Creamish solid; R_f 0.41 (9:1Chloroform:

Methanol); IR (KBr): 3320, 3143, 2822, 1672, 1532,1486, 1298, 1114, 897 cm^{-1}; MS (*m/z*): 329 (M^+); Anal. Calcd for $C_{16}H_{16}FN_5O_2$: C, 58.35; H, 4.90; N, 21.27; Found: C, 58.27; H, 4.88; N, 21.20.

3-amino-*N*-(2-bromophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2p): Creamish solid; R_f 0.42 (9:1Chloroform: Methanol); IR (KBr): 3340, 3132, 2830, 1680, 1459, 1241, 1118, 827 cm^{-1}; MS (*m/z*): 389 (M^+); Anal. Calcd for $C_{16}H_{16}BrN_5O_2$: C,49.25; H, 4.13; N, 17.95; Found: C, 49.18; H, 4.10; N, 17.86.

3-amino-4,5-dihydro-6-isopropyl-*N*-(4-nitrophenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2q): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol);IR (KBr): 3391, 2902, 2802, 1692, 1596, 1473, 1176, 974 cm^{-1}; MS (*m/z*): 356 (M^+); Anal. Calcd for $C_{16}H_{16}N_6O_4$: C, 53.93; H, 4.53; N, 23.58; Found: C, 53.87; H, 4.48; N, 23.51.

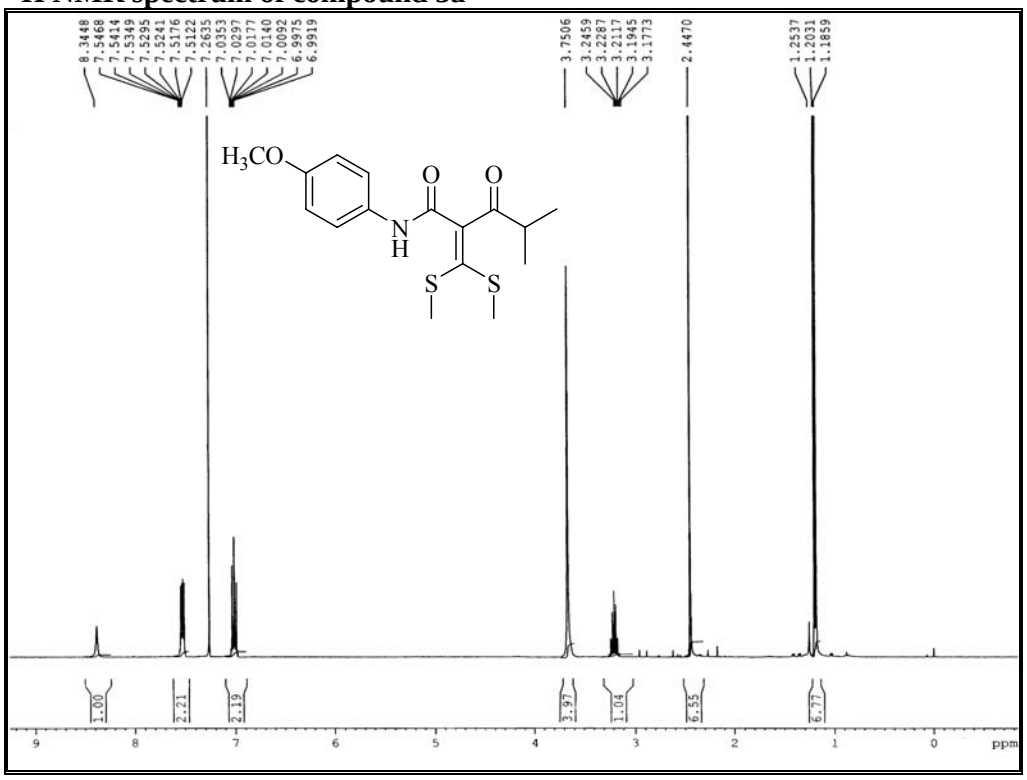
3-amino-4,5-dihydro-6-isopropyl-*N*-(2,5-dimethylphenyl)-4-oxo-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2r): Creamish solid; R_f 0.43 (9:1Chloroform: Methanol); IR (KBr): 3406, 3145, 2922, 1660, 1514, 1463,1280, 1178, 952 cm^{-1}; MS (*m/z*): 339(M^+); Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64; Found: C, 63.65; H, 6.18; N, 20.60.

3-amino-*N*-(2-chlorophenyl)-4,5-dihydro-6-isopropyl-4-oxo-1*H*-pyrazolo[4,3-*c*] pyridine-7-carboxamide (PVP-2s): Creamish solid; R_f 0.39 (9:1Chloroform: Methanol); IR (KBr): 3327, 3141, 2860, 1681, 1546, 1478,1322, 1183, 974 cm^{-1}; MS (*m/z*): 345 (M^+); Anal. Calcd for $C_{16}H_{16}ClN_5O_2$: C, 55.58; H, 4.66; N, 20.25.; Found: C, 55.51; H, 4.51; N, 20.13.

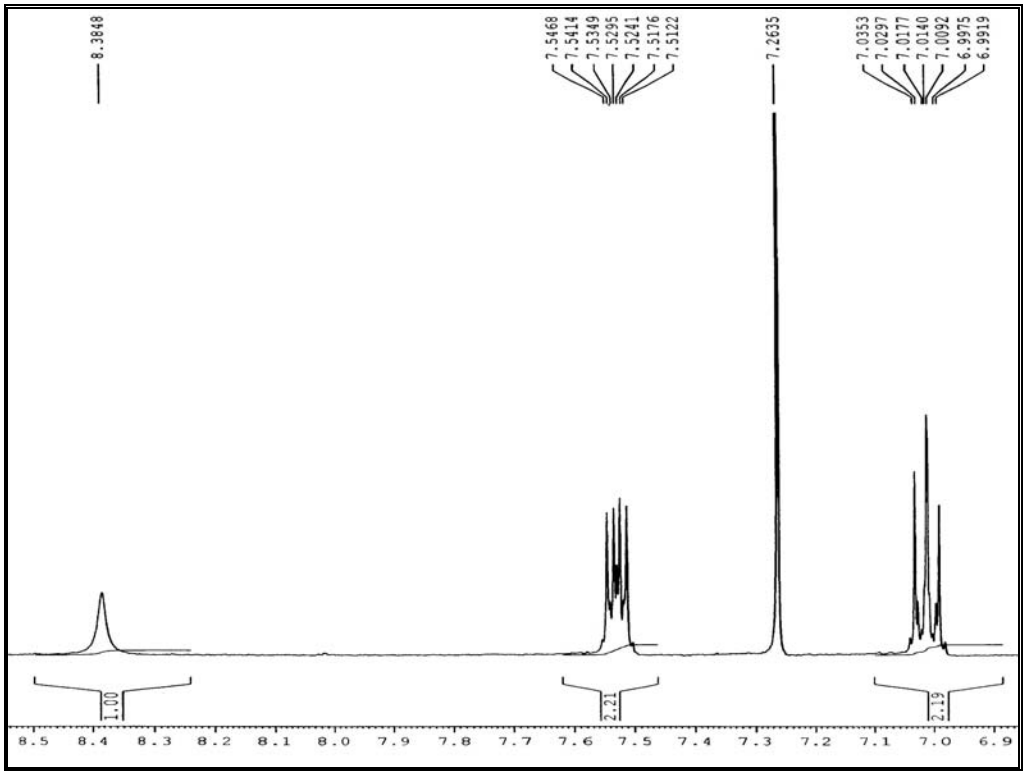
3-amino-4,5-dihydro-6-isopropyl-4-oxo-*N*-*m*-tolyl-1*H*-pyrazolo[4,3-*c*]pyridine-7-carboxamide (PVP-2m): Creamish solid; R_f 0.40 (9:1Chloroform: Methanol);IR (KBr): 3379, 3124, 2847, 1685, 1456, 1212, 1106, 854 cm^{-1}; MS (*m/z*): 325 (M^+); Anal. Calcd for $C_{17}H_{19}N_5O_2$: C, 62.75; H, 5.89; N, 21.52; Found: C, 62.69; H, 5.65; N, 21.42.

❖ **Spectral representation of synthesized compounds**

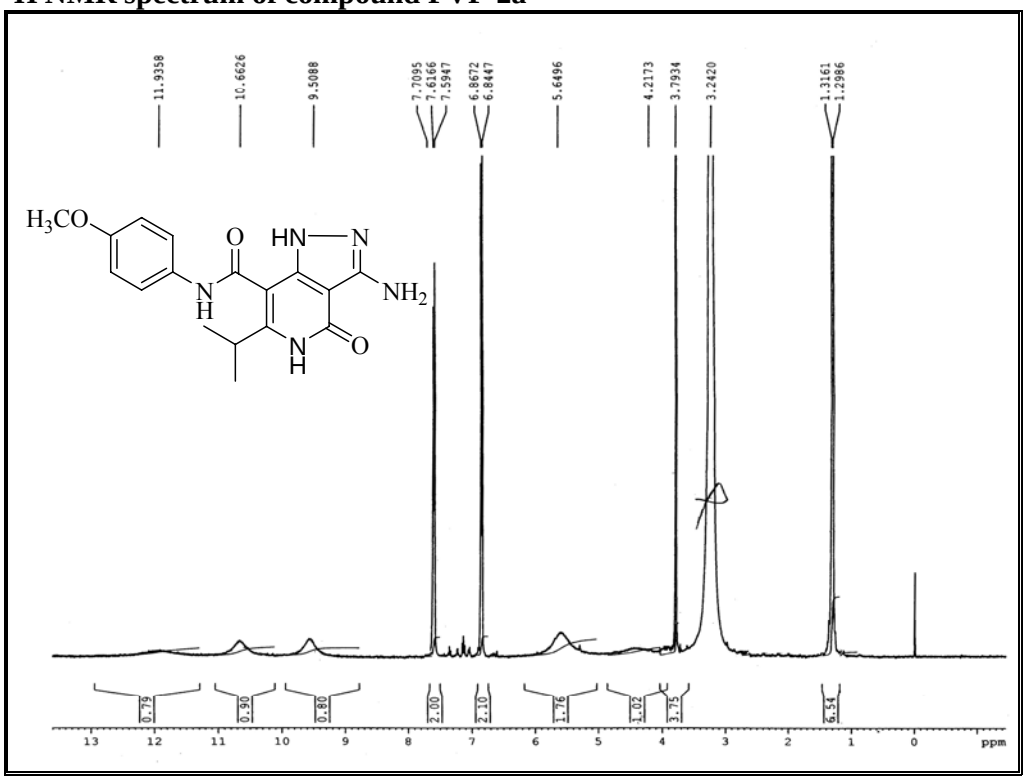
^{1}H NMR spectrum of compound 3a



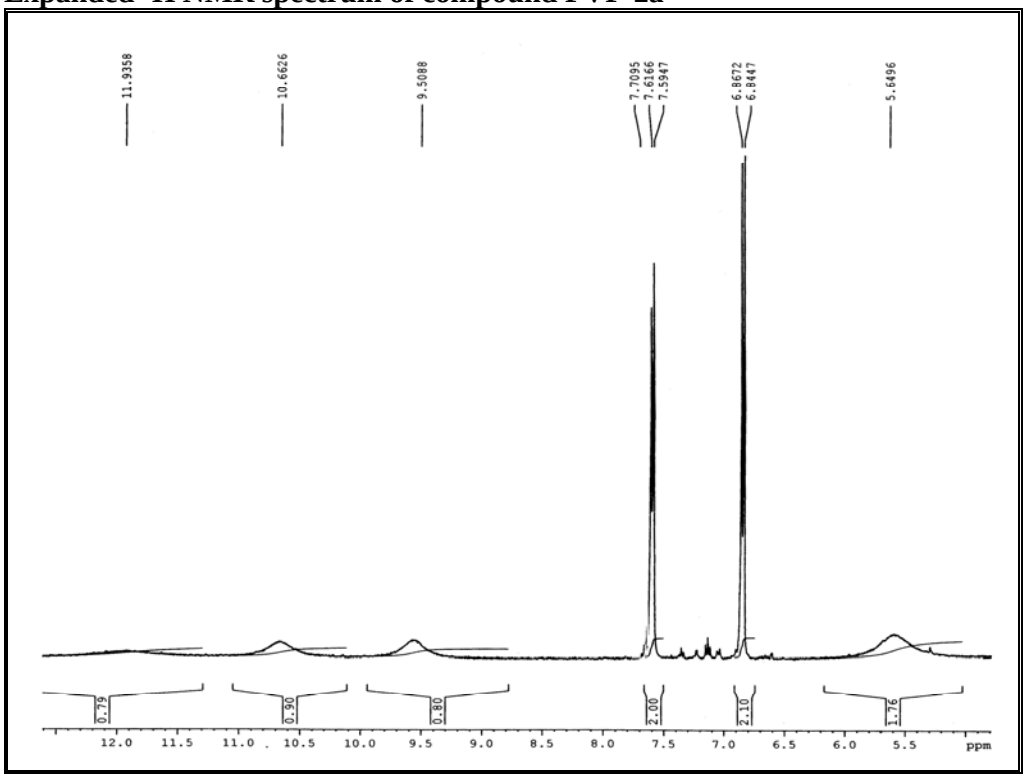
Expanded ^{1}H NMR spectrum of compound 3a



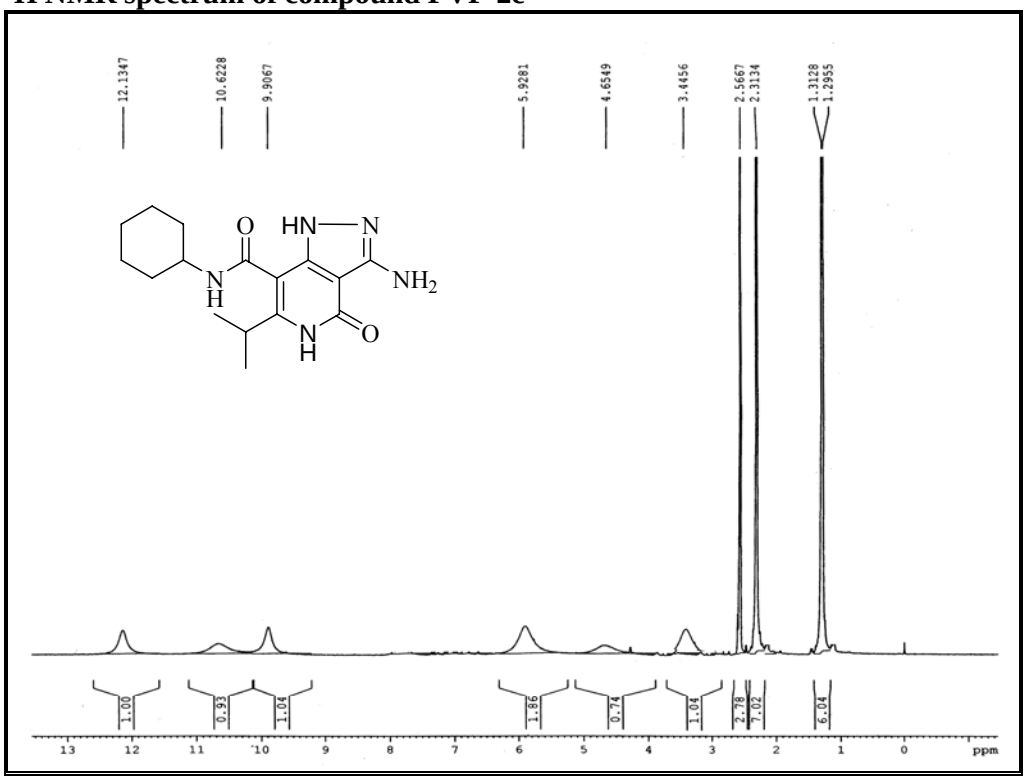
^{1}H NMR spectrum of compound PVP-2a



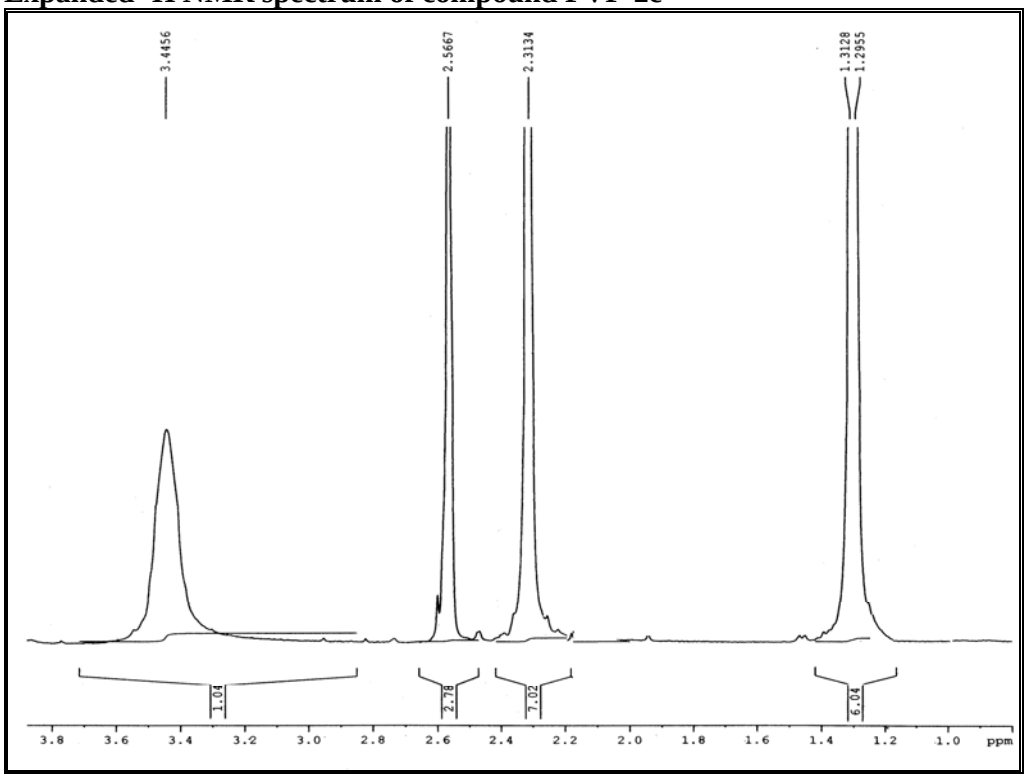
Expanded ^{1}H NMR spectrum of compound PVP-2a



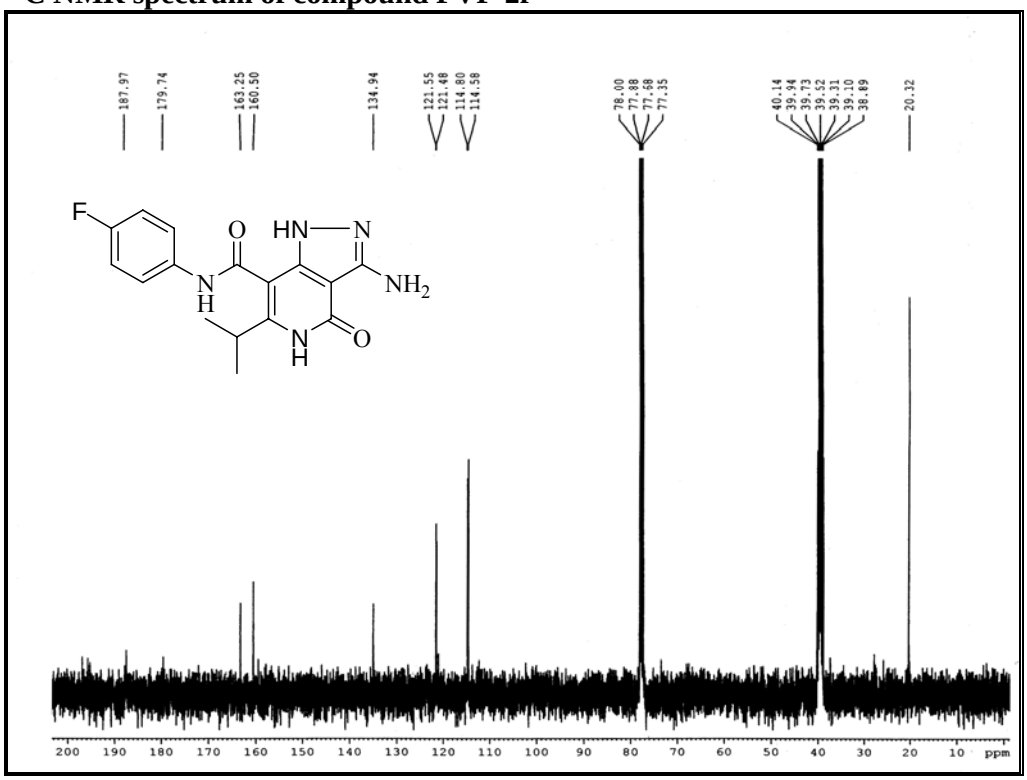
^{1}H NMR spectrum of compound PVP-2c



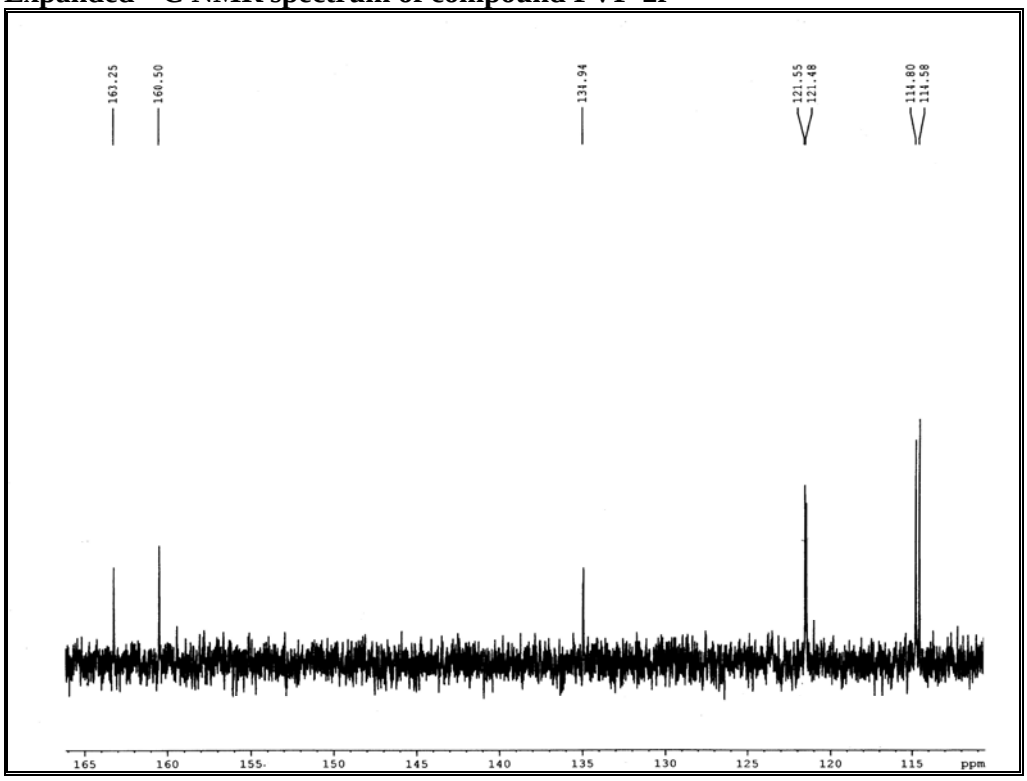
Expanded ^{1}H NMR spectrum of compound PVP-2c



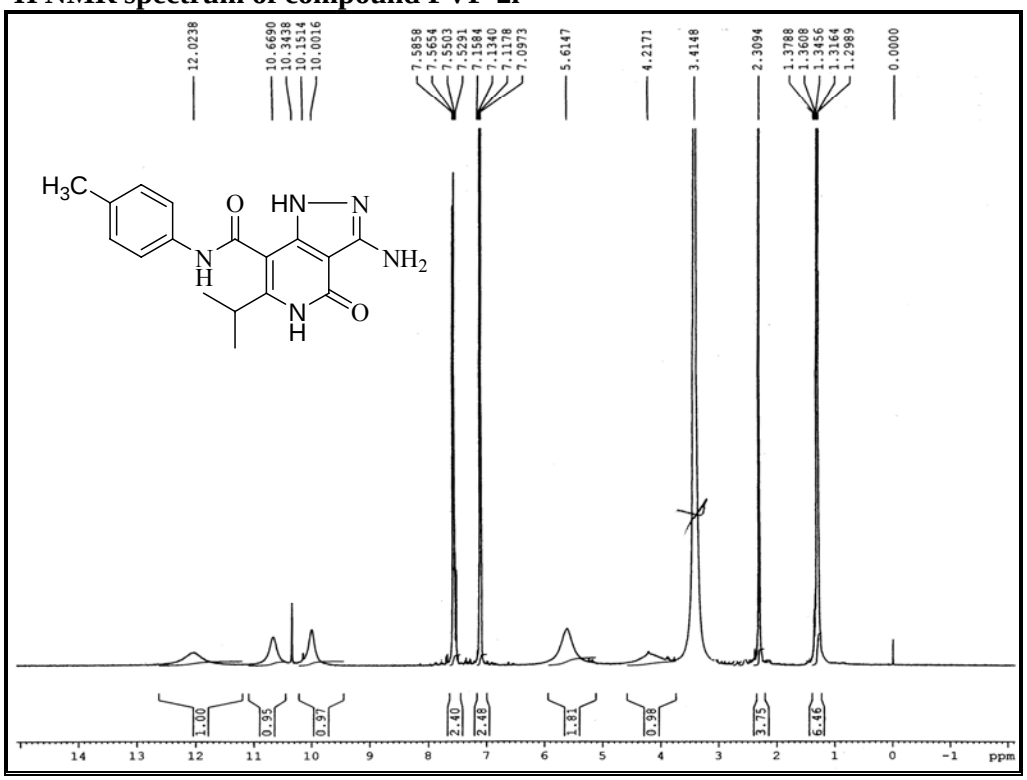
^{13}C NMR spectrum of compound PVP-2f



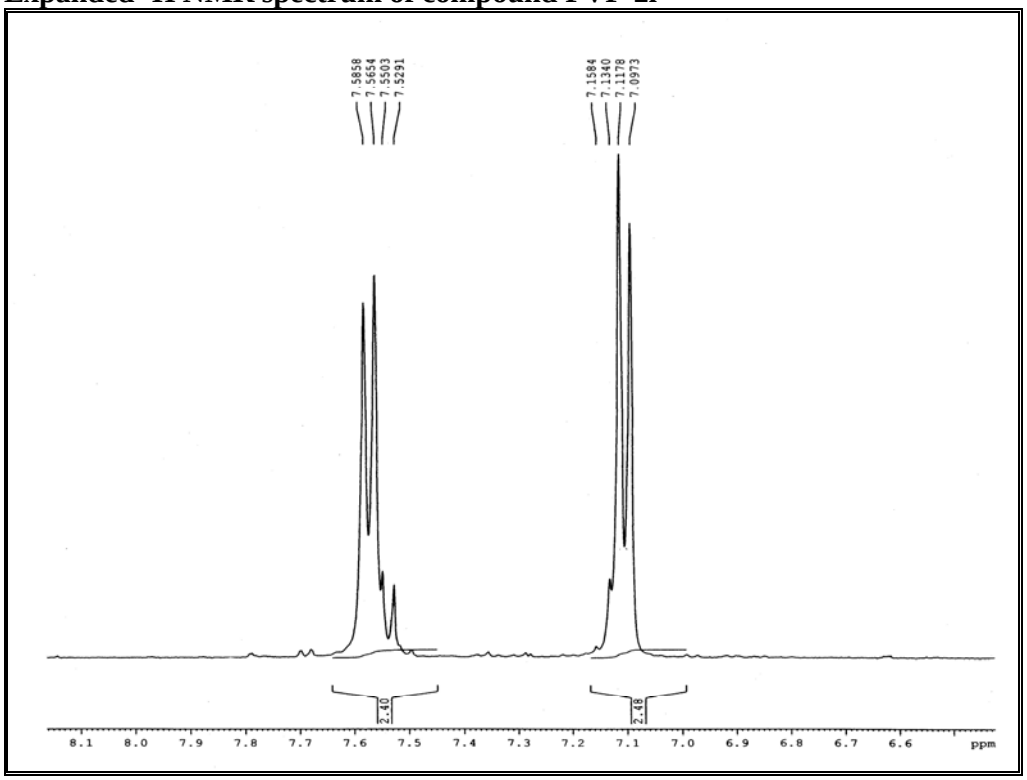
Expanded ^{13}C NMR spectrum of compound PVP-2f



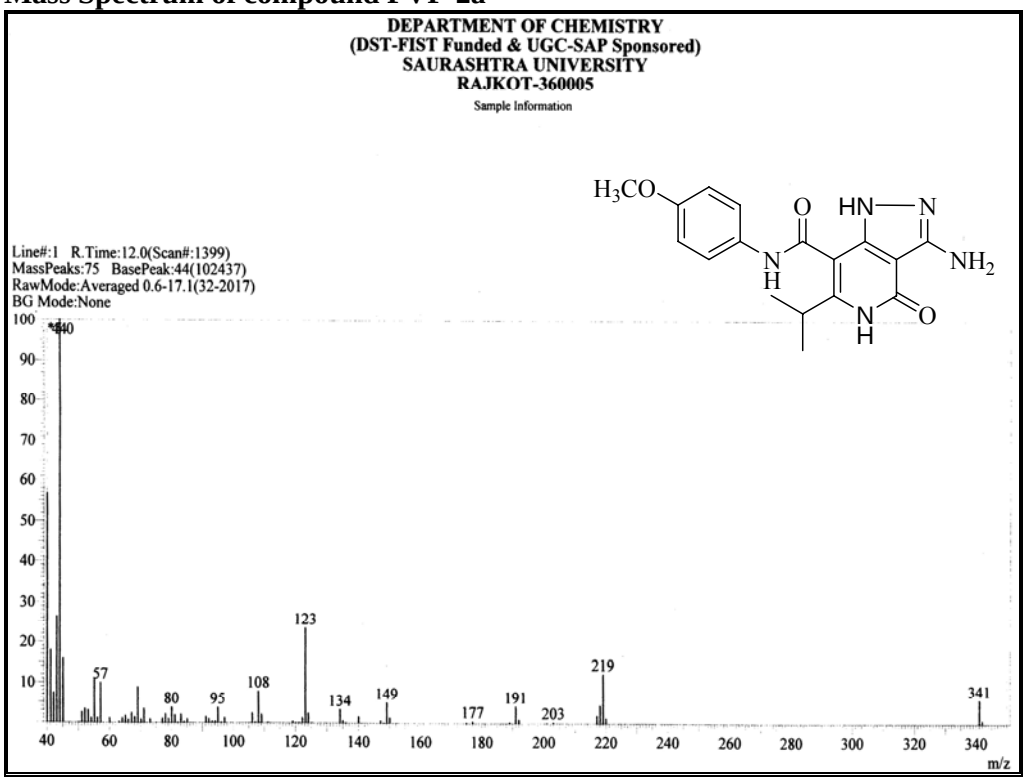
^{1}H NMR spectrum of compound PVP-2l



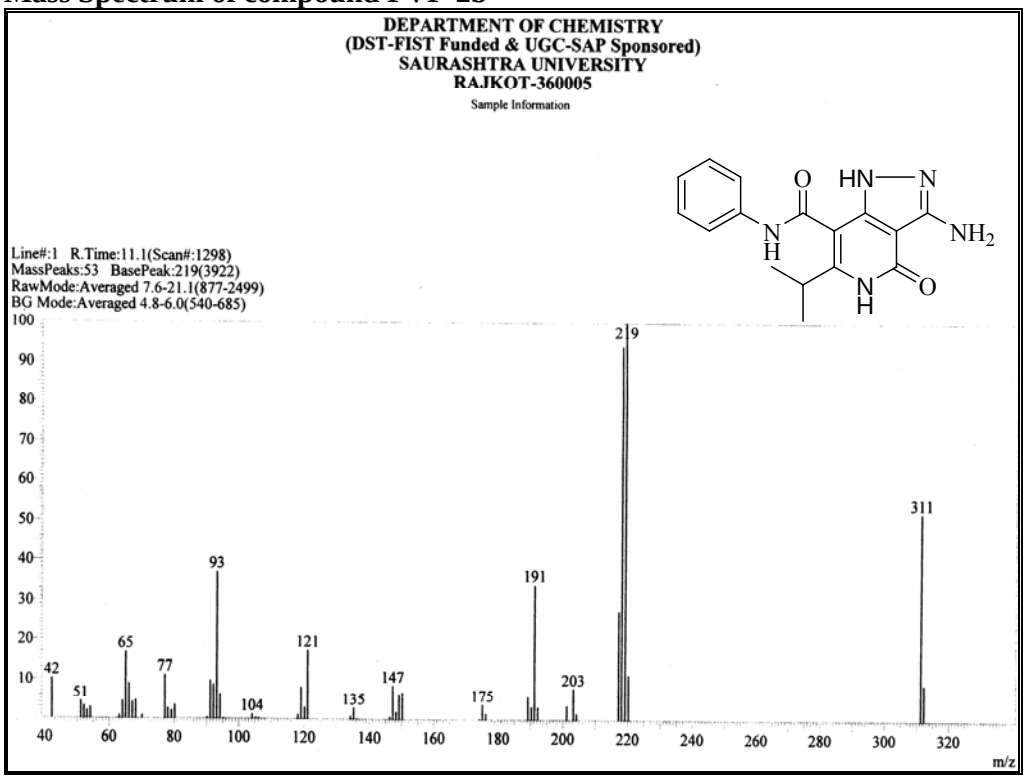
Expanded ^{1}H NMR spectrum of compound PVP-2l



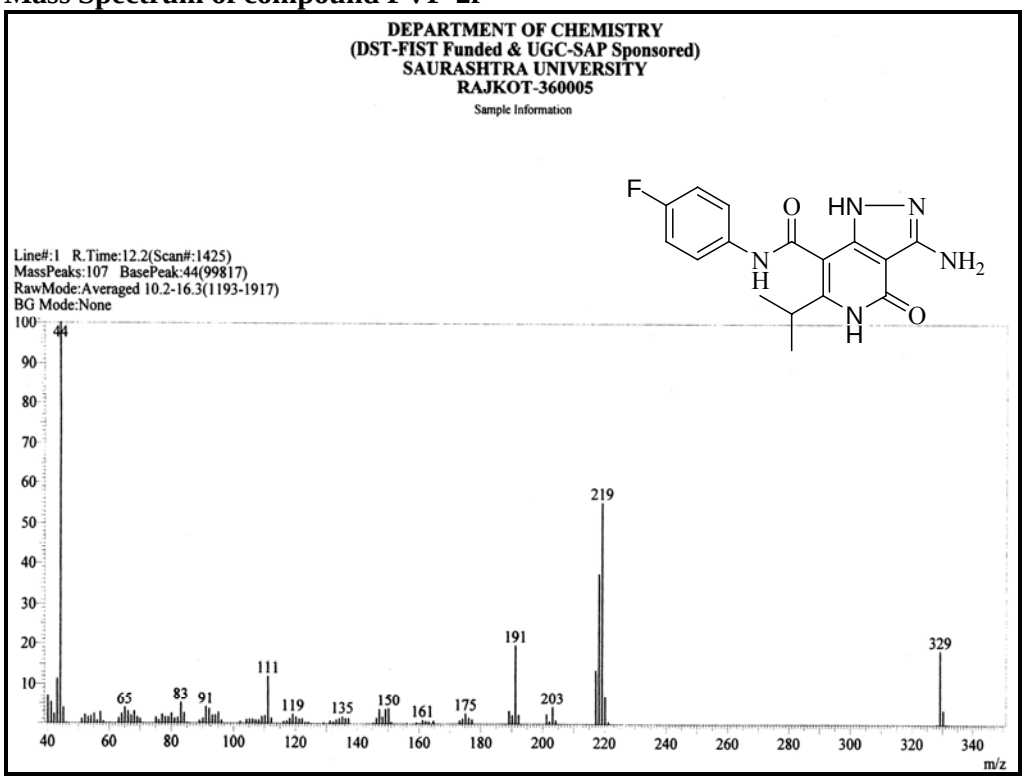
Mass Spectrum of compound PVP-2a



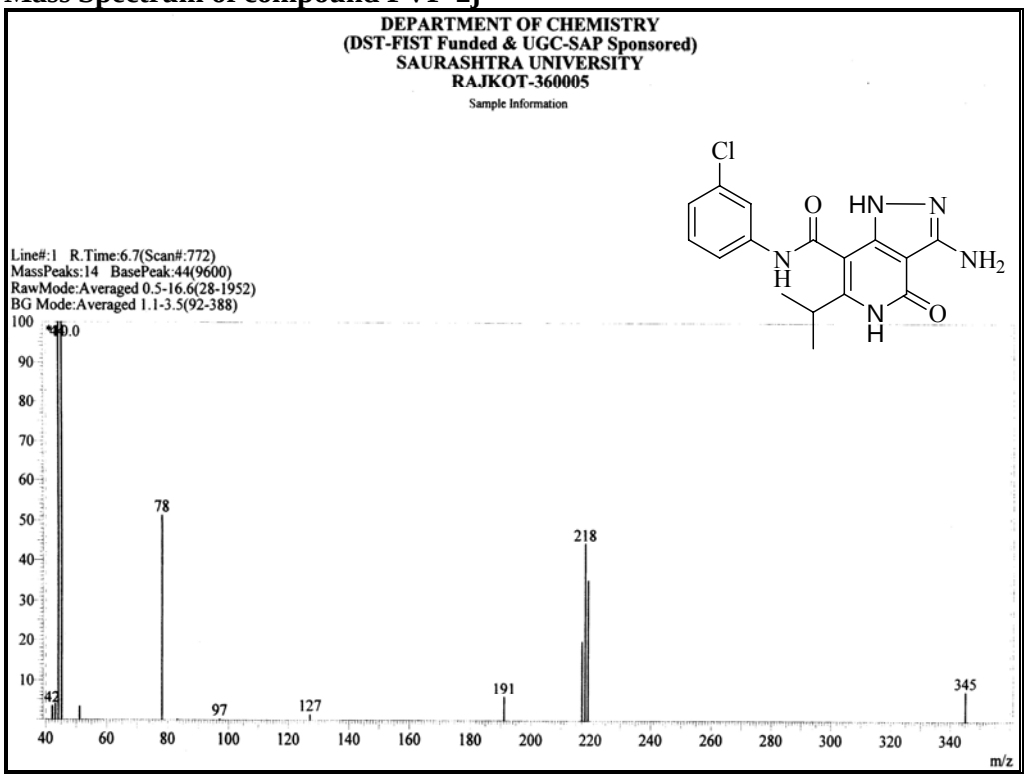
Mass Spectrum of compound PVP-2b



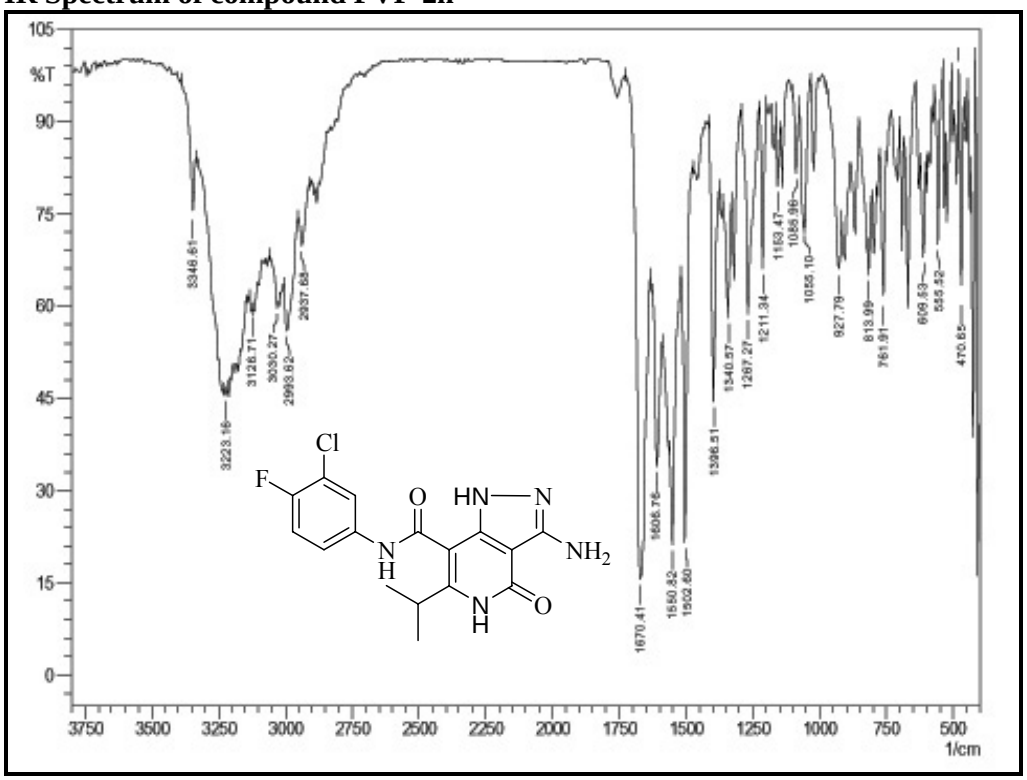
Mass Spectrum of compound PVP-2f



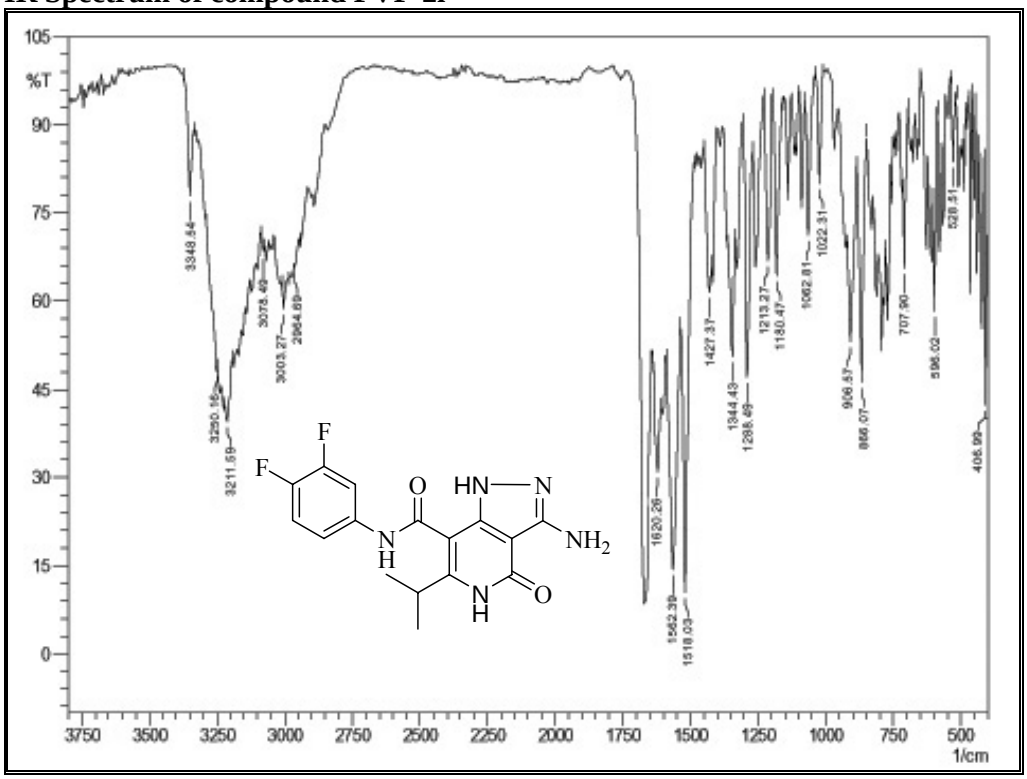
Mass Spectrum of compound PVP-2j



IR Spectrum of compound PVP-2h



IR Spectrum of compound PVP-2i



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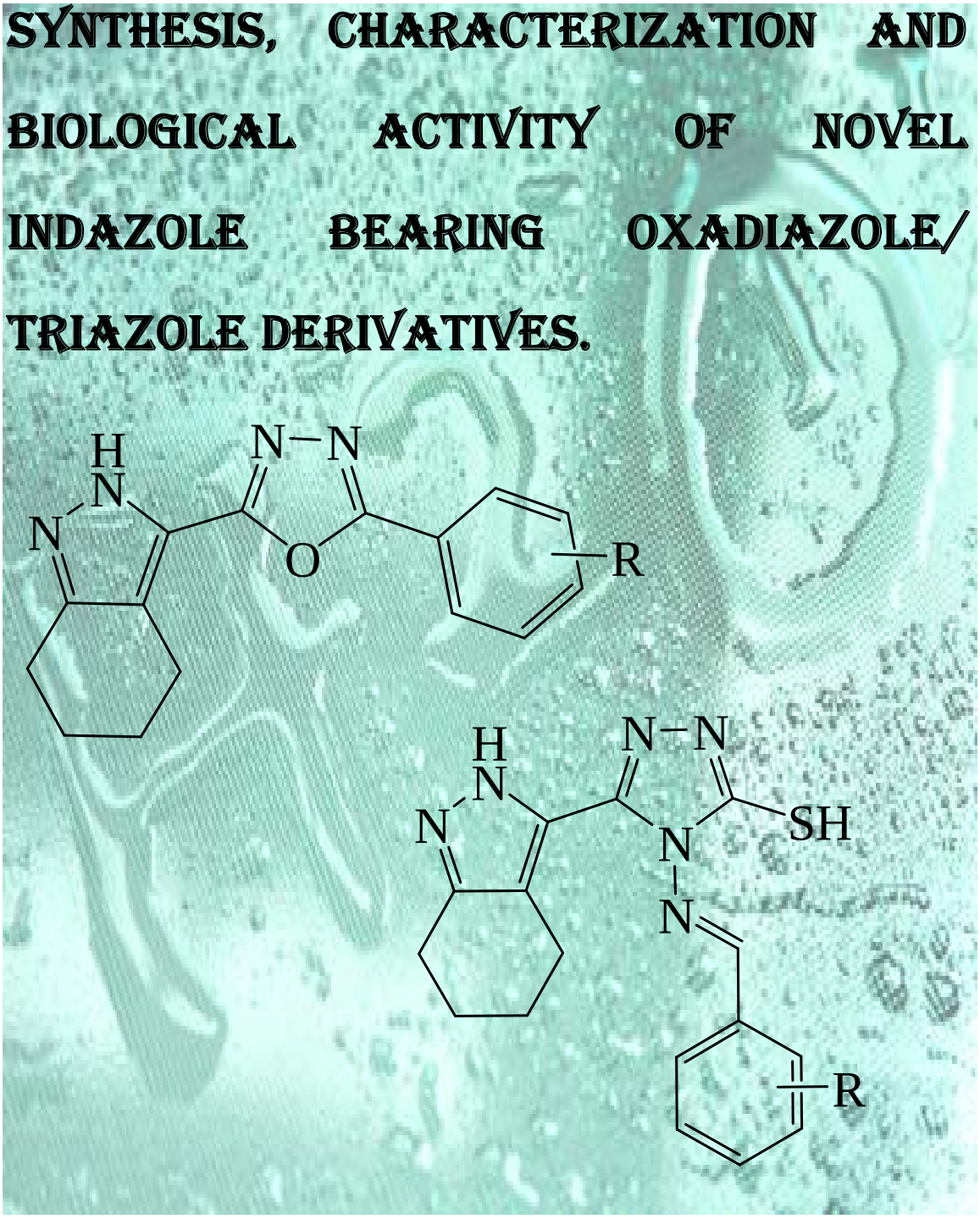
Chapter 3

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NOVEL INDAZOLE BEARING OXADIAZOLE/ TRIAZOLE DERIVATIVES.



3.1 INTRODUCTION

❖ Indazole

The systematic IUPAC name benzo[*c*]pyrazole is not used in the *Ring Index* or in *Chemical Abstract* and the heterocycle is normally referred to by its trivial name indazole or more correctly 1*H*-indazole (CAS registry number 271-244-3). Alternative names for indazole such as 1,2-benzodiazole, are not used. Benzo-fused derivatives are known as benzoindazoles. **(Figure 1)**The first indazoles were synthesized in 1880,[1] and a systematic investigation of the heterocycle was performed by V. Auwers in 1924.[2] Indeed, general synthetic pathways to indazoles were developed in the early years of the 20th century and many recent publications describe improvements of known methods. Methods for the synthesis of indazoles are described in *Houben-Weyl*,[3] and well-tested procedures for the synthesis of 1*H*-indazole,[4-7] 2-phenyl-2*H*-indazole[8] and 5-nitro-1*H*-indazole,[9] can be found in *Organic Synthesis*.

Natural products bearing an indazole structure are rare[10] and at present only two examples are known: nigellicine[11] and nigellidine.[12] However, many synthetic indazoles are known, and a number are important because of their pharmaceutical activity; some act as dopamine antagonists, anti-inflammatory, analgesic, or antipyretic agents.[13-20] Others also exhibit CNS activity,[21-23] and 6- and 7-nitroindazoles are used to study the behavior of nitric oxide in vivo.[24-26] 1-Benzyl-1*H*-indazole-3-carboxilic acids have antispermatogenetic and anticancer activity,[27-29] the latter effect being shared by other indazole derivatives.[30-32] 1-Benzoyl-1*H*-indazoles behave as antiarthritic drugs,[33] and 4-nitro- and 4-amino-2-ribofuranosyl-2*H*-indazole 3',5'-cyclic monophosphates act as potent mimics of adenosine-3',5'-cyclic monophosphates.[34] Cortivazol[35] is an indazole-based drug possessing glucocorticoid properties. Many indazoles act as enzyme inhibitors,[36-38] and some also show specific virucide,[39] bronchodilatory,[40-42] vasodilatory,[43] or neuroprotectant[44] activities; others are used in the treatment of diabetes.[45] 3-Trifluoromethyl-1*H*-indazoles possess trichomonacide properties,[46] and fused indazoles with an azasteroid ring system show antimicrobial activity.[47] Some 1*H*-indazole-4,7-quinones possess anthelmintic[48] and diuretic activity.[49] A series of indazole derivatives exhibit herbicide activity, behave

as growth inhibitors,[50-52] or are used as bactericides and fungicides in polymer based paints.[53] Guanidino-1*H*-indazoles are used as sweeteners.[54]

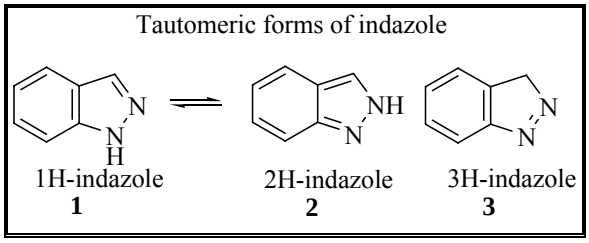


Figure 1

Although many derivatives of indazole show biological activity, no special toxicity has been reported and no special handling precautions have been recommended. The biodegradability of indazole is included in an ecological survey of heterocyclic compounds.[55]

❖ **Oxadaizole**

1,3,4-oxadiazole(**1**) is a thermally stable aromatic heterocycle and exist in two partially reduced forms; 2,3-dihydro-1,3,4-oxadiazole(1,3,4-oxadiazoline)(**2**) and 2,5-dihydro-1,3,4-oxadiazole(1,3,4-oxadiazoline)(**3**) depending on the position of the double bond. The completely reduced form of the 1,3,4-oxadiazole is known as 2,3,4,5-tetrahydro-1,3,4-oxadiazole (1,3,4-oxadiazolidine)(**4**).[56]**(Figure 2)**

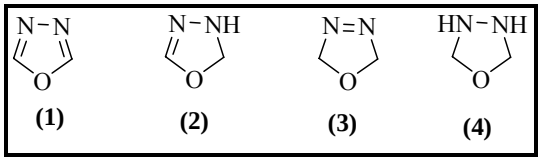


Figure 2

Bactericidal and/or fungicidal activity was reported for oxadiazole(**5a**), aminooxadiazole(**5b**)[57] and oxadiazolinethiones(**6a**)[58] (**Figure 3)** The tin derivatives (**6b**) is an effective fungicide and antimicrobial activity is shown by thiones(**6c**).[59] Antiinflammatory, sedative and analgesic properties were reported for aryloxadiazoles(**5c**).[60] Amino-oxadiazoles(**5d**) show analgesic activity and amono-oxadiazoles(**5e**) exhibit both antiinflammatory and antiproteolytic properties.[61]Anticonvulsant and nervous system depressant activity was reported for

amino-oxadiazoles(**5f**), where R is quinazolin-3-yl group.[62] Aminooxadiazole(**5g**) showlocalanaesthetic activity.[63] The oxadiazolinone(**6d**) is an orally active antiallergic agent, for example in the treatment of asthma and allergy disease and is claimed to be more potent than sodium cromoglycate.[64] Examples of the many oxadiazolones for the many herbicidal activity (week killers) are (**6e,6f**) and "oxadiazon"(**6g**), which is the subject of many regular reports in the literature. Insecticidal activity is shown by oxadiazolones(**6h,6i** the later is an aphicide), and oxadiazole(**5h**)

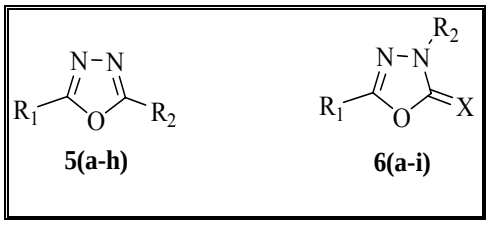


Figure 3

	R_1		R_2		R_1	R_2	X
5a	Ar		$CH_2CONHCONHR$	**6a**	heteroarylOCH_2	H	S
5b	AR		OCH_2 NHCOR	**6b**	1-methylcyclopropyl	$Sn(Ph)_3$	O
5c	trimethoxy		3,4-dimethoxyphenyl	**6c**	5-Cl-2-phenylindol-3-ylNH	H	S
5d	2-pyridyl	**or**	NR_2HCl	**6d**	3-Cl-benzo[b]thiophen-2-yl	H	O
5e	4-biphenylylmethyl		NHAr	**6e**	4-cyclohexylphenoxy	H	O
5f	Ar		$NHCH_2CONHR$	**6f**	2,4-diCl-phenoxymethyl	Bn	O
5g	Ar		$NHCO(CH_2)nNRR'HCl(n=2or3)$	**6g**	t-Bu	2,4-diCl--5-isopropoxyphenyl	O
				6h	OCH_3	o-methoxyphenyl	O
				6i	CH_3NH	2,3-diH-2,2,4-triMebenzofuran-7-yl	O

❖ Triazole

Triazoles are well known five member heterocyclic compounds and several procedures for their synthesis have been extensively studied. Such studied have been stimulated by various promising application, especially in the case of nitrogen containing heterocyclic entities. In fact certain nitrogen containing heterocyclic containing are used as pharmaceuticals e.g. analgesic, anti-inflammatory, antipyretic, agrochemicals where as some other is being studied for their medicinal interest.

The knowledge of such applications has pointed out that nitrogen containing heterocyclic's are important target to be prepared to our research. Triazoles have an important place in drug industries triazole are two types 1,2,3-triazoles (**I**) and 1,2,4-triazoles (**II**) (**Figure 4)**.

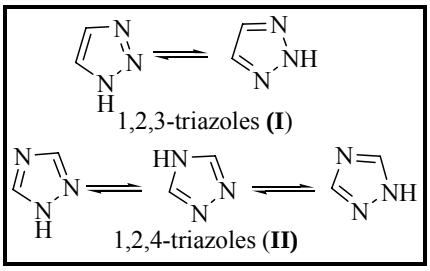


Figure 4

Hao Z.[65] and Staben Steven[66] have studied briefly with the chemistry of 1,2,4-triazoles. Bladin[67,68] is a pioneer scientist in the field of triazole, who had synthesized the first derivative of 1,2,4-triazole in 1885. 1,2,4-triazole derivatives not only known for their medicinal applications, but they are also used as analytical reagents,[69] dyes and photographic chemicals[70] corrosion inhibitors[71,72] and in the preparation of polymers.[73]

3.2 Pharmacological Profile

Wolf A. D. et al[74] reported the compounds of formula (**Figure 5)** as useful for the selective preemergence control of undesired vegetation e.g., barnyard grass, in crops such as rice, in particular paddy rice, wheat, and peanuts. These compounds also have utility for the post emergence control of weeds in certain crops, for example, rice. Furthermore, compounds of this invention can be used as directed treatments for the pre- or post-emergence control of weeds in various crops including soybeans, peanuts, cotton, garden beans and row planted rice.

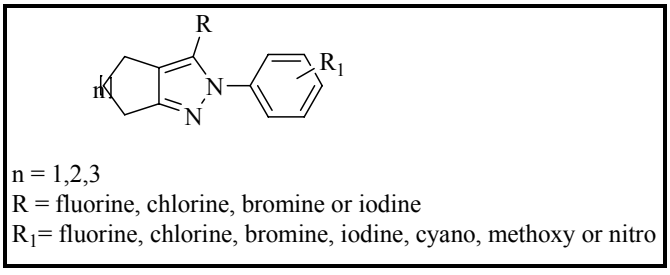


Figure 5

Metz, S. et al[75] described fused pyrazolo compounds for the treatment of inflammation, while Bauer, V. J. et al[76] described new fused bicyclic aminopyrazole and their physiologically acceptable salts possessing anti-inflammatory and analgesic properties **(Figure 6, 7)**.

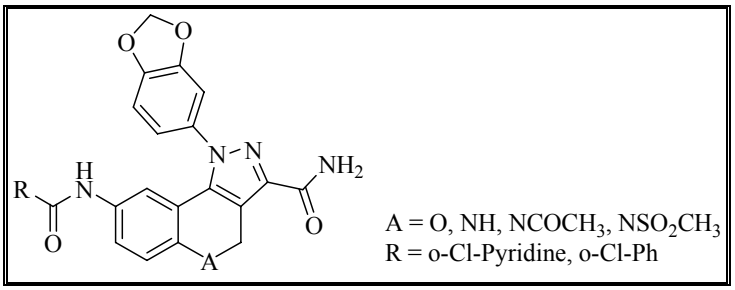


Figure 6

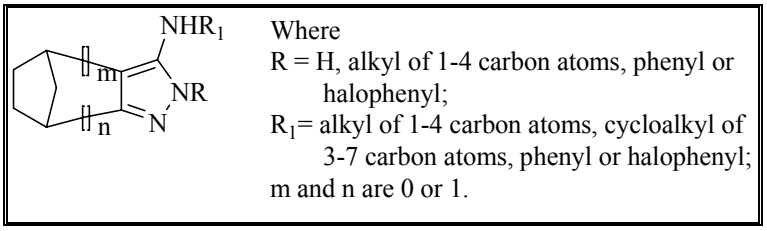


Figure 7

Corbera A. and Esteve, S.A. et al[77] had reported some tetrahydroindazole and fused pyrazole derivatives having pharmacological activity towards the sigma receptor, and their use in particular for the treatment of psychosis or pain(**Figure 8)**.

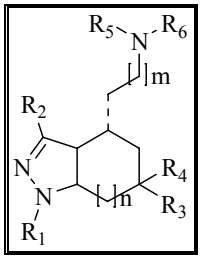


Figure 8

Peter J. Connolly et al[78] demonstrated the synthesis of some tetrahydroindazole, tetrahydrocyclopentapyrazole,(**Figure 9)** and hexahydrocycloheptapyrazole compounds and their use as HMG-COA reductase inhibitors.

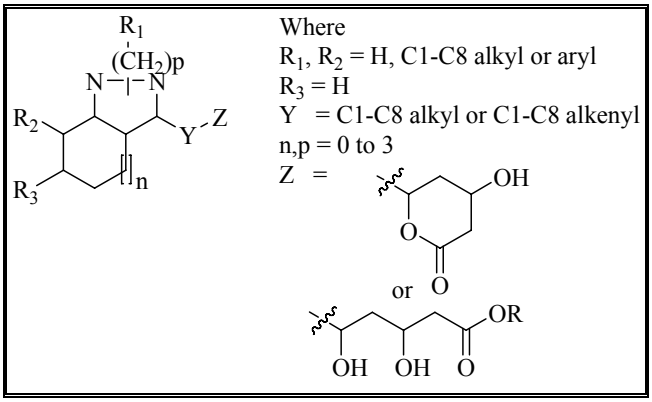


Figure 9

1,3,4-Oxadiazole is a versatile scaffold and is being consistently used as a building block in organic chemistry as well as in heterocyclic chemistry for the synthesis of different heterocycles. The synthetic versatility of 1,3,4-oxadiazole has led to the extensive use of this compound in organic synthesis.

2,5-Disubstituted-1,3,4-oxadiazole derivatives have tested for various pharmacological activities, which have been summarized as under Antibacterial,[79] Antiinflammatory,[80] Analgesic,[81] Antiviral,anticancer,[82] Antihypertensive,[83] Anticonvulsant,[84] Antiproliferative,[85] Antifungal,[86] Cardiovascular,[87] Herbicidal,[88] Hypoglycem,[89] Hypnotic and Sedative,[90] MAO inhibitor,[91] Insecticidal.[92]

Bishnoi S. R. et al[93] have screened oxadiazoles for their antimicrobial activity. A. El-Azzouny et al.[94] have synthesized 1,3,4-oxadiazole derivatives and evaluated for their analgesic, anti-inflammatory, ulcerogenic effects and inhibitory activity on plasma prostaglandin E2 (PGE2) Level.

Bhandari S. V. et al[95] have reported 1,3,4-oxadiazoles (**Figure 10)**. for their anti-inflammatory activity. Song Cao et al[96] have investigated some oxadiazoles possessing insecticidal activity. Suresh Kumar G. V. et al[97] have discovered oxadiazole derivatives and reported their antimycobacterial activity. Ali Almasired et al[98] have prepared 1,3,4- oxadiazoles of type as anticonvulsant agent. Meria Grazia Mamolo et al[99] have synthesized 3-substituted-5-(pyridine-4-yl)-3*H*-1,3,4-oxadiazole-2-one of type and studied their antimycobacterial activity.

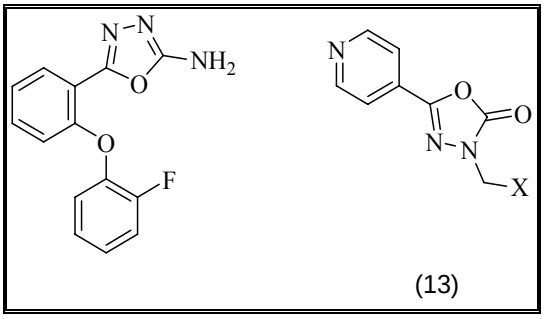


Figure 10

Krishna K. J. et al[100] have reported antimicrobial activity of oxadiazole derivatives. J. A. Christopher. et al[101] have documented anti HIV activity of 1,3,4-oxadiazole derivatives. Gilani S. J. et al[102] have synthesized some oxadiazoles as anti-inflammatory and analgesic agents. (**Figure 11)**. K. Subrahmanya Bhat et al[103] have prepared new fluorine containing 1,3,4-oxadiazoles and reported them as potential antibacterial and anticancer agents. T. P. Mohan et al.[104] have synthesized 2,5-disubstituted-1,3,4-oxadiazole derivatives and screened for their insecticidal activity.

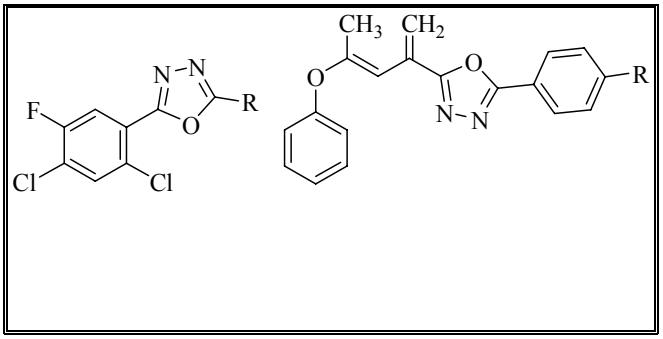


Figure 11

Ronald Kim et al[105] have discovered oxadiazole derivatives useful as protease inhibitors. Mohd Amir and Kumar Shikha[106] have documented anti-inflammatory, analgesic and ulcerogenic activity of some newly synthesized oxadiazoles. Ali A.et al.[107] have investigated some oxadiazole derivatives possessing antimicrobial and anti-HIV-1 activity. Sherif A. et al.[108] have reported oxadiazoles as potential antitumor and anti-HIV agents. Afshin Zarghi et al.[109] have synthesized R-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazoles possessing anticonvulsant activity. Mahamud Tareq et al[110] have synthesized 2,5-disubstituted-1,3,4-oxadiazoles useful as tyrosinase inhibitors. (**Figure 12)**

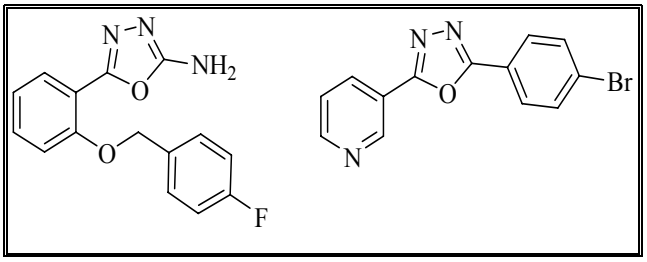


Figure 12

Triazoles are potential bioactive agents due to their wide spectrum of therapeutic importance. Drug molecule having 1,2,4-triazole nucleus (**Figure 13)**.with good activity are listed as under.

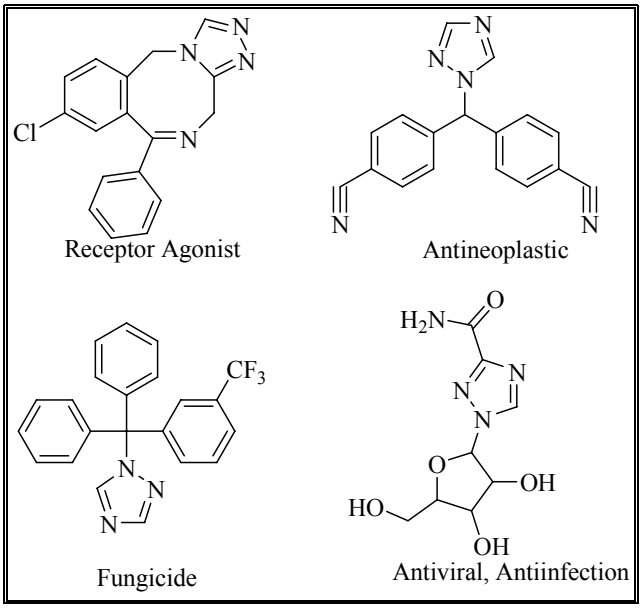


Figure 13

Literature survey reveals that various 1,2,4-triazole derivatives display significant biological activities. 3-Amino-1,2,4-triazole was the first 1,2,4-triazole to be manufactured on large scale from amino guanidine format, useful as herbicides[111] therapeutic activity of 1,2,4-triazoles are as under.

Bactericidal,[112] Diuretic,[113] Fungicidal,[114] Herbicidal,[115] Insecticidal and acaricidal,[116] Plantgrowthregulator,[117] Anticancer and Anti-HIV,[118] Antileshmanial,[119] Antitumor.[120]

Yaseen A. et al[121] have prepared 1,5-dialkyl-3-(5-marcepto-4-*N*-aryl-1*H*-1,2,4-triazolo-3-yl-methylene)-1*H*-1,2,4-triazole which exhibited remarkable activity against nine type of cancer and also anti viral activity. Bozena et al,[122] have synthesized triazole derivatives and tested for their anticonvulsant and antinoncicepative activity. Sylvie larrat et al,[123] investigated that ribavarin in combination with alpha-2-interferon is the consensus treatment for chronic hepatitis C. and E. De Clercq et al[124] screened ribavarin (**Figure 14)** for their antiviral and antimetabolic activities.

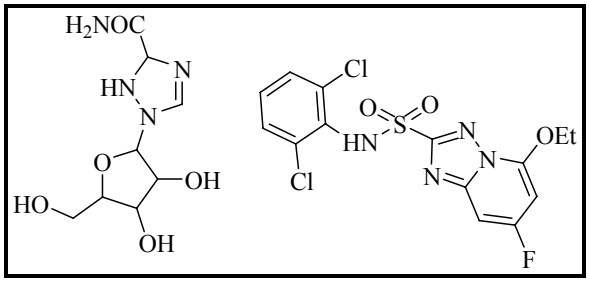


Figure 14

Daniele Binchi et al[125] have screened pure stereoisomer of two new triazole derivatives (**Figure 15)** for their antifungal activity against variety of fungi showing an activity ratio R-form and S-form up to 400.

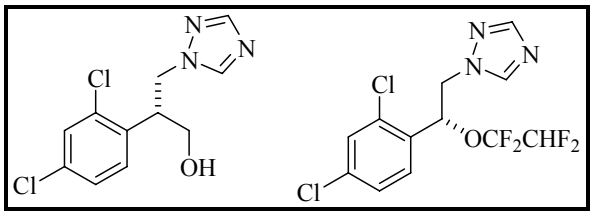


Figure 15

Krzyszotof W. et al[126] have discovered 1,2,4-triazole (**Figure 16)** and reported their antimicrobial activity. Dae-Kee Kim et al have been synthesized 1,2,4-triazole derivatives to study their pesticidal and herbicidal activity.

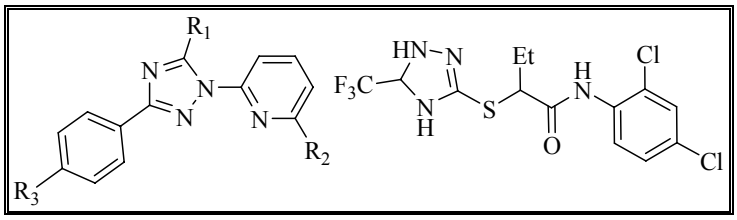


Figure 16

Sherin M. El-Feky et al[127] have reported a new series of 3,5-disubstituted triazoles (**Figure 17)** were synthesized and evaluated for *invitro* antifungal and antibacterial activity. All compounds tested showed significant antifungal activity against micromycetes compared to the commercial fungicide clotrimazole.

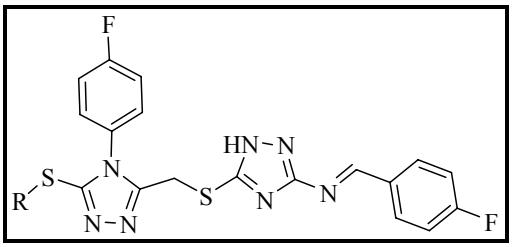


Figure 17

Hakan Bekats et al[128] have synthesized some novel 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-one (**Figure 18)** and all newly synthesized compounds were screened for their antimicrobial activities and some of which were found to possess good or moderate activities against the test microorganisms.

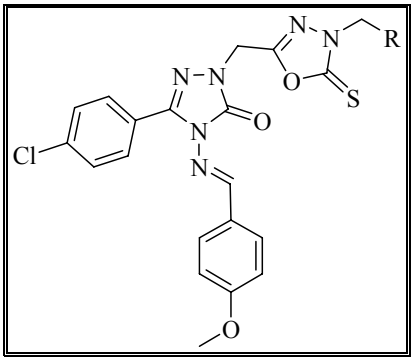


Figure 18

3.3. Alternative synthetic routes for better yield, shorter reaction time and to synthesize new analogs

❖ Creation of a C-N bond

Diazotation of an *o*-toluidine followed by capture of the generated diazonium salt is an old yet common way of accessing 1*H*-indazoles. This can be realized following two routes: the first and most common proceeds by a phase transfer-catalyzed reaction from *o*-methyl-benzendiazonium tetrafluoroborates (**Figure 19)** (method of Bartsch and Yang)[129] the second takes place via *N*-nitroso

derivatives (method of Kovach and Barnes).[130] These two procedures are well illustrated in the following example.[131]

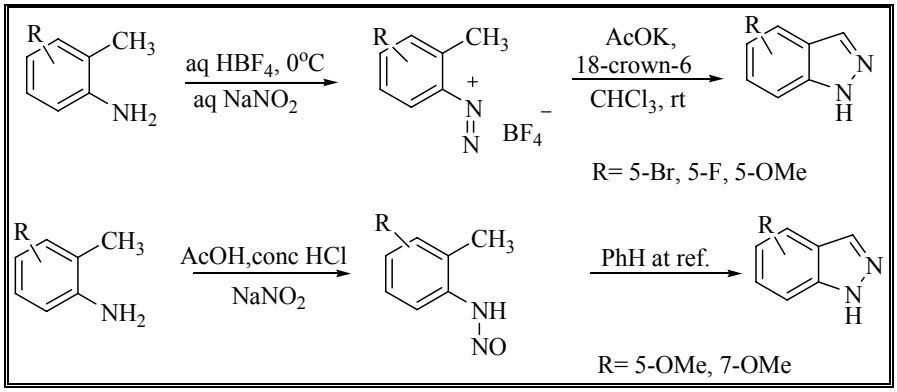


Figure 19

A different protocol proceeding *via* the intermediacy of a diazonium ion has also been reported. Thus, in the course of the preparation of an 1*H*-indazolone compound acting as norepiephedrine/serotonin reuptake inhibitor for the treatment of fibromyalgia, the construction of the 1*H*-indazolone core structure of precursor has been accomplished via the decomposition of a diazonium ion and capture of the resulting aryl cation by an *ortho*-disposed hydrazide (**Figure 20)**.[132]

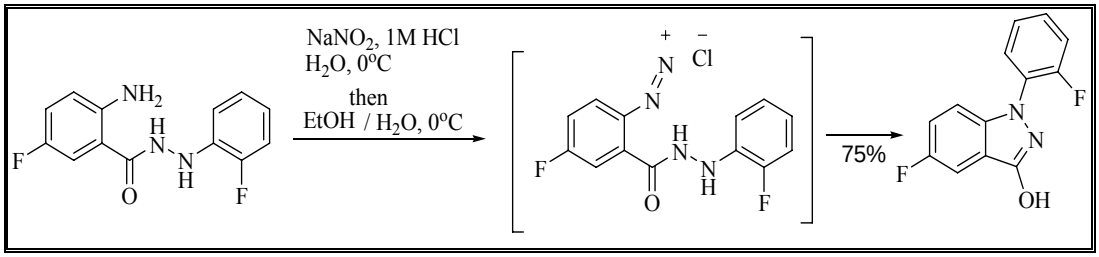


Figure 20

Reduction of a diazonium ion, or of a *N*-nitroso species, to the corresponding hydrazine and intramolecular reaction of the latter with an *ortho*-disposed carbonyl functionality is another way to reach 3-substituted-1*H*indazoles. Following this protocol, 5-bromo and 5-methoxy-3-carboxy-1*H*-indazoles (**Figure 21)** have been prepared from properly substituted isatines .[133]

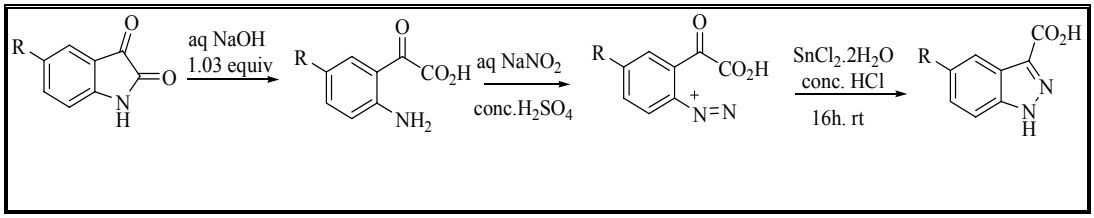


Figure 21

Another example can be found in the work of Zhang et al.[134] which, in the course of a study aimed at preparing bicyclic benzamides as novel 5-HT1F receptor agonists, have reported the preparation of 1*H*-indazole (**Figure 22).** It is worth noting that this example features an indole to indazole conversion[135] and reduction of the diazo intermediate with SO_2.[136]

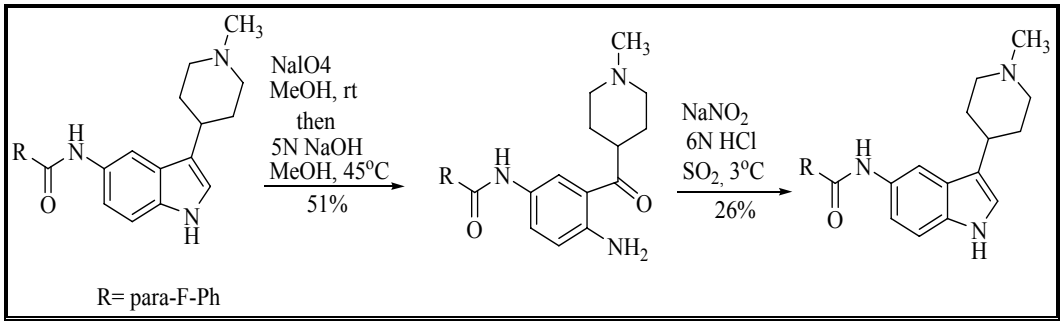


Figure 22

The synthesis of a series of 1*H*-indazol-3-ones with creation of the N-N bond has been achieved via the intramolecular trapping of an *N*-acylnitrenium intermediate by an *ortho*-disposed amino group[137]. Starting from an *o*-aminobenzamide the *N*-acylnitrenium cation was best generated by action of the hypervalent iodine reagent PIFA in DCM at 0°C (**Figure 23).**

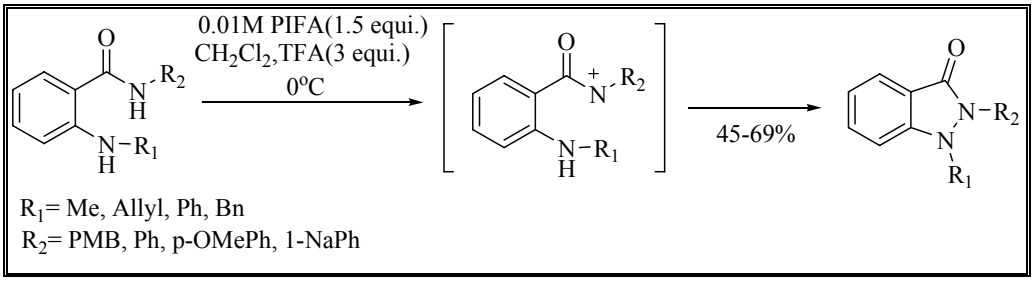


Figure 23

The chemistry of 2*H*-indazoles has not been explored as well as the chemistry of 1*H*-indazoles. However, the discovery that *N*-2 substituted 2*H*-indazole compounds may exhibit biological activities has generated recent interest in their simple and efficient preparation.

A synthesis of 2-aryl-2*H*-indazoles *via* a palladium-mediated intramolecular amination reaction of *N*-aryl-*N*-(*o*bromobenzyl)-hydrazines has been reported by Song and Yee.[138] The best conditions to effect the transformation are heating in toluene at 90°C for 15h in the presence of $Pd(OAc)_2$ (5 mol%), dppf (7.5 mol%), and *t*-BuONa (15 0 mol%). Yields were comprised in the 50 to 60% range. The catalytic system is equally effective for electron-rich and electron-deficient substituent's on both phenyl rings. In a mechanistic point of view the formation of the sp_2 C-N bond is followed by the spontaneous oxidation of the dihydroindazole intermediates to give the 2-aryl-2*H*-indazole products (**Figure 24)**.

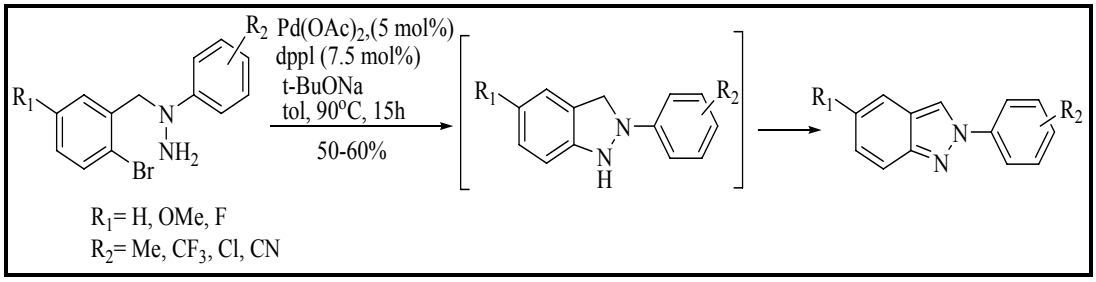


Figure 24

Most 1,3,4-oxadiazoles are best obtained by synthesis from acyclic precursors. Such reactions are 'one bond' or 'two bond' cyclization. Different methods for the synthesis have been cited in literature.[139-141]

Anil N. Mayekar et al[142] reported a series of new 1,3,4 oxadiazole (**Figure 25)** derivatives having 6-bromonaphthalene moiety are synthesized a hydrazide was treated with various substituted aromatic acids in presence of $POCl_3$ to give 2-{[(6-bromo-2-naphthyl)oxy]methyl}-5-aryl-1,3,4-oxadiazole.

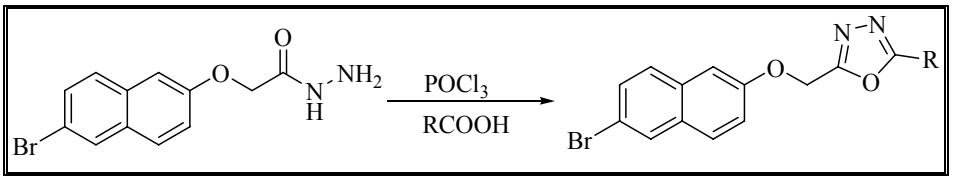


Figure 25

Chandrakantha, B. et al[143] have synthesized oxadiazoles (**Figure 26)** by the reaction of hydrazide and aromatic acid in presence of $POCl_3$.

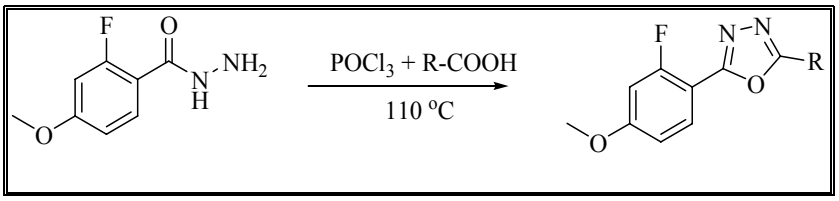


Figure 26

D. Ramesh and B. Sreenivasan[144] have synthesized 1,3,4-oxadiazoles (**Figure 27)** from semicarbazide in presence of $POCl_3$.

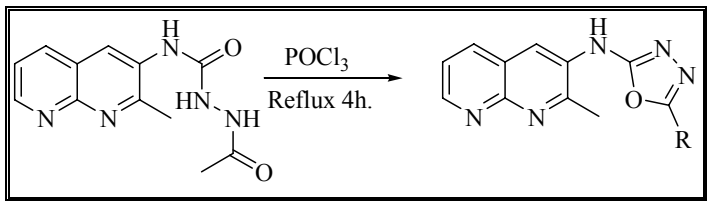


Figure 27

K. Mogilaiah and B. Sakram[145] have prepared 1,3,4-oxadiazole (**Figure 28)** from acetophenone-2-trifluoromethyl-1,8-naphthyridine-3-carbonyl hydrazone in presence of acetic anhydride.

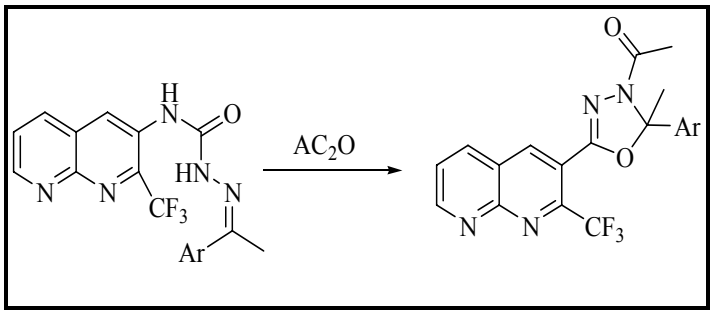


Figure 28

Yu Yuve et al[146] have reported microwave assisted synthesis protocol with 91 % of the yield (**Figure 29)**.

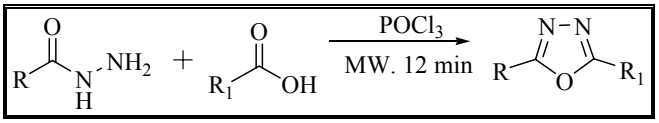


Figure 29

Silica sulfuric acid catalyst used for the rapid and ecofriendly synthesis of 1,3,4-oxadiazoles (**Figure 30)** at ambient temperature by M. Dabiri et al.[147]

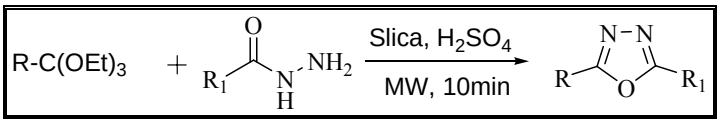


Figure 30

A.K. Mishra et al[148] have reported synthesis and antimicrobial activity of some newer oxadiazole/triazole derivatives (**Figure 31)** starting from 2-substituted-1*H*-benzimidazole.

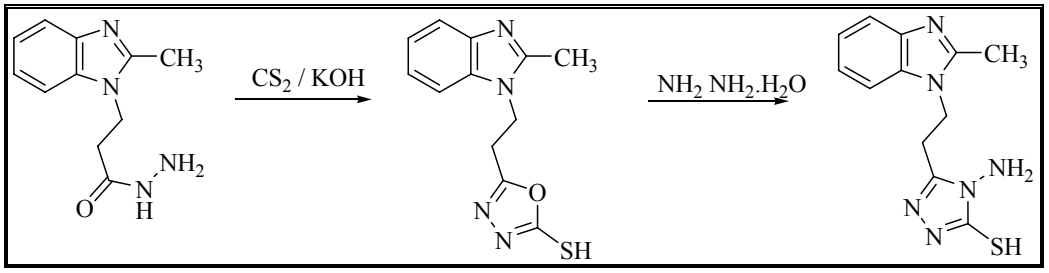


Figure 31

Reid and Heindel et al[149]reported that the reaction of aryl acid hydrazide with CS_2 /KOH and hydrazine hydrate yielded triazoles (**Figure 32)**.

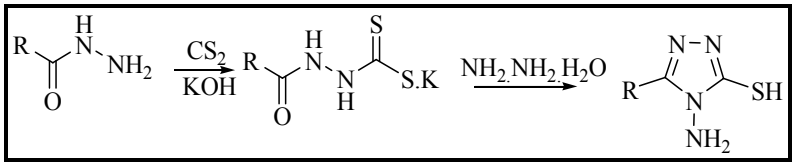


Figure 32

K. Paulvannam et al[150] have developed an improved synthesis of 1,3,5-trisubstituted 1,2,4-triazoles (**Figure 33)** via Ag_2CO_3 mediated cyclization of triazenes. The reaction was complete within 3h and the products were isolated in moderate to high yields.

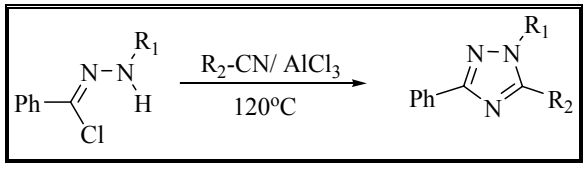


Figure 33

K. S. Bhat et al[151] have synthesized 4-amino-3-(2,4-dichloro-5-fluorophenyl)1,2,4-triazole-5-thiol (**Figure 34)** with the help of thiocarbohydrazide and 2,4 dichloro-5-fluoro benzoic acid.

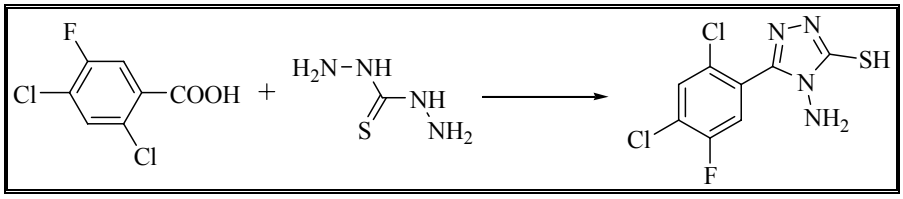


Figure 34

Sumesh E. et al[152] also synthesized triazole derivatives by the reaction of 4-hydroxy-8-(trifluoromethyl)quinoline-3-carbohydrazide and give 1,2,4-triazole (**Figure 35)**.

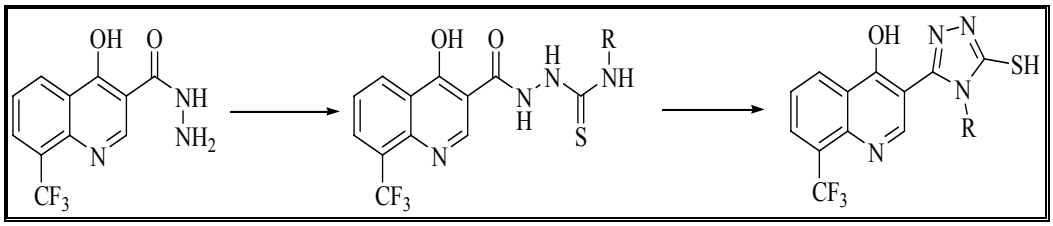


Figure 35

L. Labanauskas et al[153] have prepared triazoles (**Figure 36)** by the addition reaction of thiosemicarbazide with substituted benzoyl chloride in the presence of pyridine. Then the substituted thiosemicarbazide cyclised in water in the presence of alkaline catalyst.

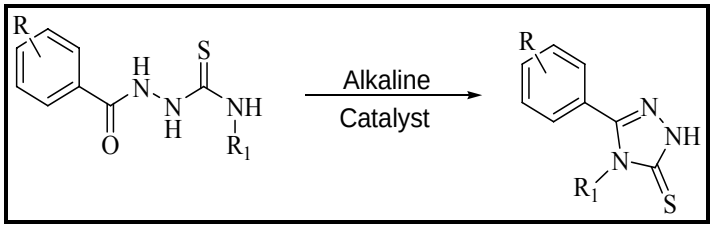


Figure 36

3.4 CURRENT RESEARCH WORK

Our group is involved in design, synthesis and biological screening of heterocyclic compounds. On conducting literature survey, it was found that tetrahydroindazole is not more explored though it has great importance in the field of medicinal chemistry. On the observation of medicinal importance of tetrahydroindazole, its derivatization is necessary.

The 3-carboxamide derivatives of 1*H*- and 2*H*-indazole possess good medicinal values. We sought to develop some 3-carbohydrazide 4,5,6,7-tetrahydro-2*H*-indazole derivatives. As per mentioned in literature, ethyl-2-oxo-2-(2-oxocyclohexyl)acetate was prepared by reacting cyclohexanone and diethyl oxalate with the help of sodium ethoxide in ethanol at 0-5 °C. Subsequent treatment of ethyl-2-oxo-2-(2-oxocyclohexyl)acetate with hydrazine hydrate in ethanol resulted into ethyl 4,5,6,7-tetrahydro-2*H*-indazole-3-carboxylate, while without solvent in excess hydrazine hydrate on reflux resulted into 4,5,6,7-tetrahydro-2*H*-indazole-3-carbohydrazide.

The synthesis of novel indazole bearing oxadiazole derivatives (**PVP-3Aa-o**) has been achieved by the reaction of hydrazide of *2H*-indazole with acid in the presences of $POCl_3$. However the reaction of hydrazide of *2H*-indazole with carbon disulfide and base afforded the potassium salt of hydrazide which on reaction with hydrazine hydrate and followed by aldehyde afforded desired triazole derivatives (**PVP-3Ba-o**). All newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis and screened for antimicrobial activity.

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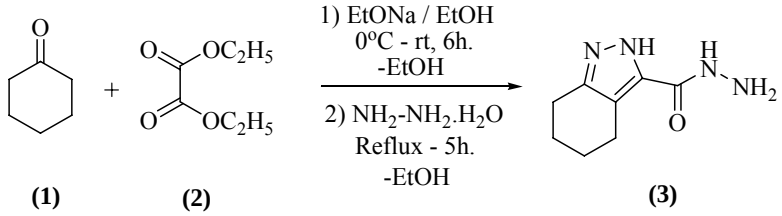
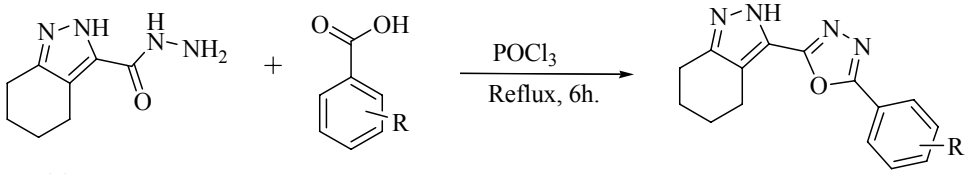
3.5. RESULTS AND DISCUSSION

Scheme:-1 Synthesis of substituted oxadiazole and triazole derivatives.



(1) **(2)** **(3)**

Scheme:-2

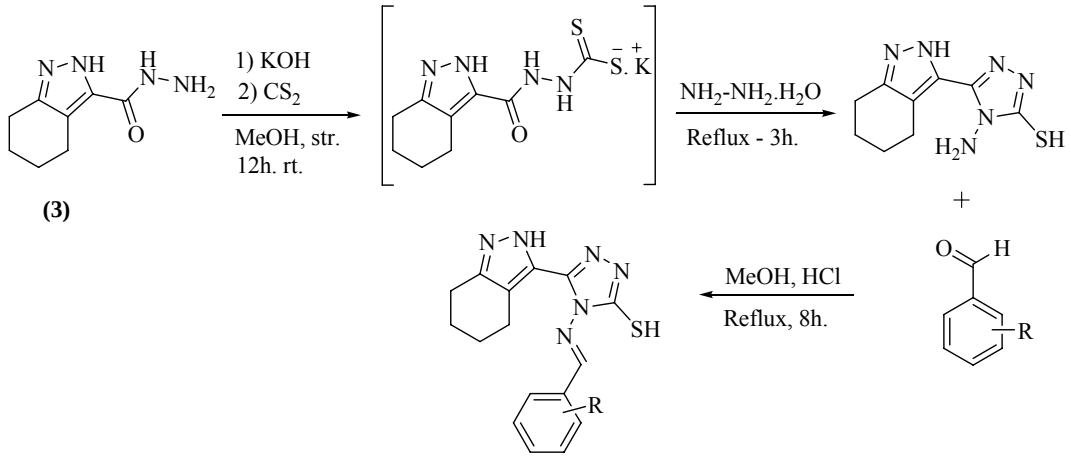


(3)

PVP- 3Aa-o

R= CH_3, OCH_3, Cl, NO_2

Scheme:-3



(3)

PVP- 3Ba-o

R= CH_3, Cl, F, OH, OCH_3

Table 1: Synthesis of substituted oxadiazole and triazole derivatives.

Entry	R	Yield %	M.P. ^{o}C
PVP-3Aa	4-ClC_6H_4	90	180-182
PVP-3Ab	4-$CH_3C_6H_4$	91	176-178
PVP-3Ac	2-$CH_3C_6H_4$	84	186-188
PVP-3Ad	3-ClC_6H_4	90	192-194
PVP-3Ae	2-ClC_6H_4	86	187-189
PVP-3Af	2-OHC_6H_4	90	183-185
PVP-3Ag	2-OH,3-$NO_2C_6H_3$	90	195-197
PVP-3Ah	2-ClC_7H_6	86	188-190
PVP-3Ai	C_7H_6	89	185-187
PVP-3Aj	3-$NO_2C_6H_4$	89	195-197
PVP-3Ak	4-$NO_2C_6H_4$	88	201-203
PVP-3Al	C_8H_7	90	178-180
PVP-3Am	4-$OCH_3C_6H_4$	90	183-185
PVP-3An	2-OH,4-$NO_2C_6H_3$	87	186-188
PVP-3Ao	C_6H_5	85	195-197
PVP-3Ba	2-ClC_6H_4	90	238-240
PVP-3Bb	4-OHC_6H_4	90	240-242
PVP-3Bc	4-$OCH_3C_6H_4$	89	246-248
PVP-3Bd	4-FC_6H_4	85	250-252
PVP-3Be	4-ClC_6H_4	87	248-250
PVP-3Bf	4-$CH_3C_6H_4$	89	243-245
PVP-3Bg	3-BrC_6H_4	90	257-259
PVP-3Bh	3-OHC_6H_4	85	245-247
PVP-3Bi	2-OHC_6H_4	88	256-258
PVP-3Bj	C_6H_5	90	260-262
PVP-3Bk	4-$NO_2C_6H_4$	90	248-250
PVP-3Bl	3-$NO_2C_6H_4$	89	249-251
PVP-3Bm	3,4-di-$OCH_3C_6H_3$	85	252-254
PVP-3Bn	2-$CH_3C_6H_4$	88	258-260
PVP-3Bo	2,5-di-$OCH_3C_6H_3$	90	254-256

In mechanism, the amine group acts as nucleophile which attack on carbonyl carbon of acetyl chloride and form iminium by removal of chloride. Followed by the proton migration and removal of water molecule it forms oxadiazole.

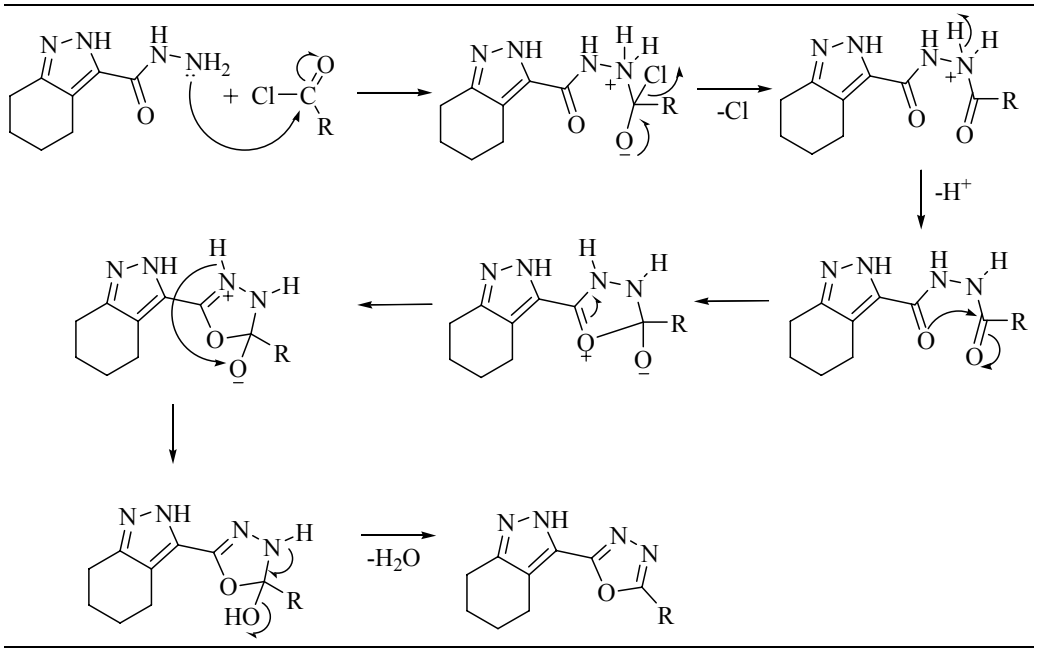


Figure 37: Proposed mechanism for the formation of oxadiazole

3.6. ANTIMICROBIAL SENSITIVITY TESTING

WELL DIFFUSION / AGAR CUP METHOD (Lt. General Raghunath D. 1998, Ashok Rattan, 1998; Patel R., Patel K. 2004,)

In vitro effectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective then many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive then using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Requirements

1. Young broth culture of a standard test organism
2. Sterile Mueller Hinton Agar plate
3. Solution of antimicrobial substance
4. Cup borer
5. Alcohol etc.

Inoculum preparation

Inoculum was prepared by selecting 4-5 colonies from slope of stock culture of the indicator organism and emulsifying them in a suitable broth. The inoculated broth was incubated at 37ºC till it equals turbidity of a 0.5 McFarland standard. This happens in 2-8 h.

Procedure

1. Inoculate test organism on the top of Mueller Hinton Agar plate with help of sterile swab. (it can be inoculated in melted agar also)
2. The swab was dipped in the inoculum and surface of plate was streaked with swab.
3. Streaking was repeated for 3 times and each time the plate was rotated at angle of 60º.
4. Sterilize the cup-borer make four cups of the diameter of 8-10 mm. at equal distance in the plate previously inoculated with seed culture.
5. The depth of well was 2.5-5.0 mm.
6. The wells have been clearly punched so the surrounding medium is not lifted when the plug was removed out.
7. The plates were incubated at 37ºC for 24 h. Then the zone of inhibition measured and the size of zone cited in table.

Antibiotic Sensitivity Assay

(Concentration250/500/ 1000 µG/ml)

Sr. No.	COD E No.	*Pseudomonas aeruginosa*			*Proteus vulgaris*			*Escherichia coli*			*Staphylococcus aureus*			*Candida albicans*		
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
1.	**3Aa**	1.1	1.2	1.3	R	1	1.2	1.1	1.2	1.4	1.1	1.2	1.5	1.1	1.5	1.9
2.	**3Ab**	1.3	1.4	1.9	1.3	1.7	2.1	1.2	1.5	2	1.1	1.5	1.9	1.1	1.4	1.6
3.	**3Ac**	1.2	1.3	1.5	1.1	1.4	1.6	1.3	1.4	1.9	1.2	1.6	2	1.2	1.5	2
4.	**3Ad**	R	R	R	1.1	1.3	1.7	1.1	1.3	1.6	R	R	R	1.1	1.4	1.8
5.	**3Ae**	1.4	1.6	2	1	1.2	1.4	R	R	R	1.1	1.2	1.5	1.2	1.5	2
6.	**3Af**	1	1.1	1.3	R	1.1	1.3	R	R	R	R	1	1.4	R	1.1	1.5
7.	**3Ag**	R	1	1.1	1.1	1.6	1.8	1.4	1.5	2	1.1	1.2	1.3	1.1	1.3	1.7
8.	**3Ah**	R	R	R	1.1	1.3	1.5	1.2	1.3	1.7	R	1	1.3	1	1.2	1.8
9.	**3Ai**	1.1	1.2	1.5	1.2	1.4	1.7	1.3	1.5	2	1.1	1.2	1.5	1.1	1.2	1.7
10.	**3Aj**	1.3	1.4	1.8	R	1.1	1.3	R	1	1	1.2	1.4	1.7	1	1.3	1.8
11.	**3Ak**	1.3	1.5	1.7	R	1	1.2	1.1	1.3	1.6	1.1	1.3	1.5	1	1.2	1.5
12.	**3Al**	1.2	1.4	1.6	1.1	1.4	1.8	1.2	1.5	1.9	R	1.2	1.7	1.1	1.5	2
13.	**3Am**	1.4	1.6	2	1.3	1.7	2	1.1	1.3	1.5	R	1	1.2	R	1.1	1.4
14.	**3An**	1.1	1.1	1.3	1.1	1.3	1.8	1.4	1.6	2	1.1	1.2	1.4	1	1.2	1.7
15.	**3Ao**	R	R	R	R	R	R	1.2	1.5	1.7	1.6	1.8	1.4	1.5	2	1.1
16.	**3Ba**	1.1	1.2	1.4	1	1.3	1.7	1.4	1.5	2	1.3	1.5	1.2	1.3	1.7	R
17.	**3Bb**	1.3	1.4	1.8	1.2	1.5	1.9	1.2	1.7	2.1	1.4	1.7	1.3	1.5	2	1.1
18.	**3Bc**	1.1	1.5	1.7	1.1	1.3	1.5	1.3	1.4	1.7	R	1.3	R	1	1	1.2
19.	**3Bd**	1.1	1.4	1.6	1	1.2	R	1.2	1.3	1.6	1.2	1.4	1.6	1.1	1.2	1.7
20.	**3Be**	1.4	1.7	2	1.2	1.5	1.8	1.2	1.2	1.5	1.4	1.7	2.1	1	1.2	1.8
21.	**3Bf**	1.3	1.6	2	1.3	1.6	2	R	R	R	1.1	1.8	2.3	1.1	1.4	1.8
22.	**3Bg**	1.2	1.6	1.8	1.1	1.3	1.4	R	R	R	1.2	1.5	1.7	1.2	1.6	2.1
23.	**3Bh**	R	1	1.1	1	1.2	1.8	1.1	1.1	1.3	1.2	1.3	1.1	1.2	1.4	2
24.	**3Bi**	R	1.1	1.3	1.1	1.3	1.7	R	1.6	2	1	R	1	1	1.1	1.5
25.	**3Bj**	1.1	1.4	1.7	1	1.1	1.3	1.1	1.1	1.3	1.2	1.5	1.1	1.2	1.3	1.5
26.	**3Bk**	1.1	1.1	1.3	R	1.1	1.4	1.2	1.4	1.7	1.4	1.7	1	1.4	1.5	2

27.	**3Bl**	1.1	1.2	1.3	R	1	1.2	1.1	1.2	1.4	1.1	1.2	1.5	1.1	1.5	1.9
28.	**3Bm**	1.3	1.4	1.9	1.3	1.7	2.1	1.2	1.5	2	1.1	1.5	1.9	1.1	1.4	1.6
29.	**3Bn**	1.2	1.3	1.5	1.1	1.4	1.6	1.3	1.4	1.9	1.2	1.6	2	1.2	1.5	2
30.	**3Bo**	1.3	1.1	1.3	1.1	1.3	1.7	1.1	1.3	1.6	1.3	1.1	1.3	1.1	1.4	1.8
31.	**A**	1.8			1.8			1.9			1.9			-		
32.	**CPD**	2.2			2.1			2.1			2.2			-		
33.	**GF**	1.8			1.9			2.0			2.0			-		
34.	**GRF**	-			-			-			-			2.6		
35.	**FLC**	-			-			-			-			2.8		

Note: Zone of inhibition interpretation is as follows.

1. **Zone SIZE <1.0 C.M.- RESISTENT(R)**
2. **ZONE SIZE 1.0 To 1.5 – INTERMEDIATE**
3. **ZONE SIZE >1.5 - SENSITIVE**

STD Antibiotic Sensitivity Assay Concentration 40 µG/ml

A: AMPICILLIN
CPD: CEFPODOXIME
GF: GATIFLOXACIN
GRF: GRESIOFULVIN
FLC: FLUCONAZOLE

3.7. CONCLUSION

In summary, we have described the synthesis of novel indazole bearing oxadiazole derivatives and triazole derivatives. The reaction of hydrazide of *2H*-indazole with substituted carboxylic acid in the presences of $POCl_3$ afforded desired oxadiazole derivatives (**3A**). However the reaction of hydrazide of *2H*-indazole with carbon disulfide and base afforded the potassium salt of hydrazide which on reaction with hydrazine hydrate and followed by aldehyde afforded desired triazole derivatives (**3B**) in excellent yields. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-3Aa-o** and **3Ba-o** showed moderate to potent activity. The compounds **PVP-3Ab, 3Be** and **3Bf** showed comparatively good activity against all the bacterial strains.

3.8 EXPERIMENTAL SECTION

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. Melting points were measured in open capillaries and are uncorrected.

- **General procedure for the synthesis 4,5,6,7-tetrahydro-2*H*-indazole-3-carbohydrazide (3).**

To the stirred solution of sodium ethoxide (0.2mol), a mixture of cyclohexanone (0.2 mol) and diethyl oxalate (0.2 mol) was added drop wise below 5-10 ºC. Vigorous stirring was required to prevent complete solidification of the reaction mixture. After completion of the reaction. The reaction mixture was decomposed by the careful addition of cold dilute sulfuric acid solution. The ethyl 2-ketocyclohexylglyoxalate separated as heavy oil. Ethyl 2-oxo-2-(2-oxocyclohexyl) acetate is added into access 80% hydrazine hydrate and refluxed for 5 to 6 h. The reaction mixture was allowed to cool at room temperature and the precipitate obtained was filtered, dried and recrystallized from ethanol white crystals. Yield-85%.

- **General procedure for the synthesis of 4,5,6,7-tetrahydro-3-(5-aryl-1,3,4-oxadaizol-2-yl)-2*H*-indazole (3Aa-o).**

Equimolar amount of 4,5,6,7-tetrahydro-2*H*-indazole-3-carbohydrazide and appropriate carboxylic acid were taken in $POCl_3$. The reaction mixture was refluxed for 6 h. and allowed to cool at room temperature and poured into crushed ice and stand by over night. The solid was filtered, dried and recrystallized from ethanol to give analytical pure product in 85-90% yield.

- **General procedure for the synthesis of 4-amino-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol**

To a mixture of potassium hydroxide (0.15 mol) and 4,5,6,7-tetrahydro-2*H*-indazole-3-carbohydrazide (0.1 mol) in methanol, carbon disulphide (0.15 mol) was added. This mixture was stirred for 12 h. It was than diluted with dry ether and thus the solid obtained was filtered and washed with ether and dried .There is a no need to further purify the salt for further reaction. A suspension of the potassium salt (0.1 mol), hydrazine hydrate (0.2 mol) was refluxed with stirring for 3 h. The color of the reaction mixture changed to green, hydrogen sulfide was evolved and a homogeneous solution resulted. Dilute the solution with cold water and neutralized with glacial acetic acid, precipitated a white solid. The product was filtered, washed with cold water and crystallized from dioxane yield 80%

- **General procedure for the synthesis of 4-(arylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (3Ba-o).**

Equimolar amount of triazole and appropriate aldehyde were taken in methanol and added 2 drops of con. HCl as a catalyst. The reaction mixture was refluxed for 8 h. and allowed to cool at room temperature. The solid was filtered, dried and recrystallized from ethanol to give pure yellow crystals in 85-90% yield.

❖ **Spectral data of the synthesized compounds**

3-(5-(4-chlorophenyl)-1,3,4,-oxadiazole-2-yl)-4,5,6,7-tetrahydro-2*H*-indazole (PVP-3Aa): Creamish solid; R_f 0.33 (6:4 hexane-EtOAc); IR (KBr): 3227, 3186, 3078, 2941, 1668, 1579, 1467, 1249, 1161 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63; Found: C, 59.48; H, 4.15; N, 18.52.

4,5,6,7-tetrahydro-3-(5-*p*-tolyl-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Ab): Creamish solid; R_f 0.35 (6:4 hexane-EtOAc); IR (KBr): 3149, 2980, 2862, 1653, 1509, 1461, 1237, 1051 cm^{-1}; ^{1}H NMR: δ 1.80 (m, 4H, 2xCH_2), 2.39 (s, 3H, CH_3), 2.69 (m, 2H, CH_2), 2.88 (m, 2H, CH_2), 7.31-7.35 (d, 2H, Ar-H), 7.93-7.95 (d, 2H, Ar-H), 10.58 (s, 1H, NH); ^{13}C NMR: δ 21.22, 21.44, 21.67, 22.36, 22.82, 117.22, 120.92, 126.84, 126.95, 129.71, 129.77, 133.15, 142.24, 142.46, 160.13, 163.93; MS (*m/z*): 280 (M^+); Anal. Calcd for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99; Found: C, 68.48; H, 5.65; N, 19.82.

4,5,6,7-tetrahydro-3-(5-*o*-tolyl-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Ac): Creamish solid; R_f 0.30 (6:4 hexane-EtOAc); IR (KBr): 3186, 3149, 3078, 2950, 1668, 1579, 1467, 1161, 1030 cm^{-1}; ^{1}H NMR: δ 1.80 (m, 4H, 2xCH_2), 2.68 (m, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.87- 2.88 (m, 2H, CH_2), 7.24 -7.28 (t, 1H, Ar-H), 7.32-7.41 (m, 2H, Ar-H), 7.90 -7.92 (d, 1H, Ar-H), 10.28 (s, 1H, NH); MS (*m/z*): 280 (M^+); Anal. Calcd for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99; Found: C, 68.58; H, 5.45; N, 19.92.

3-(5-(3-chlorophenyl)-1,3,4,-oxadiazole-2-yl)-4,5,6,7-tetrahydro-2*H*-indazole (PVP-3Ad): Creamish solid; R_f 0.29 (6:4 hexane-EtOAc) ; IR (KBr): 3207, 3123, 2959, 1658, 1546, 1472, 1265, 1041 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63; Found: C, 59.78; H, 4.20; N, 18.52.

3-(5-(2-chlorophenyl)-1,3,4,-oxadiazole-2-yl)-4,5,6,7-tetrahydro-2*H*-indazole (PVP-3Ae): Creamish solid; R_f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3207, 3123, 2959, 1668, 1526, 1265, 1049 cm^{-1}; MS (*m/z*): 300 (M^+); Anal. Calcd for $C_{15}H_{13}ClN_4O$: C, 59.91; H, 4.36; N, 18.63; Found: C, 59.76; H, 4.18; N, 18.60.

2-(5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-1,3,4,-oxadiazole-2-yl)phenol (PVP-3Af): yellow solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3207, 3123, 2990, 2862, 1653, 1509, 1461, 1061 cm^{-1}; MS (*m/z*): 282 (M^+); Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85; Found: C, 63.78; H, 5.05; N, 19.82.

2-(5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-1,3,4,-oxadiazole-2-yl)-6-nitrophenol (PVP-3Ag): Creamish solid; R_f 0.36 (6:4 hexane-EtOAc); IR (KBr): 3223, 3149, 2990, 2862, 1653, 1509, 1437, 1061 cm^{-1}; MS (*m/z*):327 (M^+); Anal. Calcd for $C_{15}H_{13}N_5O_4$: C, 55.05; H, 4.00; N, 21.40; Found: C, 55.11; H, 4.05; N, 21.32.

3-(5-(2-chlorobenzyl)-1,3,4,-oxadiazole-2-yl)-4,5,6,7-tetrahydro-2*H*-indazole (PVP-3Ah): Creamish solid;R_f 0.31 (6:4 hexane-EtOAc); IR (KBr): 3227, 3193, 2966, 1628, 1522, 1456, 1217, 1041 cm^{-1}; MS (*m/z*): 314 (M^+); Anal. Calcd for $C_{16}H_{15}ClN_4O$: C, 61.05; H, 4.80; N, 17.80; Found: C, 61.01; H, 4.75; N, 17.63.

3-(5-benzyl)-1,3,4,-oxadiazole-2-yl)-4,5,6,7-tetrahydro-2*H*-indazol (PVP-3Ai): Creamish solid; R_f0.33 (6:4 hexane-EtOAc); IR (KBr): 3227, 3173, 2989, 1648, 1586, 1468, 1251, 1061 cm^{-1}; MS (*m/z*): 280 (M^+); Anal. Calcd for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99; Found: C, 68.43; H, 5.65; N, 19.82.

4,5,6,7-tetrahydro-3-(5-(3-nitrophenyl)-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Aj): Creamish solid; R_f0.36 (6:4 hexane-EtOAc); IR (KBr): 3442, 3226, 3143, 2986, 1642, 1566, 1447, 1241, 1051 cm^{-1}; MS (*m/z*): 311 (M^+); Anal. Calcd for $C_{15}H_{13}N_5O_3$: C, 57.87; H, 4.21; N, 22.50; Found: C, 57.73; H, 4.14; N, 22.42.

4,5,6,7-tetrahydro-3-(5-(3-nitrophenyl)-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Ak): Creamish solid; R_f 0.29 (6:4 hexane-EtOAc); IR (KBr): 3206, 3163, 2996, 1672, 1566, 1478, 1241, 1049 cm^{-1}; MS (*m/z*): 311 (M^+); Anal. Calcd for $C_{15}H_{13}N_5O_3$: C, 57.87; H, 4.21; N, 22.50; Found: C, 57.83; H, 4.18; N, 22.52.

4,5,6,7-tetrahydro-3-(5-styryl-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Al): Creamish solid; R_f0.28 (6:4 hexane-EtOAc); IR (KBr): 3226, 3143, 2988, 1632, 1546, 1424, 1231, 1061 cm^{-1}; MS (*m/z*): 292 (M^+); Anal. Calcd for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17; Found: C, 69.73; H, 5.44; N, 19.12.

4,5,6,7-tetrahydro-3-(5-(4-methoxyphenyl)-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Am): Creamish solid;R_f 0.30 (6:4 hexane-EtOAc); IR (KBr): 3217, 3153, 2950, 1613, 1539, 1431, 1061 cm^{-1}; MS (*m/z*): 296 (M^+); Anal. Calcd for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91; Found: C, 64.78; H, 5.35; N, 18.82.

2-(5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-1,3,4,-oxadiazole-2-yl)-5-nitrophenol (PVP-3An): Creamish solid; R_f 0.32 (6:4 hexane-EtOAc); IR (KBr): 3187, 3153, 2980, 1623, 1569, 1431, 1051 cm^{-1}; MS (*m/z*): 327 (M^+); Anal. Calcd for $C_{15}H_{13}N_5O_4$: C, 55.05; H, 4.00; N, 21.40; Found: C, 55.08; H, 4.05; N, 21.52.

4,5,6,7-tetrahydro-3-(5-phenyl-1,3,4,-oxadiazole-2-yl)-2*H*-indazole (PVP-3Ao): Creamish solid; R_f 0.34 (6:4 hexane-EtOAc); IR (KBr): 3227, 3120, 2980, 1623, 1509, 1461, 1051 cm^{-1}; MS (*m/z*): 266 (M^+); Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04; Found: C, 67.58; H, 5.25; N, 21.12.

4-(2-chlorobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Ba): yellow solid; R_f 0.53 (6:4 hexane-EtOAc); IR (KBr): 3414, 3171, 3143, 2937, 2856, 1589, 1492, 1276, 1049 cm^{-1}; MS (*m/z*): 358 (M^+); Anal. Calcd for $C_{16}H_{15}ClN_6S$: C, 53.55; H, 4.21; N, 23.42; Found: C, 53.46; H, 4.15; N, 23.32.

4-(((3-mercato-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazol-4-yl)imino) methyl)phenol (PVP-3Bb): yellow solid; R_f 0.54 (9:1Chloroform: Methanol); IR (KBr): 3394, 3115, 3068, 2989, 1648, 1597, 1458, 1261, 1061 cm^{-1}; ^1H NMR: δ 1.76- 1.81 (m, 4H, 2xCH_2), 2.59 -2.67 (m, 4H, 2xCH_2), 6.90- 6.95 (m, 2H, Ar-H), 7.65-7.74 (m, 2H, Ar-H), 9.80 (s, 1H, CH), 10.20 (s, 1H, OH), 13.62 (s, 1H, NH), 13.87 (s, 1H, SH); MS (*m/z*): 340 (M^+); Anal. Calcd for $C_{16}H_{16}N_6OS$: C, 56.45; H, 4.74; N, 24.69; Found: C, 56.43; H, 4.45; N, 24.62.

4-(4-methoxybenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bc): yellow solid; R_f 0.56 (9:1Chloroform: Methanol); IR (KBr): 3394, 3115, 2939, 2850, 1597, 1458, 1168, 1051 cm^{-1}; ^1H NMR: δ 1.72-1.79 (m, 4H, 2xCH_2), 2.57 -2.65 (m, 4H, 2xCH_2), 3.87 (s, 3H, OCH_3), 6.99- 7.01 (d, 2H, Ar-H), 7.82-7.87 (d, 2H, Ar-H), 9.33 (s, 1H, CH), 12.65 (s, 1H, NH), 13.87 (s,

1H, SH); MS (*m/z*): 354 (M^+); Anal. Calcd for $C_{17}H_{18}N_6OS$: C, 57.61; H, 5.12; N, 23.71; Found: C, 57.52; H, 5.15; N, 23.65.

4-(4-fluorobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bd): yellow solid; R_f 0.54 (9:1Chloroform: Methanol); IR (KBr): 3414, 3252, 3173, 2919, 1586, 1478, 1241, 1051 cm^{-1}; MS (*m/z*): 342 (M^+); Anal.Calcd for $C_{16}H_{15}FN_6S$: C, 56.13; H, 4.42; N, 24.54; Found: C, 56.20; H, 4.25; N, 24.42.

4-(4-chlorobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Be): yellow solid; R_f 0.55 (9:1Chloroform: Methanol); IR (KBr): 3442, 3327, 3073, 2989, 1653, 1586, 1259, 1056 cm^{-1}; ^{13}C NMR: δ 20.10, 20.83, 22.03, 22.47, 22.60, 22.74, 72.15, 115.24, 122.0, 127.68, 127.92, 128.22, 128.37, 128.70, 128.77, 129.87, 130.76, 133.97, 134.02, 137.62, 140.66, 149.36, 161.72, 164.29; MS (*m/z*): 358 (M^+); Anal. Calcd for $C_{16}H_{15}ClN_6S$: C, 53.55; H, 4.21; N, 23.42; Found: C, 53.48; H, 4.19; N, 23.32.

4-((4-methylbenzylidene)amino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bf): yellow solid; R_f0.51 (9:1Chloroform: Methanol); IR (KBr): 3422, 3317, 3073, 2939, 1586, 1427, 1276, 1067 cm^{-1}; MS (*m/z*): 338 (M^+); Anal. Calcd for $C_{17}H_{18}N_6S$: C, 60.33; H, 5.36; N, 24.83; Found: C, 60.30; H, 5.30; N, 24.72.

4-(3-bromobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bg): yellow solid; R_f 0.52 (9:1Chloroform: Methanol); IR (KBr): 3442, 3173, 2989, 2876, 1586, 1465, 1261, 1061 cm^{-1}; MS (*m/z*): 403 (M^+); Anal. Calcd for $C_{16}H_{15}BrN_6S$: C, 47.65; H, 3.75; N, 20.84; Found: C, 47.58; H, 3.69; N, 20.82.

3-(((3-mercato-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazol-4-yl)imino) methyl)phenol (PVP-3Bh): yellow solid; R_f 0.52 (9:1Chloroform: Methanol); IR (KBr): 3393, 3327, 3173, 2989, 1586, 1487, 1256, 1044 cm^{-1}; MS (*m/z*): 340 (M^+); Anal. Calcd for $C_{16}H_{16}N_6OS$: C, 56.45; H, 4.74; N, 24.69; Found: C, 56.35; H, 4.70; N, 24.72.

2-(((3-mercato-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazol-4-yl)imino) methyl)phenol (PVP-3Bi): yellow solid; R_f 0.53 (9:1Chloroform: Methanol); IR (KBr): 3402, 3327, 3063, 2989, 2850, 1586, 1458, 1287, 1053 cm^{-1}; MS (*m/z*): 340 (M^+); Anal. Calcd for $C_{16}H_{16}N_6OS$: C, 56.45; H, 4.74; N, 24.69; Found: C, 56.40; H, 4.68; N, 24.65.

4-(Benzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bj): yellow solid; R_f 0. 54 (9:1Chloroform: Methanol); IR (KBr): 3327, 3173, 2959, 1586, 1441, 1245, 1050 cm^{-1}; MS (*m/z*): 324 (M^+); Anal. Calcd for $C_{16}H_{16}N_6S$: C, 59.24; H, 4.97; N, 25.91; Found: C, 59.20; H, 4.89; N, 25.82.

4-(4-nitrobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bk): yellow solid; R_f 0.53 (9:1Chloroform: Methanol); IR (KBr): 3397, 3173, 2939, 1586, 1492, 1241, 1068 cm^{-1}; MS (*m/z*): 369 (M^+); Anal. Calcd for $C_{16}H_{15}N_7O_2S$: C, 52.02; H, 4.09; N, 26.54; Found: C, 51.90; H, 4.12; N, 26.62.

4-(3-nitrobenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bl): yellow solid; R_f 0.53 (9:1Chloroform: Methanol); IR (KBr): 3414, 3173, 3142, 2989, 2856, 1586, 1256, 1061 cm^{-1}; MS (*m/z*): 369 (M^+); Anal. Calcd for $C_{16}H_{15}N_7O_2S$: C, 52.02; H, 4.09; N, 26.54; Found: C, 51.98; H, 4.15; N, 26.42.

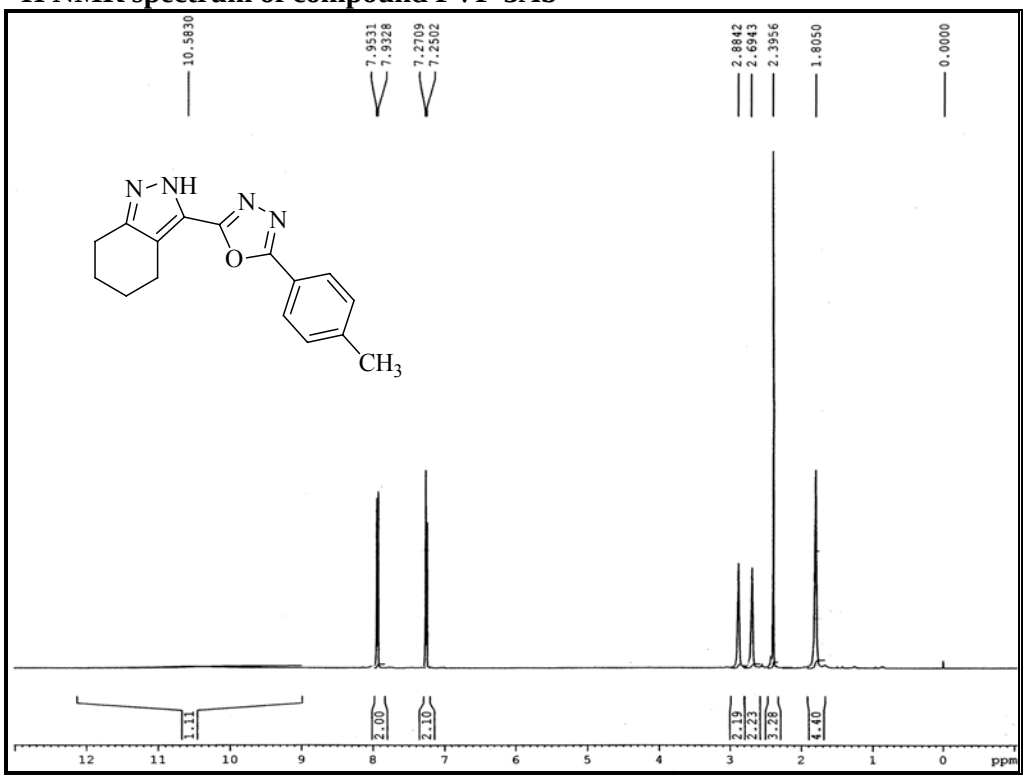
4-(3,4-dimethoxybenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bm): yellow solid; R_f 0.53 (9:1Chloroform: Methanol); IR (KBr): 3459, 3327, 3193, 2999, 1586, 1437, 1257, 1065 cm^{-1}; MS (*m/z*): 384 (M^+); Anal. Calcd for $C_{18}H_{20}N_6O_2S$: C, 56.23; H, 5.24; N, 21.86; Found: C, 56.12; H, 5.15; N, 21.75.

4-((2-methylbenzylidene)amino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bn): yellow solid; R_f 0.55 (9:1Chloroform: Methanol); IR (KBr): 3442, 3327, 3173, 2989, 1586, 1475, 1231, 1049 cm^{-1}; MS (*m/z*): 338 (M^+); Anal. Calcd for $C_{17}H_{18}N_6S$: C, 60.33; H, 5.36; N, 24.83; Found: C, 60.20; H, 5.29; N, 24.72.

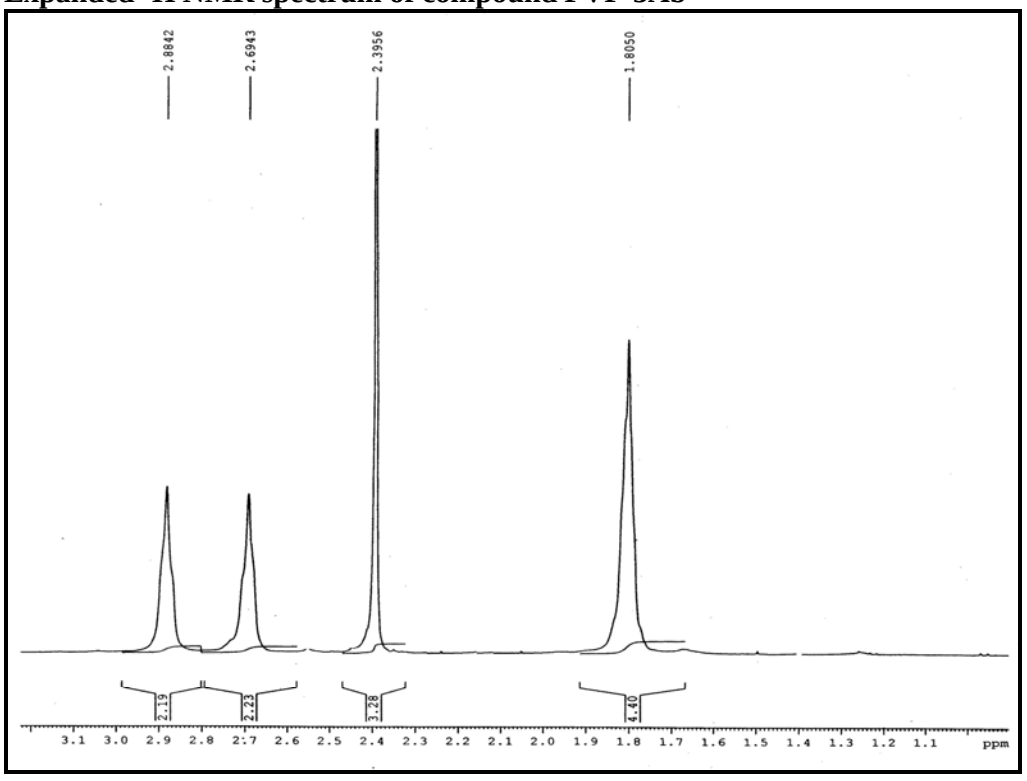
4-(2,5-dimethoxybenzylideneamino)-5-(4,5,6,7-tetrahydro-2*H*-indazole-3-yl)-4*H*-1,2,4-triazole-3-thiol (PVP-3Bo): yellow solid; R_f 0.53 (9:1Chloroform: Methanol); IR (KBr): 3459, 3327, 3193, 2999, 1648, 1586, 1437, 1051 cm^{-1}; MS (*m/z*): 384 (M^+); Anal. Calcd for $C_{18}H_{20}N_6O_2S$: C, 56.23; H, 5.24; N, 21.86; Found: C, 56.20; H, 5.27; N, 21.85.

❖ **Spectral representation of synthesized compounds**

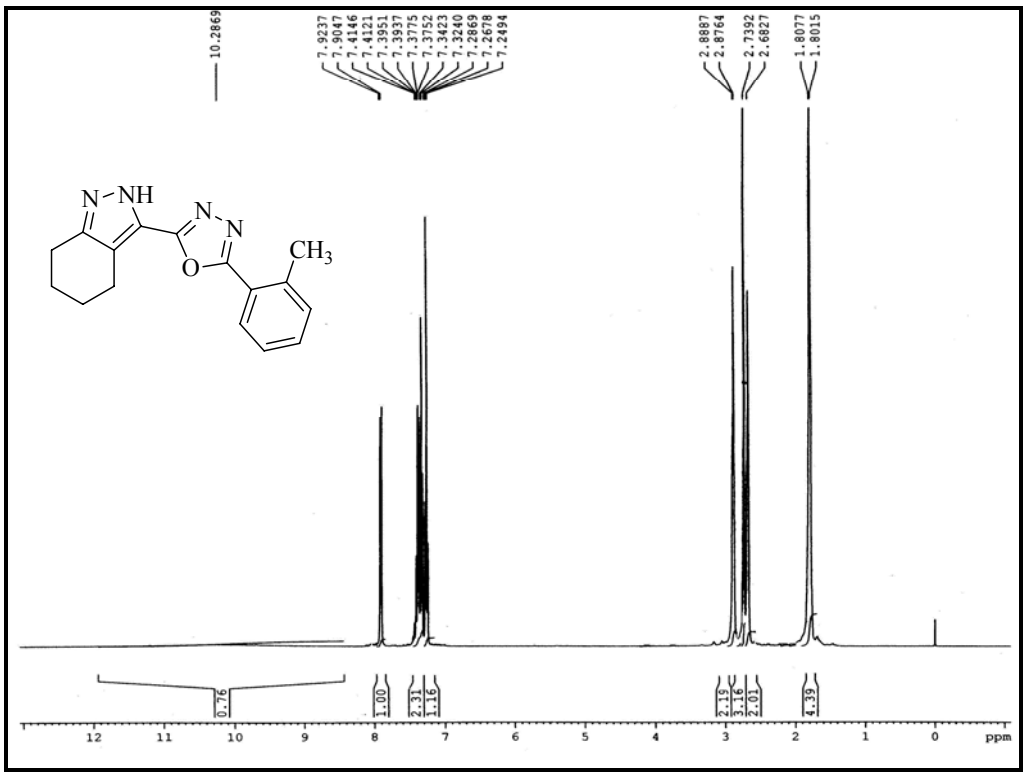
^{1}H NMR spectrum of compound PVP-3Ab



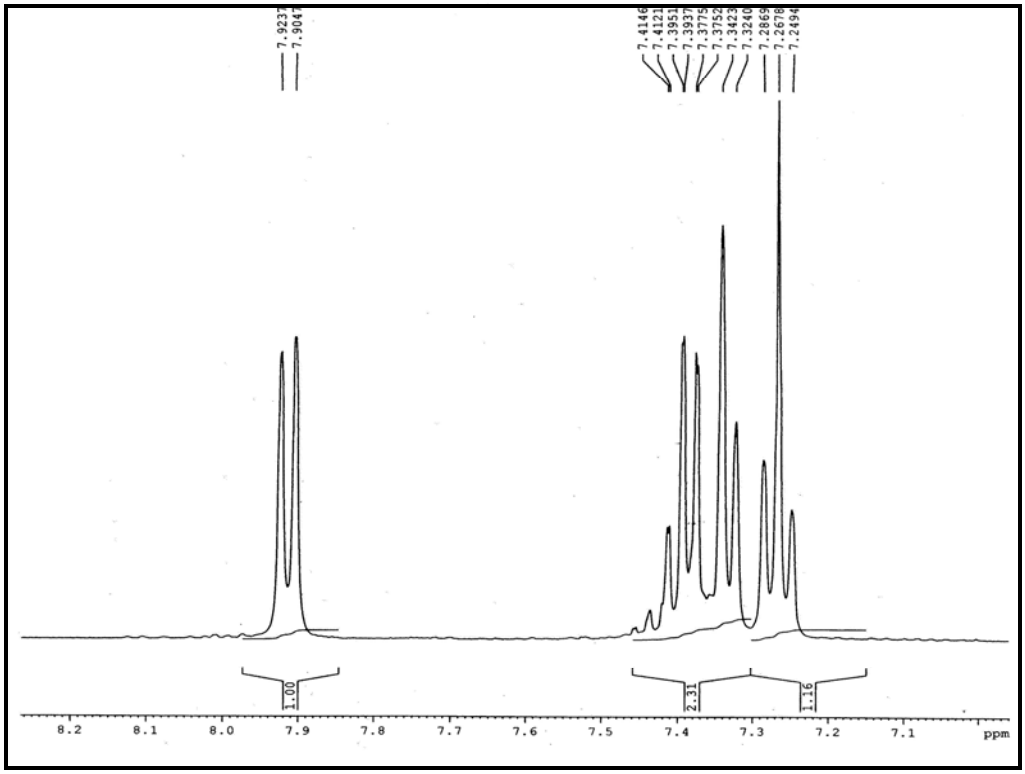
Expanded ^{1}H NMR spectrum of compound PVP-3Ab



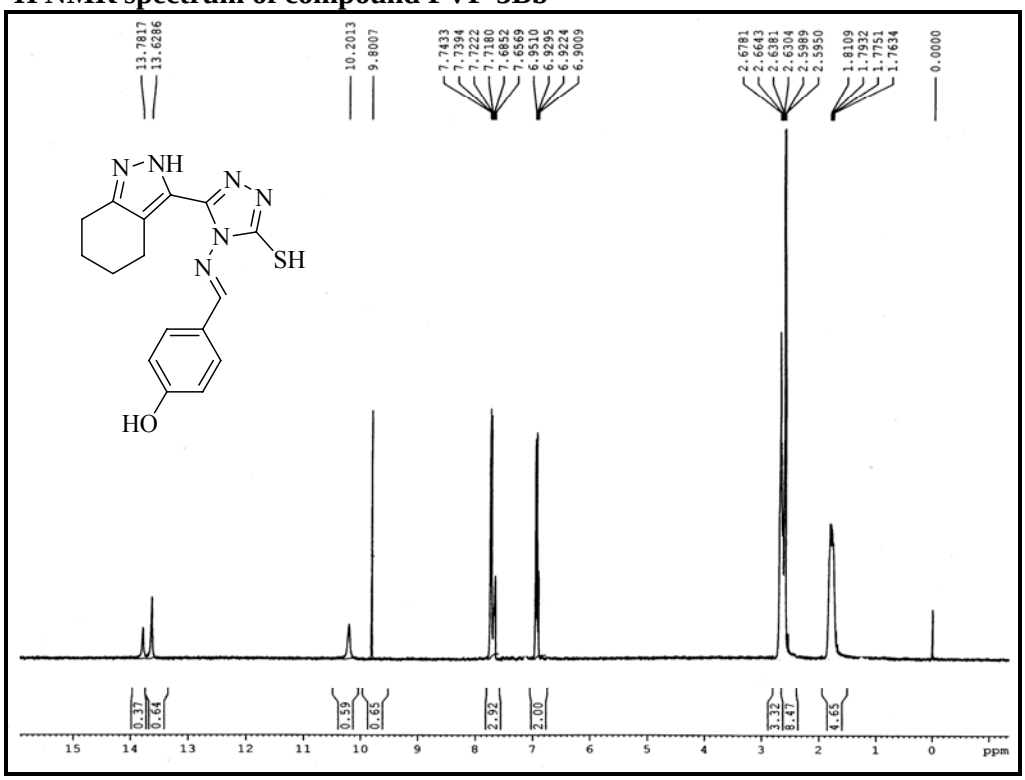
^{1}H NMR spectrum of compound PVP-3Ac



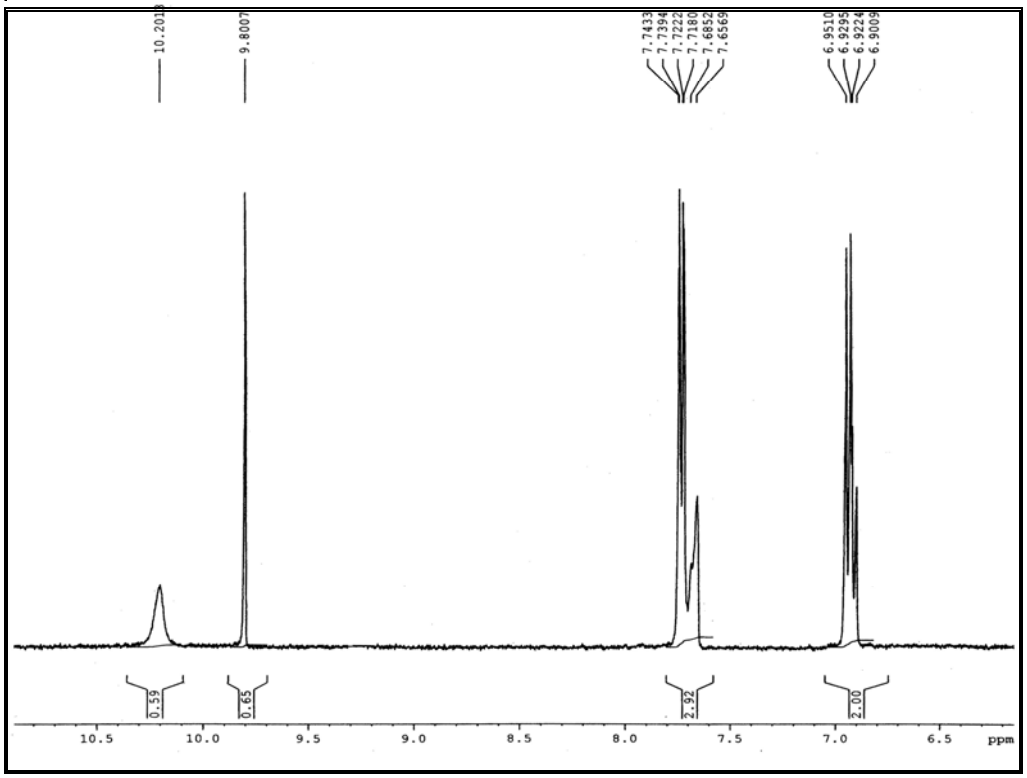
Expanded ^{1}H NMR spectrum of compound PVP-3Ac



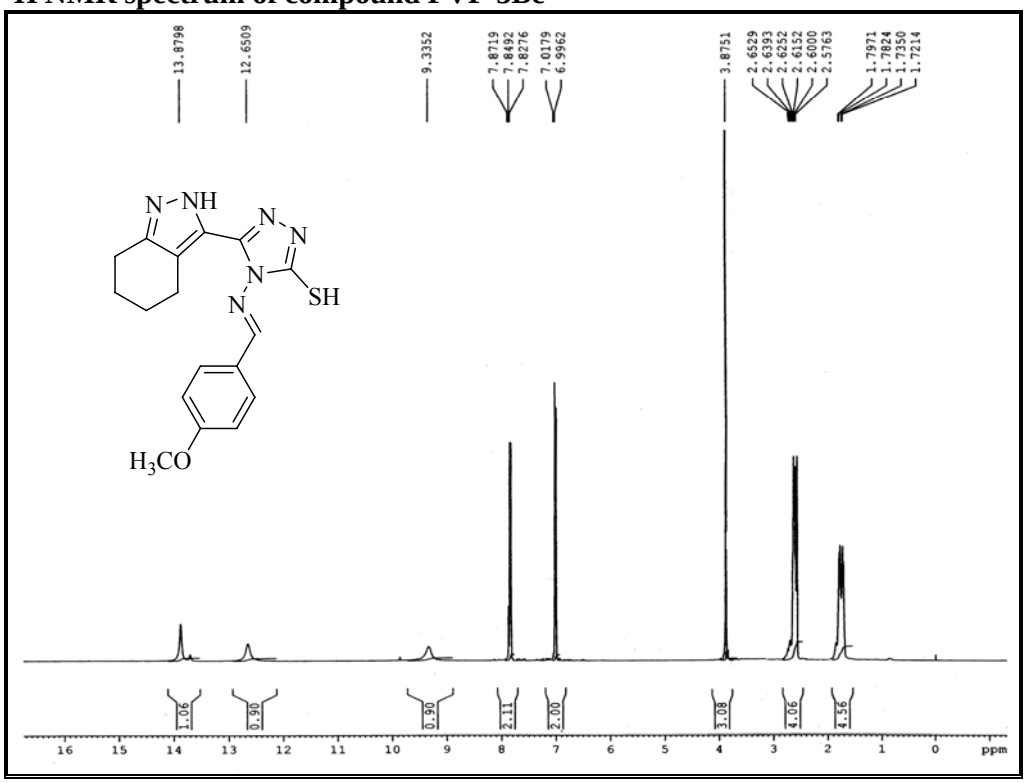
^{1}H NMR spectrum of compound PVP-3Bb



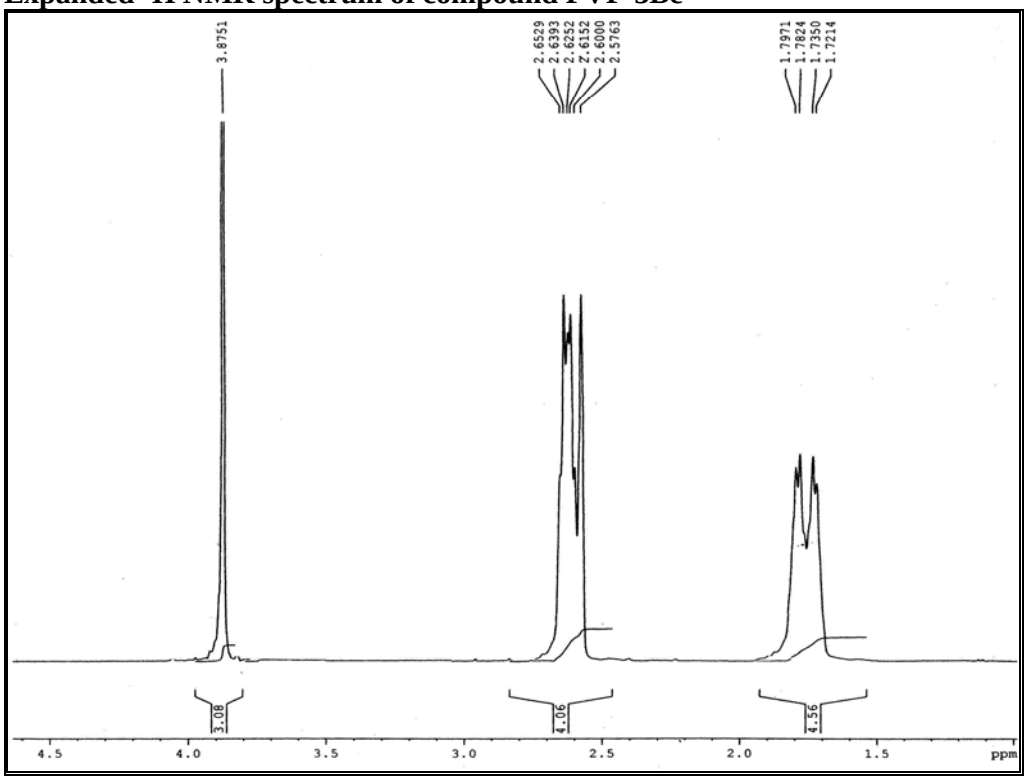
Expanded ^{1}H NMR spectrum of compound PVP-3Bb



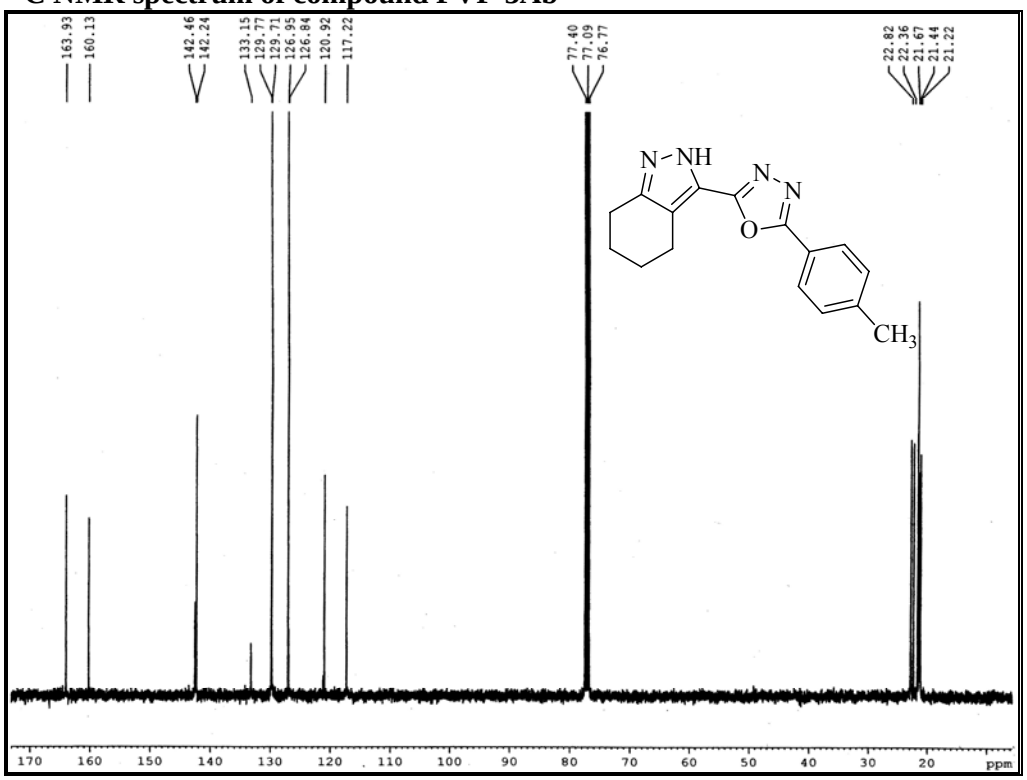
^{1}H NMR spectrum of compound PVP-3Bc



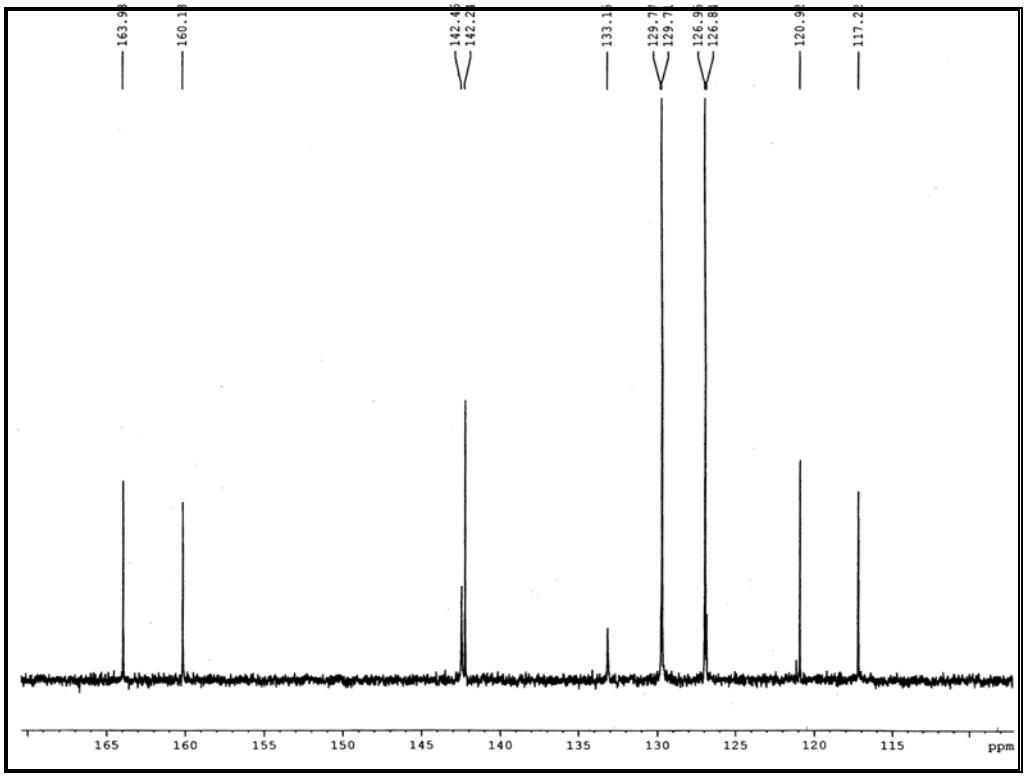
Expanded ^{1}H NMR spectrum of compound PVP-3Bc



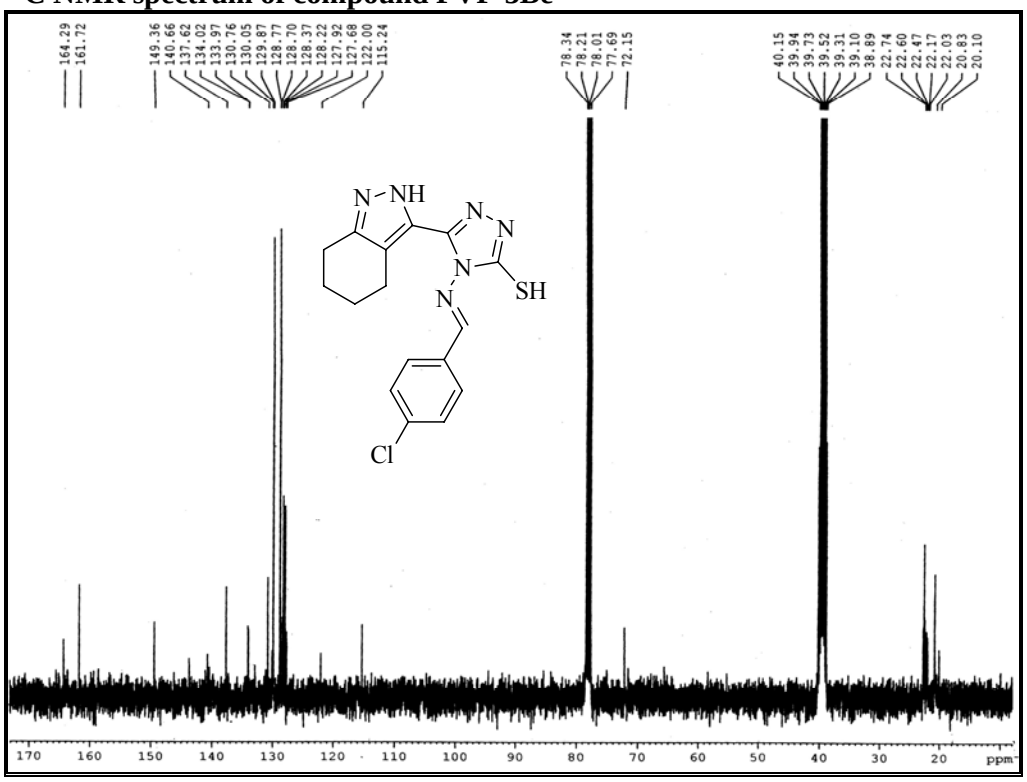
^{13}C NMR spectrum of compound PVP-3Ab



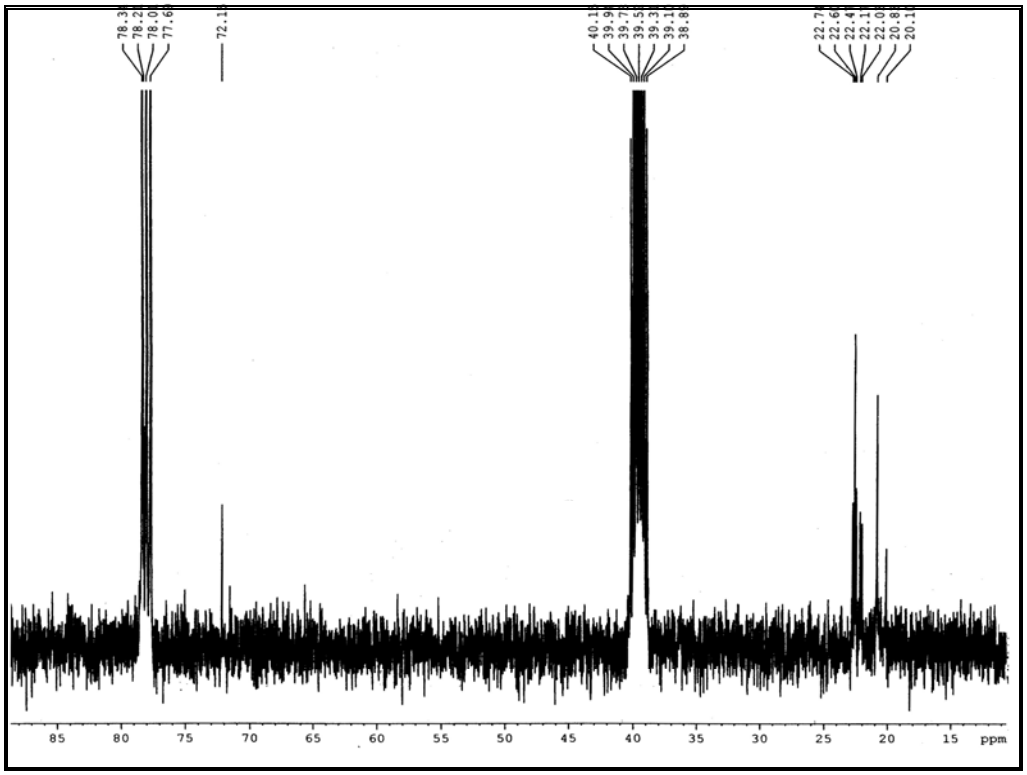
Expanded ^{13}C NMR spectrum of compound PVP-3Ab



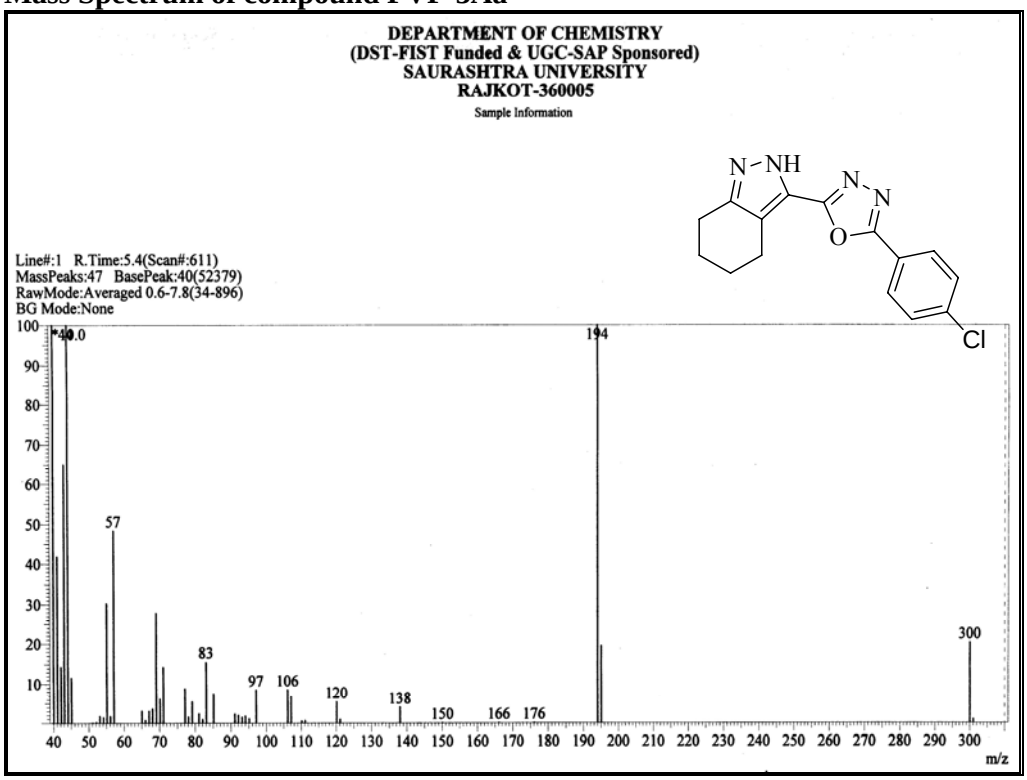
^{13}C NMR spectrum of compound PVP-3Be



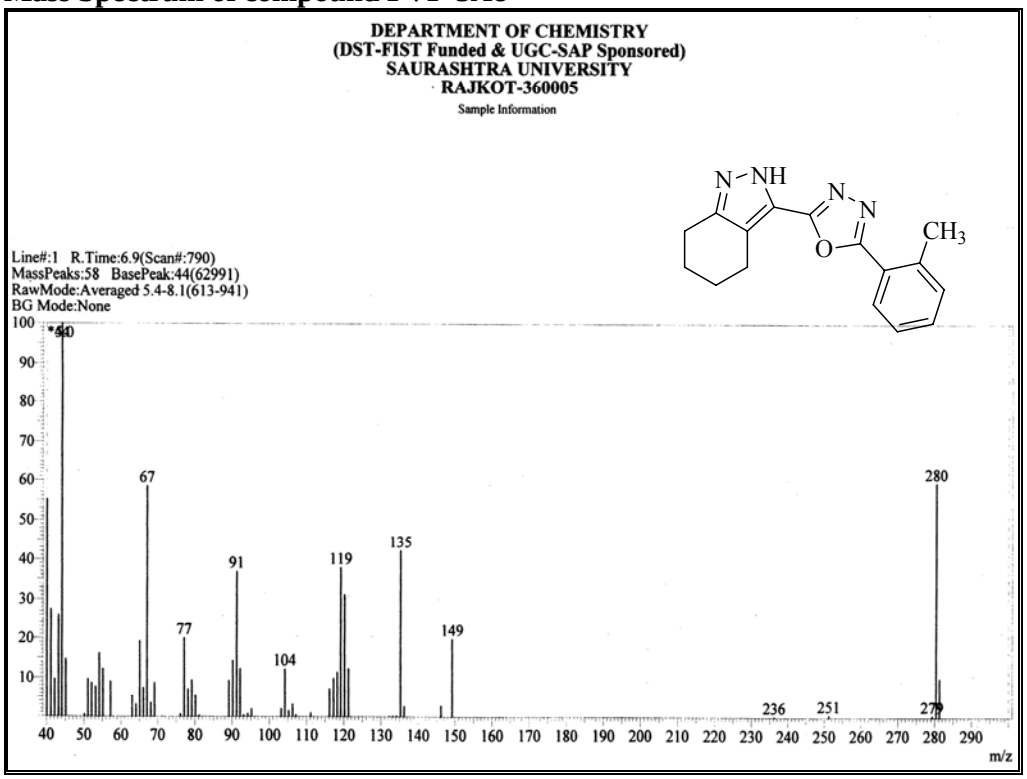
Expanded ^{13}C NMR spectrum of compound PVP-3Be



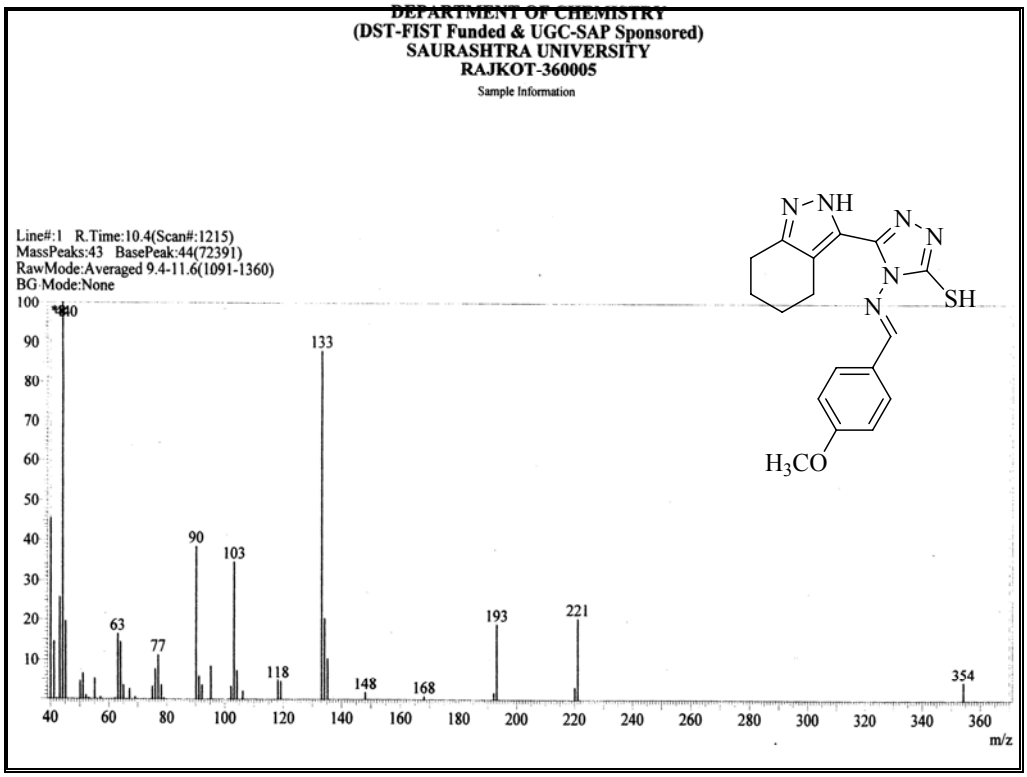
Mass Spectrum of compound PVP-3Aa



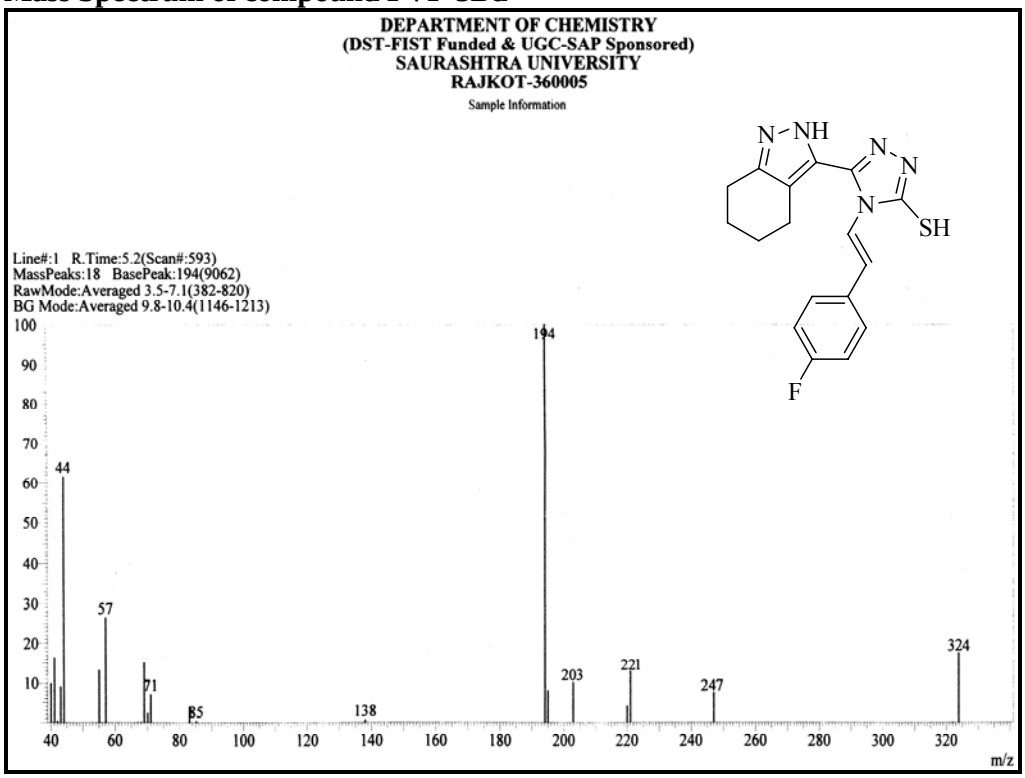
Mass Spectrum of compound PVP-3Ac



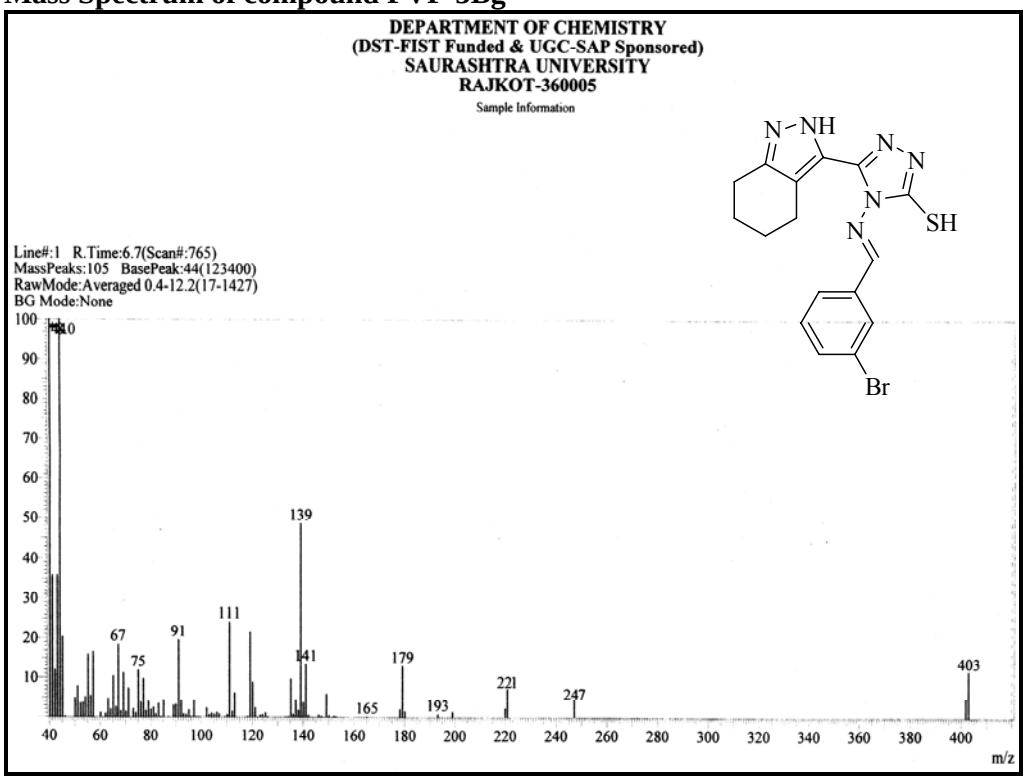
Mass Spectrum of compound PVP-3Bc



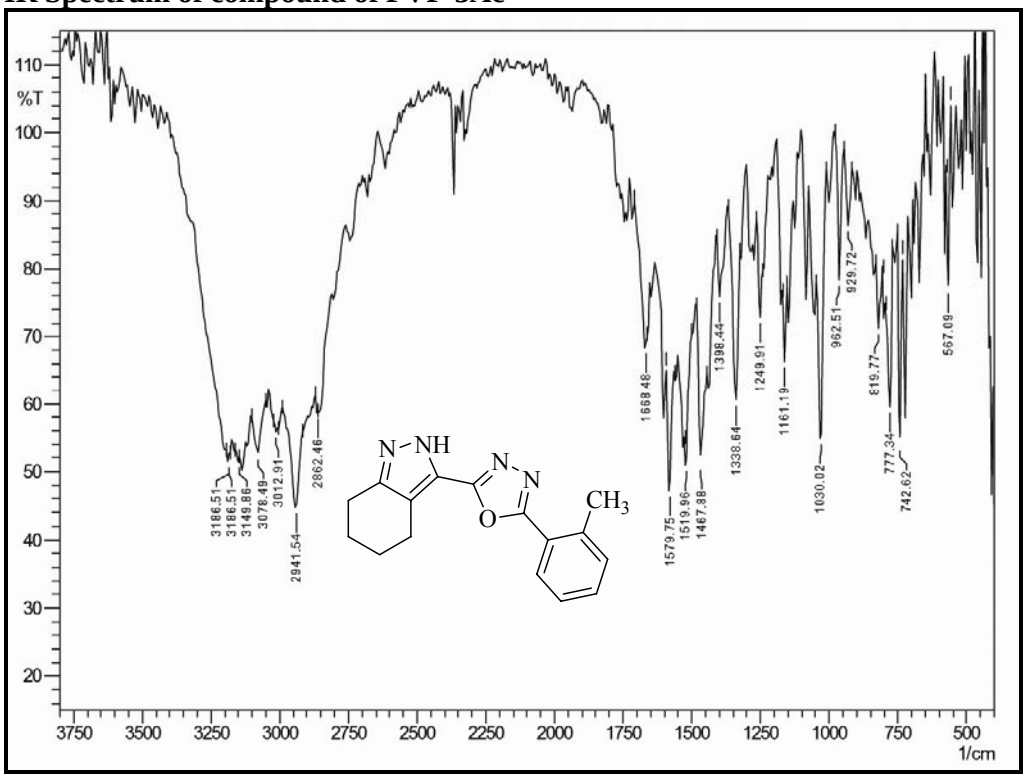
Mass Spectrum of compound PVP-3Bd



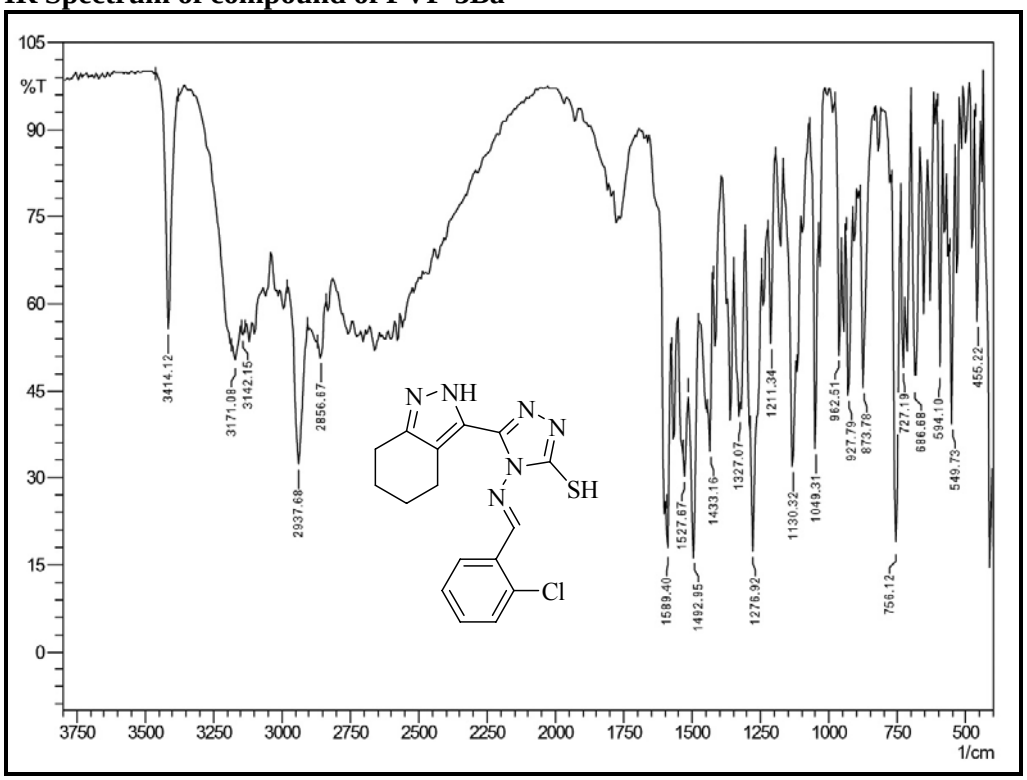
Mass Spectrum of compound PVP-3Bg



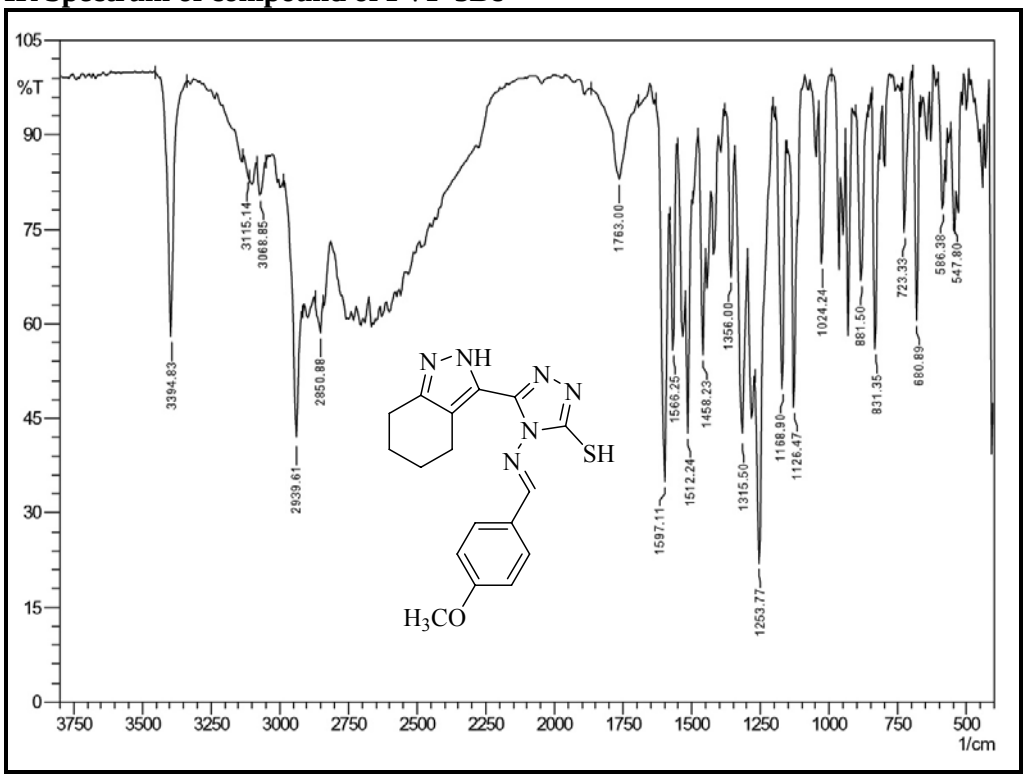
IR Spectrum of compound of PVP-3Ac



IR Spectrum of compound of PVP-3Ba



IR Spectrum of compound of PVP-3Bc



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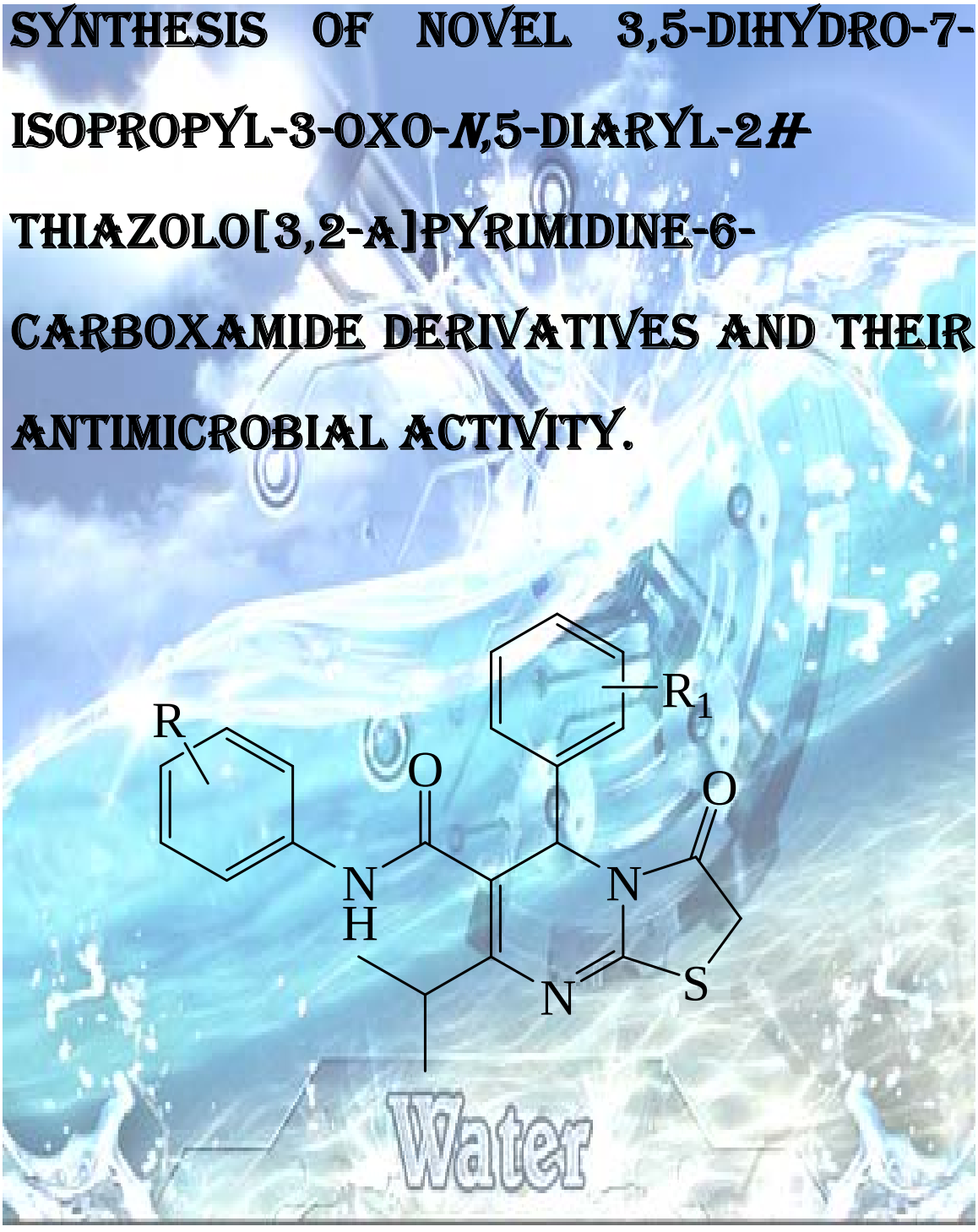
Chapter 4

SYNTHESIS OF NOVEL 3,5-DIHYDRO-7-ISOPROPYL-3-OXO-*N*,5-DIARYL-2*H*-THIAZOLO[3,2-A]PYRIMIDINE-6-CARBOXAMIDE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY.



4.1 INTRODUCTION

Biginelli P. reported the synthesis of functionalized 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) via three-component condensation reaction of an aromatic aldehydes, urea and ethyl acetoacetate **(Figure 1)**. In the past decade, this multicomponent reaction has experienced a remarkable revival, mainly due to the interesting pharmacological properties associated with this dihydropyrimidine scaffold.[1]

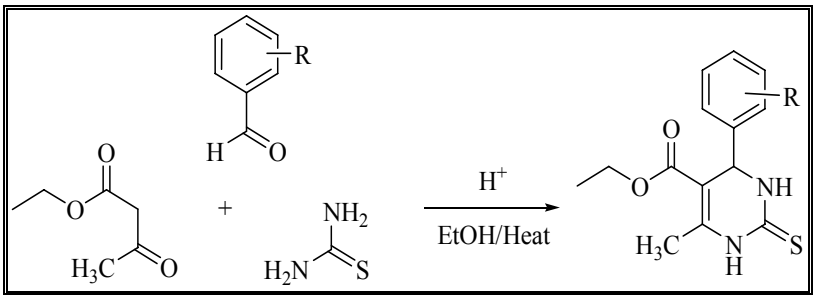


Figure 1

Biginelli reaction is not only important to synthesize analogs of DHPM ring using different building block as potent bioactive heterocycles, but diversed fused and non-fused heterocycles can be synthesized by careful applications**(Figure 2)**.

❖ **Various scaffolds derived from DHPMs**

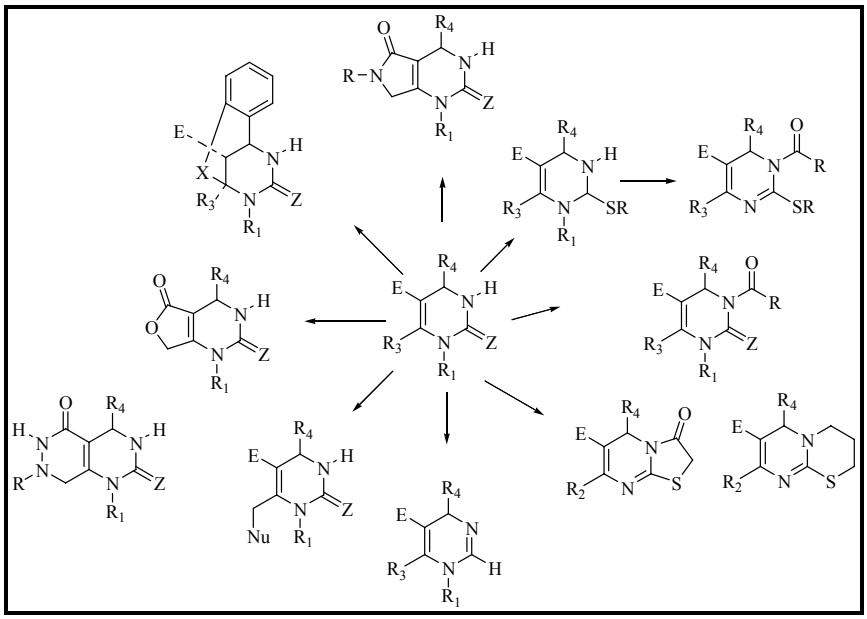


Figure 2.

As displayed in above figure, it can be understood that a number of new moieties can be generated from DHPM ring.

4.2 Pharmacological Profile

Kappe et al reported that *N*3-acylated DHPMs can be rapidly synthesized in a high throughput fashion by combining microwave-assisted acylations with microwave-assisted scavenging techniques. Scavenging experiments can be carried out employing either supported nucleophilic amine sequestration reagents or water.[2] *N*-acylated DHPMs are pharmacologically very important scaffolds as most of bioactive DHPMs are *N*-acylated. *N*-acylation of DHPM can be performed **(Figure 3)** as shown below.

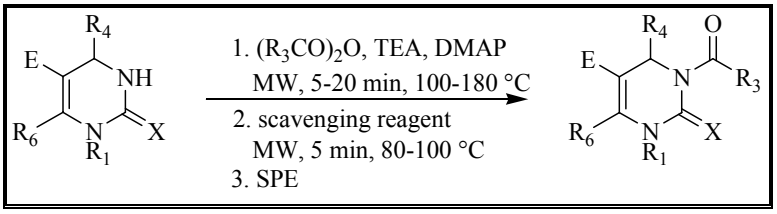


Figure 3

*N*3-Substituted DHPMs **(Figure 4)** have been identified to possess potent pharmacological profiles. Following compound exhibited high binding affinity and subtype selectivity for the cloned human R1a receptor.[3]

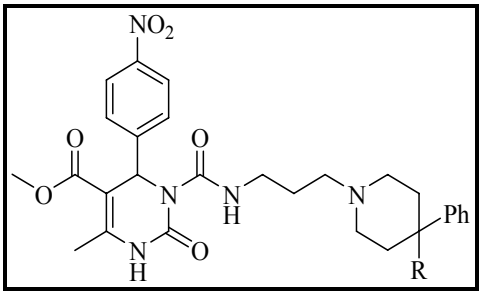


Figure 4

Systematic modifications of above compounds led to identification of highly potent and subtype-selective compounds with high binding affinity (*K*i) 0.2 nM) for R1a receptor and greater than 1500-fold selectivity over R1b and R1d adrenoceptors. The

compounds were found to be functional antagonists in human, rat, and dog prostate tissues **(Figure 5, 6)**.

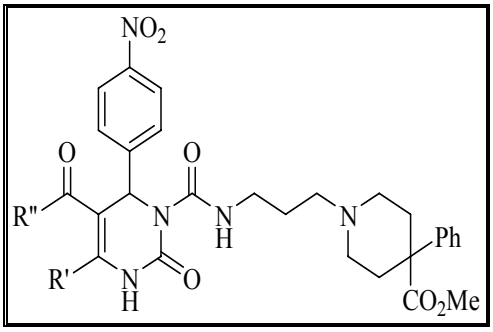


Figure 5

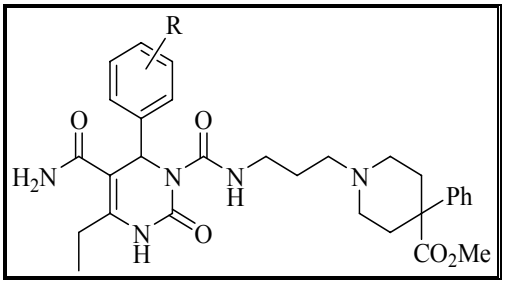


Figure 6

Modifications to the C5 position also play important role in potency of DHPM ring. 4-aryldihydropyrimidinones attached to an aminopropyl-4-arylpiperidine via a C-5 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. **(Figure 7)**. 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.[4]

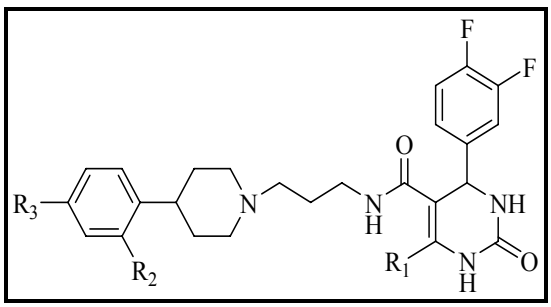


Figure 7

Mithun Ashok et al[5] have reported a new series of new 2-(arylidine)-5-(4-methylthiophenyl)-6-carboethoxy-7-methyl-5*H*-thiazolo[3,2-a]pyrimidine-3(1*H*)-ones. The newly synthesized compounds (**Figure 8)** were screened for their anti-bacterial and antifungal activities and have exhibited moderate to excellent growth inhibition of bacteria and fungi.

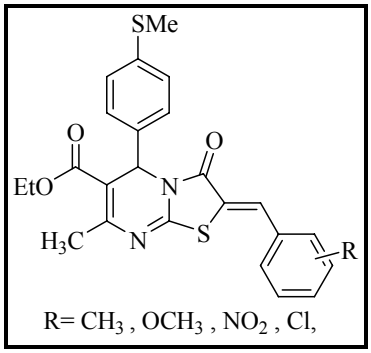


Figure 8

Hui Zhi et al[6] have developed a novel AchE inhibitors. A docking screening model of AchE inhibitor was used to evaluate a series of 5*H*-thiazolo[3,2-a]pyrimidine derivatives **(Figure 9)**. The virtual screening hits were analyzed in drug likeness and physic chemical features. Therefore were focused to those compounds. To investigate the relationship between the bioactivities and the structure, 10 target compounds with the 5*H*-thiazolo[3,2-a]pyrimidine scaffold were synthesized as potential AchE inhibitors.

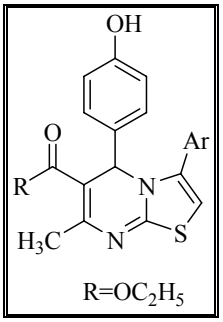


Figure 9

4.3 Alternative synthetic routes for better yield and shorter reaction time to synthesize new analogs

❖ Intramolecular Heck cyclization of DHPMs

The intramolecular Heck reaction can be observed in DHPM skeleton. The starting material for the intramolecular Heck reaction, DHPM was prepared by selective N3-acylation of 4-(*o*-bromophenyl)-dihydropyrimidone with acryloyl chloride[7] **(Figure 10)**.

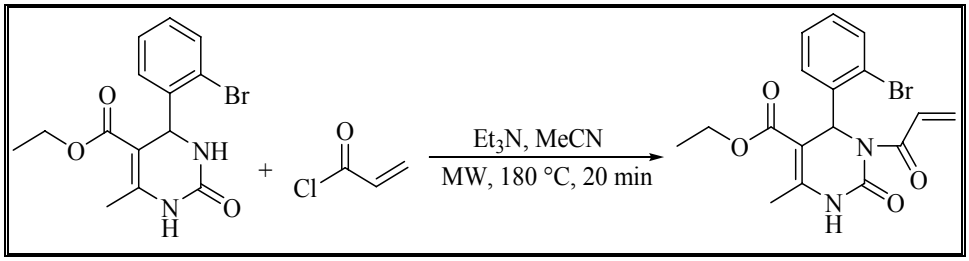


Figure 10

Applying intramolecular Heck reaction, tricyclic ring system can be obtained as shown below[8] **(Figure 11)**.

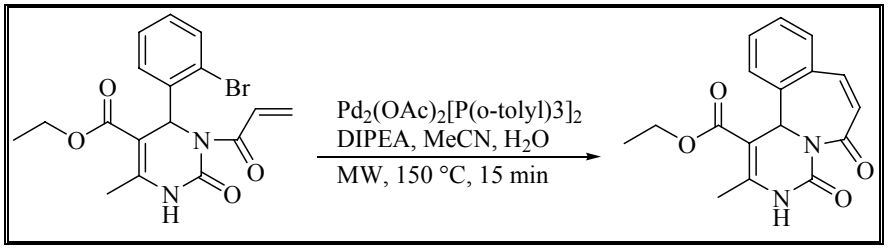


Figure 11

The computational experiments reveal that the formation of a tricyclic ring system did not flatten out the overall geometry. On the contrary, the aryl ring was still locked in a pseudoaxial position, resembling other nonfused 4-aryl-dihydropyrimidines.[9,10] In fact, here, **(Figure 12)** the intramolecular Heck strategy allows locking of the aryl ring in the proposed bioactive, that is, the pseudoaxial, orientation.[11]

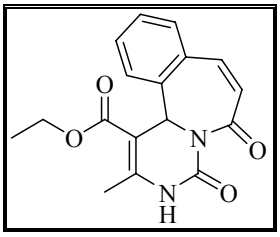


Figure 12

❖ **N3-Arylation of DHPMs**

N3-arylated DHPM analogues cannot be obtained by classical Biginelli condensation strategies involving *N*-arylureas. Here, the corresponding *N*1-substituted derivates will be formed exclusively.[12,13]

Wannberg et al reported protocol using concentrated mixture of 20 mol % of CuI as catalyst, 1.5 equiv of Cs_2CO_3 as base, and 5 mol equiv of DMF as solvent. The reactions were conducted at 180 °C for 40 min with a set of eight differently substituted aryl iodides **(Figure 13)**.

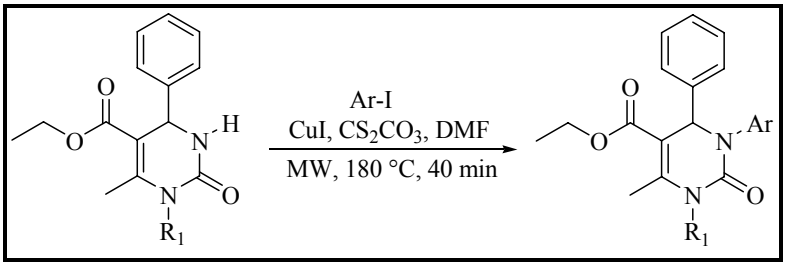


Figure 13

❖ **Bicyclic systems derived from DHPMs**

Many bicyclic systems can be synthesized from DHPM scaffold. Pyrazolo[4,3-d]pyrimidine derivatives synthesized by reacting sodium azide with *N*-Me, 6-Br-Me DHPM. The possible mechanism of this transformation is shown in below and involves decomposition of the diazide to vinyl diazo derivative, which undergoes spontaneous 1,5-electrocyclization to 3*H*-pyrazole. Subsequent migration of the ester substituent from the tetrahedral carbon to N2 (thermal van Alphen-Hüttel rearrangement) yields pyrazolo[4,3-d]pyrimidine. The structure confirming the position of the ester group at N_2 was established by an X-ray analysis.

Use of the 4-chloroacetoacetate building block in a Biginelli-type condensation is very useful to get variety of bicyclic systems. The resulting functionalized DHPM appeared to be an ideal common chemical template for the generation of a variety of interesting bicyclic scaffolds such as **(Figure 14,15)** furo[3,4-*d*]-pyrimidines, pyrrolo[3,4-*d*]pyrimidines, and pyrimido[4,5-*d*]pyridazines.[a]

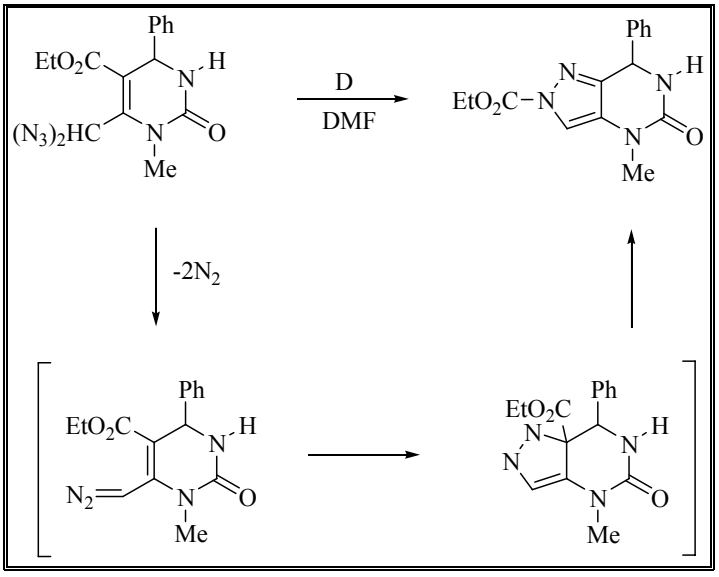


Figure 14

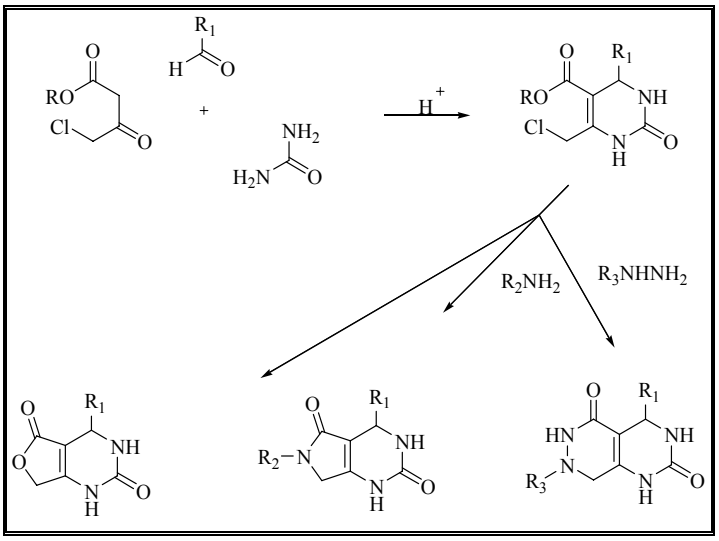


Figure 15

Solid-phase and solution-phase protocols for the synthesis of furo[3,4-*d*]pyrimidines, pyrrolo[3,4-*d*]-pyrimidines, and pyrimido[4,5-*d*]pyridazines are reported. The multistep solid-phase sequence involves the initial high-speed, microwave-promoted acetoacetylation of hydroxymethylpolystyrene resin with methyl 4-chloroacetoacetate. The immobilized 4-chloroacetoacetate precursor was subsequently subjected to threecomponent Biginelli-type condensations employing

urea and a variety of aromatic aldehydes. The resulting 6-chloromethyl-functionalized resin-bound dihydropyrimidones served as common chemical platforms for the generation of the desired heterobicyclic scaffolds using three different traceless cyclative cleavage strategies. The corresponding furo[3,4-*d*]pyrimidines were obtained by microwave flash heating in a rapid, thermally triggered, cyclative release. Treatment of the chloromethyl dihydropyrimidone intermediates with a variety of primary amines followed by high-temperature microwave heating furnished the anticipated pyrrolo[3,4-*d*]pyrimidine scaffolds via nucleophilic cyclative cleavage. In a similar way, reaction with monosubstituted hydrazines resulted in the formation of pyrimido[4,5-*d*]pyridazines. All compounds **(Figure 16)** were obtained in moderate to good overall yields and purities.[b]

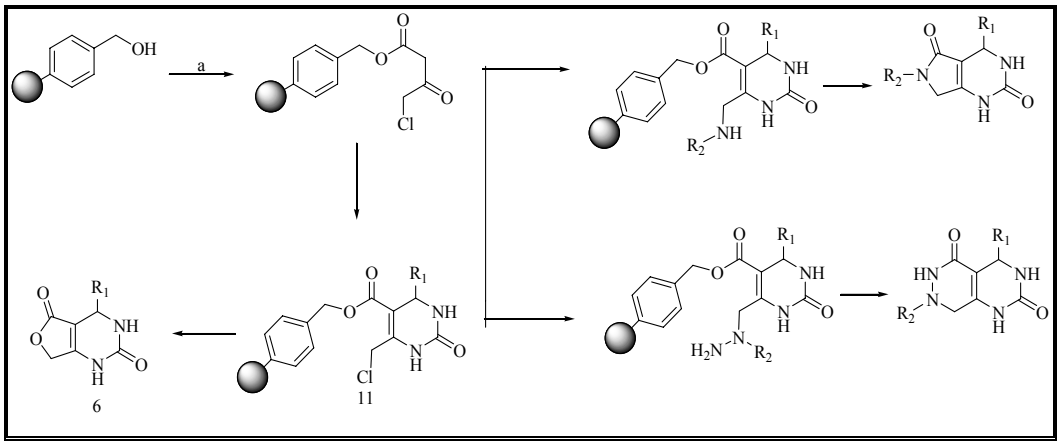


Figure 16

Preparation of thiazolo[3,2-a]pyrimidine derivatives is very well reported in literature. Two approaches is generally employed for synthesis.

❖ **Azole approach:**

Various methods for **(Figure 17)** synthesis of thiazolo[3,2-a]pyrimidine derivatives using thiazole as starting material.

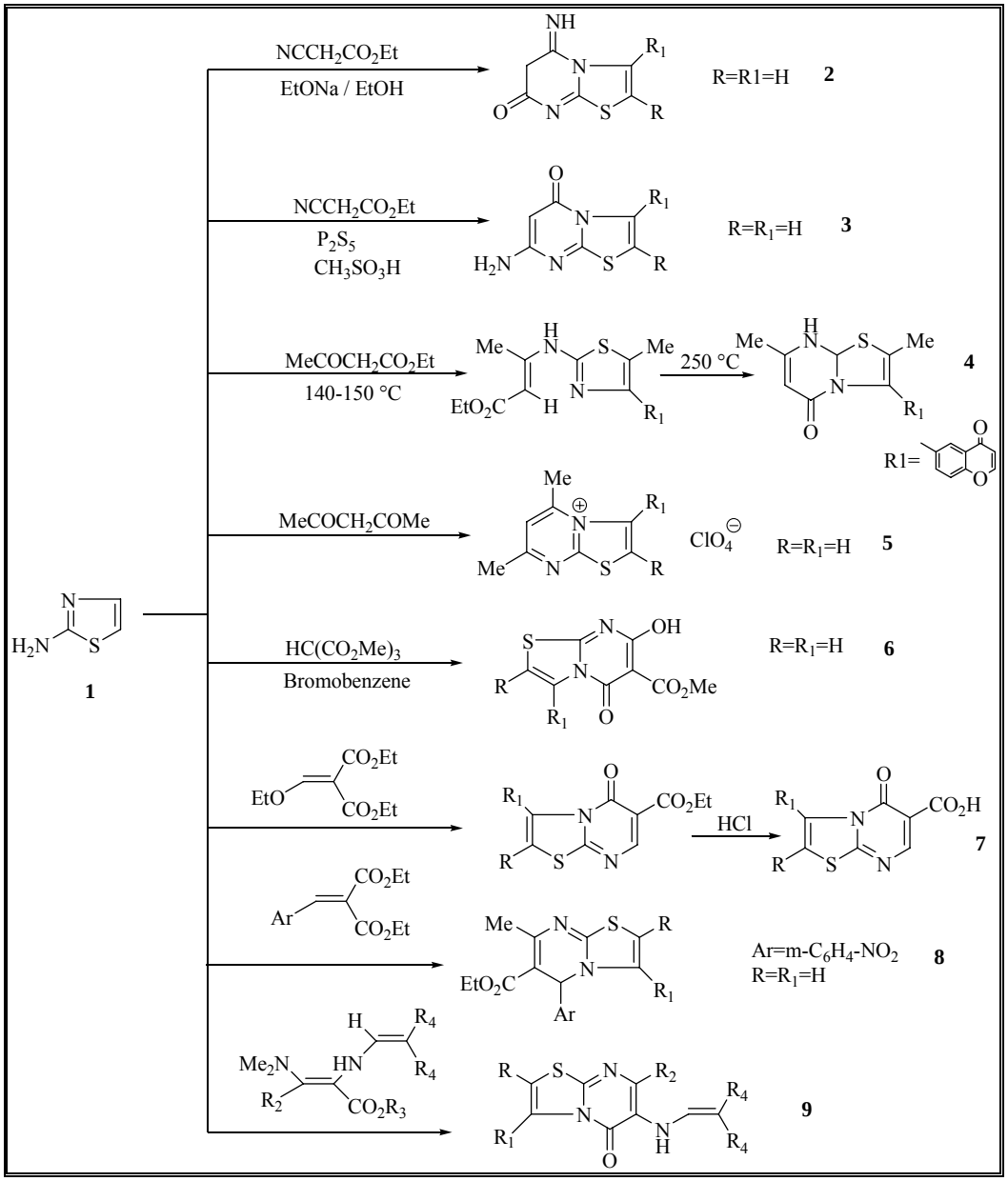


Figure 17

Literature survey on synthetic methodology for thiazolo[3,2-a]pyrimidine derivatives can be summarized in chart 1 & 2 where various methods are illustrated for synthesis of this class of compounds.

Thiazolo[3,2-a]pyrimidine **2** was prepared in 30% yield by the reaction of 2-aminothiazole **1** with ethyl cyanoacetate in a sodium ethoxide/ethanol mixture or using polyphosphoric acid or acetic acid. However, oxothiazolopyrimidine **3** was obtained upon treatment with phosphorous pentoxide and methanesulfonic acid.

The reaction of **1** with ethyl acetoactate at 140-150°C resulted in the formation of compound that was then converted to the Z-isomer upon heating at 250°C and cyclized to give **4**. 2-Aminothiazole **5** cyclized with acetylacetone at l00"C, in the presence of methanesulfonic acid-phosphorus pentoxide or formic acid-phosphorus pentoxide, followed by treatment with 70% perchloric acid, to give the thiazolopyrimidin-4-ium salt **5**. The ester **6** was obtained from 2-aminothiazole **1** with an excess of methyl methanetricarboxylate in 61 % yield. Cyclocondensation of **1** with diethyl ethoxymethylene malonate in acetic acid followed by hydrolysis of the ester gave **7.** Similarly, 2-aminothiazole **1** reacted with benzylidine in ethanol to give **8**. Stanovink *et al.,* [13-171 reported the synthesis of a series of thiazolopyrimidine derivatives upon reacting 2-aminothiazole with a variety of different reagents. Thus, dimethylaminobut- 2-enoate (or pentenoate), reacted with **1** to give thiazolopyrimidines **23.**[14-27]

The reaction of 2-aminothiazole **1** with 2-hydropolyfluoroalk-2-enoate in basic medium gave two isomers, 7-oxo **2** and its isomeric 5-oxo **3. (Figure 18)** The structure of both **2** and **3** was established through ^{1}H NMR, ^{13}C NMR and mass spectra[28]**.** 2-Aminothiazole derivatives**,** (R' = H**,** CO_2Et; R_2 = Ph, aryl, Me), reacted with the acetylenic derivative and ester derivative in ethanol and polyphosphoric acid, respectively, to give the isomeric oxothiazolopyrimidine derivatives **4** and **5,** in 5-32% and 8-97 % yield, respectively[29]. Condensation of 2-aminothiazole **1** in absolute ethanol with the sodium salt of ethyl oximinocyanoacetate gave after acidification (pH 6) with diluted hydrochloric acid, the nitroso derivative **6** in 92% yield[30]. Treatment of the 2-aminothiazaole derivatives **5** with the hydrazone derivatives gave the oxothiazolo [3,2-a] pyrimidine derivatives **7.**[31]

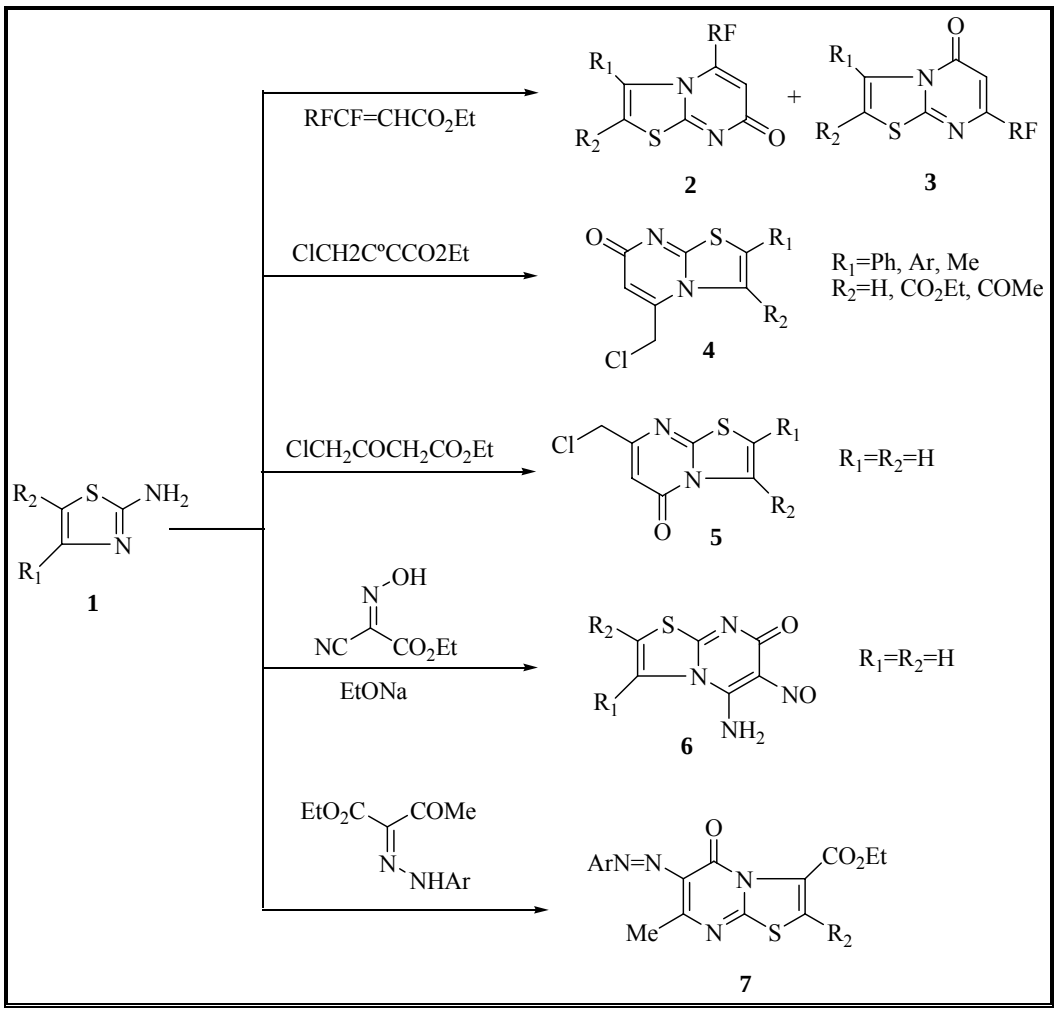


Figure 18

2-Amino-2-thiazoline reacted with 2-acylamino-3-dimethylamino-propenoates in acetic acid to yield 6-acylamino-5-oxo-2,3-dihydro-5-thiazolo[3,2-a]pyrimidines in 73 and 12% yields, respectively[32] **(Figure 19)**.

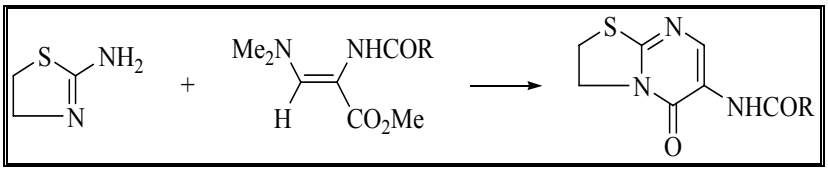


Figure 19

Moreover, 2-amino-2-thiazoline reacted with an aromatic aldehyde and diethyl malonate, to give **(Figure 20)** a mixture of thiazolidino[3,2-a]pyrimidines. Furthermore, malononitrile reacted to give following product.[33-34]

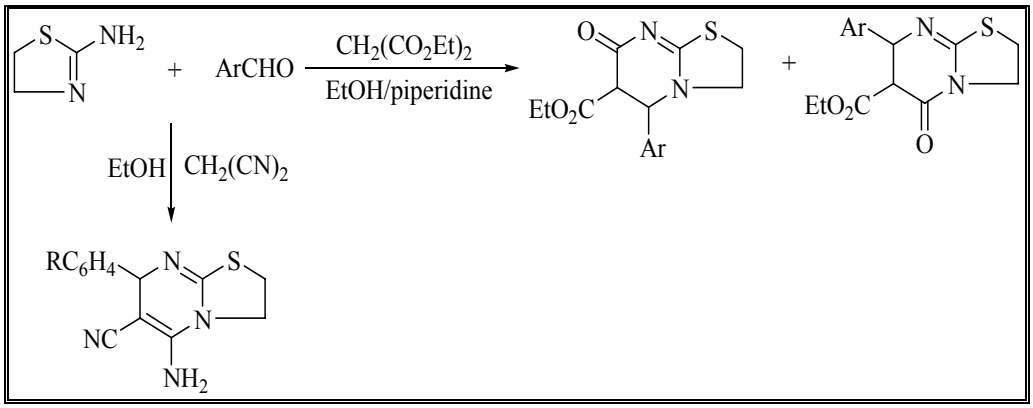


Figure 20

2-Amino-2-thiazoline reacted with potassium 2-ethoxycarbonyl-2-fluorovinyl alcoholate in a sodium methoxide/methanol mixture to give 6-fluoro-2,3-dihydro-5-oxothiazolo[3,2-a]pyrimidine.[35] **(Figure 21)**

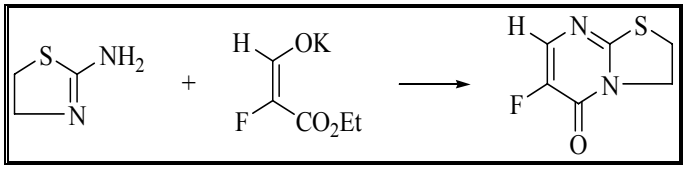


Figure 21

2-(Methylthio)-2-thiazoline reacted with /3-alanine to give a 5-oxothiazolo[3,2-a]-pyrimidine **(Figure 22)** derivative in 23% yield.[36]

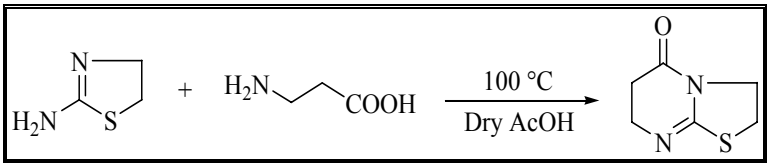


Figure 22

❖ **Azine approach**

Pyrmidinethione derivatives were alkylated with monochloroacetic acid or chloroacetyl chloride and then cyclized to give thiazolopyrimidine derivatives.[37-50] Thus, pyrimidinethione reacted in DMF[37] or in an acetic anhydride/pyridine mixture[39] to give thiazolo-pyrimidines **(Figure 23)**. Alkylation in the presence of an aromatic aldehyde gave the ylidene. Similarly, pyrimidinethione derivatives reacted with monochloroacetic acid in acetic acid/acetic anhydride/sodium acetate mixture or with chloroacetyl chloride in dry dioxane to give the corresponding thiazolopyrimidines.[41-42]

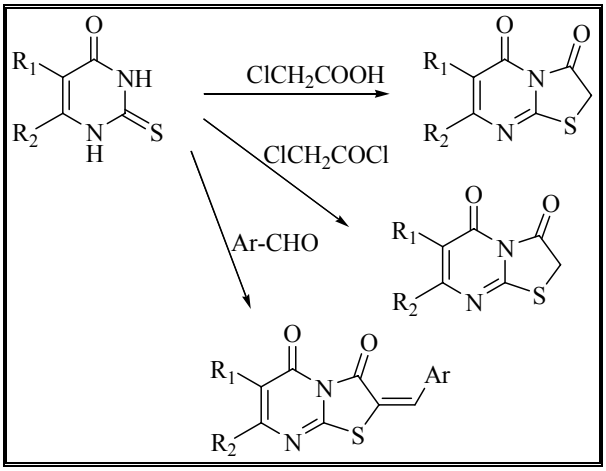


Figure 23

Treatment of mercaptopyrimidine derivative with 2-chloroethanol in DMF gave the asymmetrical thioether which underwent cyclization on refluxing with a mixture of acetic anhydride-pyridine, to give the oxothiazolopyrimidine.[51] **(Figure 24)**

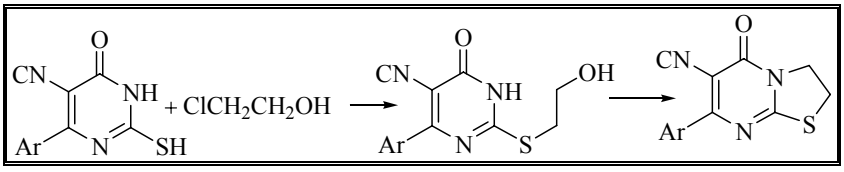


Figure 24

1,3-dibromopropan-2-ol reacted with mercaptopyrimidine derivative to give product through the non isolated intermediates**. (Figure 25)** The same reaction product was obtained by reacting with I-bromomethyloxirane.[52]

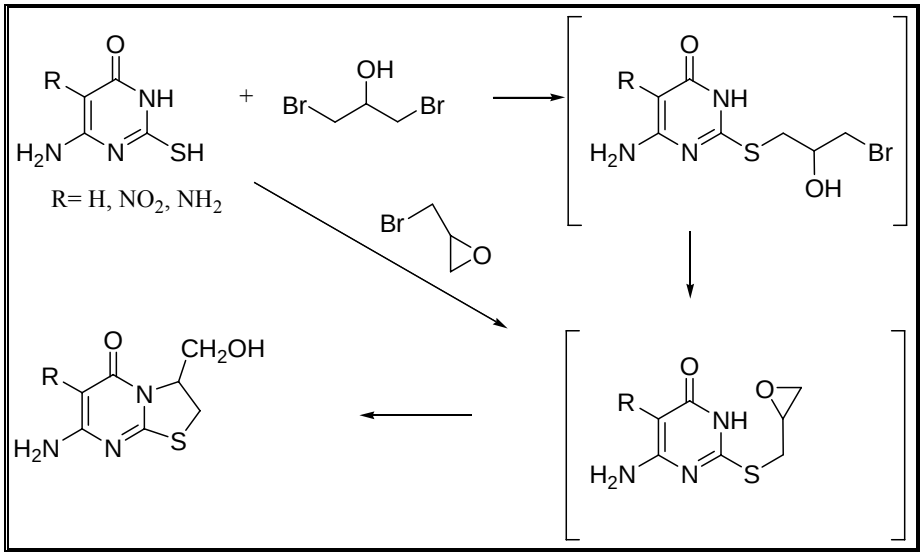


Figure 25

Several derivatives of 4,5-disubstituted imidazole, 2,4,5-trisubstituted pyrimidine, 2-substituted purine, thiazolo[3,2-*a*]purine, [1,3]thiazino[3,2-*a*]purine, thiazolo[2,3-*i*]purine, [1,3]thiazino-[2,3-*i*]purine, and 6-substituted pyrazolo[3,4-*d*]pyrimidine were **(Figure 26,27,28)** synthesized and tested as inhibitors of the xanthine oxidase enzyme[53].

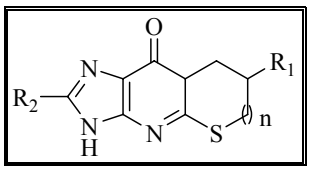


Figure 26

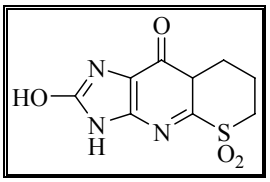


Figure 27

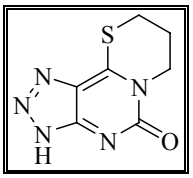


Figure 28

Dihydropyrimidines are well-known calcium channel blockers. According to the literature analogous derivatives are anti-inflammatories. Thus Bo´szing and co-workers decided to synthesize the pyrimidothiazines and assay these compounds for the same profile. Acute anti-inflammatory activity was tested by inhibition of the carrageenan-induced paw edema in rats.[54] **(Figure 29)**

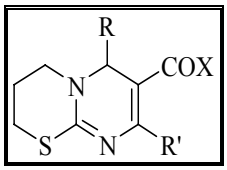


Figure 29

Adam et al filed US patent for phenyl substituted thiazolo pyrimidine derivatives synthesized from DHPM. **(Figure 30)** These compounds and their slats are novel and are distinguished by valuable therapeutic properties. Specifically it has been found that the compounds of general formula given below are metabotropic glutamate receptor antagonists. These compounds are capable of high affinity binding to group II mGluR receptors.[55]

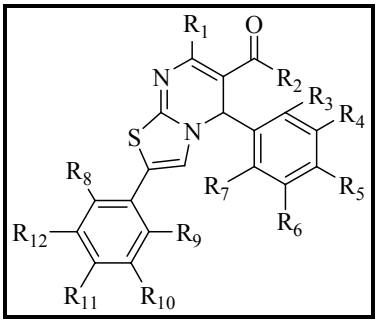


Figure 30

Compounds displayed by general formulae given below exhibit excellent adenosine A_3 receptor antagonism **(Figure 31)** where A is an optionally substituted benzene ring. B may be substituted and R_1 is optionally substituted cyclic group.[56]

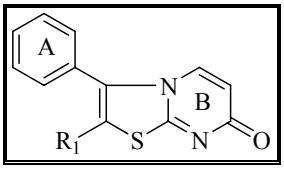


Figure 31

Amr, A. E. G. E. and Maigali, S. S. described the analgesic and antiparkinsonian activity of some thiazolopyrimidine derivatives as shown below. **(Figure 32)** Out of them compound of type **III** are potent antiparkinsonian agents.[57]

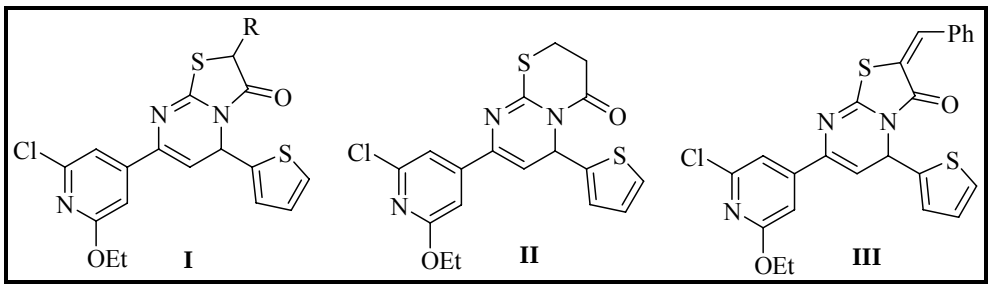


Figure 32

CDC25 phosphatases play critical roles in cell cycle regulation and are attractive targets for anticancer therapies. Several small non-peptide molecules are known to inhibit CDC25, but many of them appear to form a covalent bond with the enzyme or act through oxidation of the thiolate group of the catalytic cysteine. Matthieu Montes et al reported thiazolopyrimidine structure based compound **(Figure 33)** as CDC25 phosphatases inhibitor.[58]

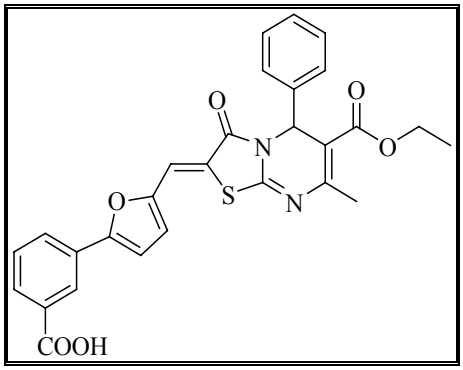


Figure 33

Helmut M. Hugel et al[59] **(Figure 34)** have been developed the application of multicomponent reaction involving the combination of multiple starting materials with multiple functional groups leading to the higher efficiency and environmentally friendly construction of target molecules.

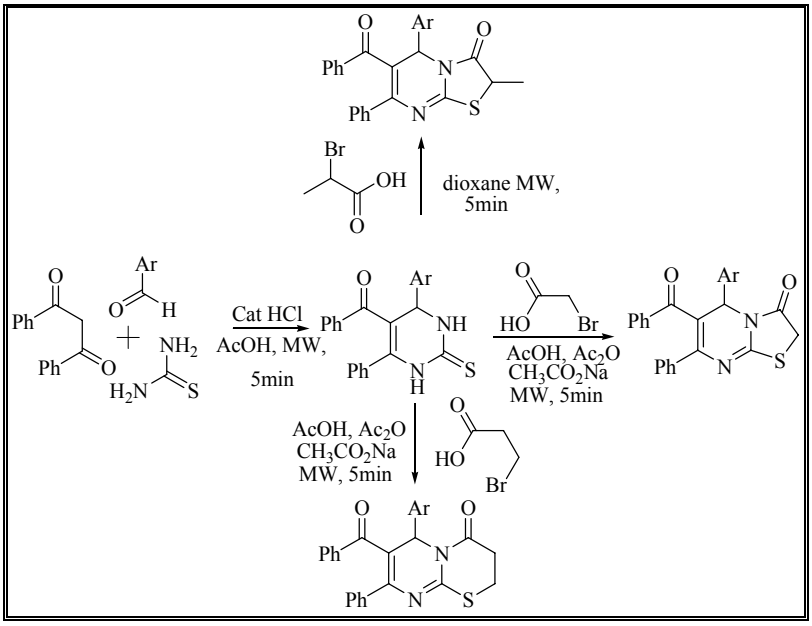


Figure 34

2-thioxopyrimidine derivatives were prepared by the Biginelli reaction protocol thus the 5 min MW irradiation of a 1,3-diphenyl-1,3-propanedione, arylaldehyde and thiourea in gl. acetic acid plus a few drops of cons. HCl gave the products in 75%-80% yields. The 2-thione DHPM were transformed into thiazolopyrimidines and pyrimidothiazine derivatives with bromo acids and MW irradiation. When compared to conventional heating the MW technology completed the two step synthesis much faster.

I.V. Kulakov et al[60] have synthesized thiazolo[3,2-a]pyrimidines by the reaction of 4-aryl-substituted 3,4-dihydropyrimine(1H)-2-thiones and methyl chloroacetate in boiling toluene afford target molecule **(Figure 35)** in good yield. Their structures were shown by ^{1}H NMR and X-ray crystallography.

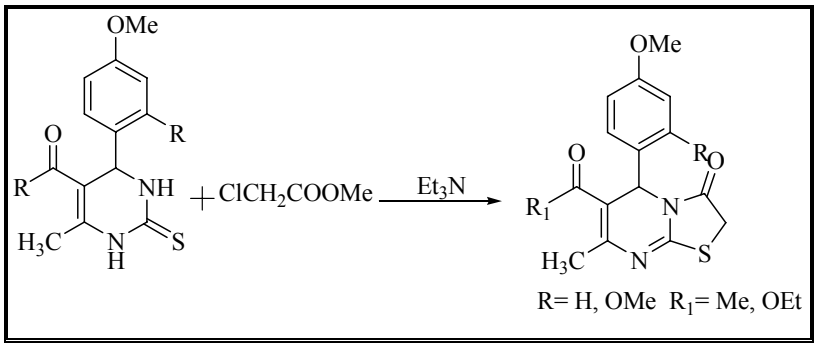


Figure 35

4.4 CURRENT RESEARCH WORK

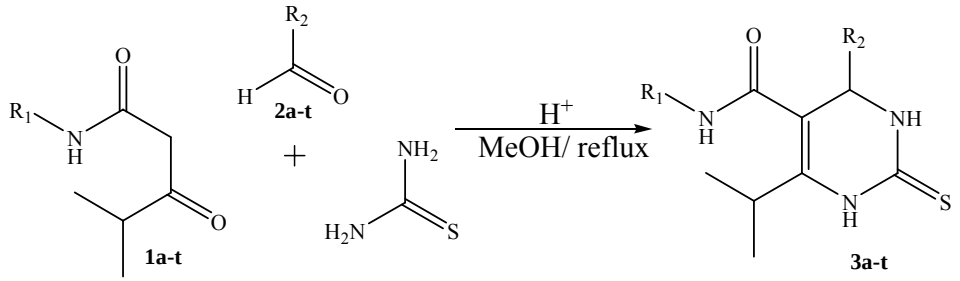
Importance of dihydropyrimidine ring to develop variety of bicyclic systems briefly surveyed in section 4.1. Reports reveals that N_3-substitution in dihydropyrimidine ring is enhance therapeutic activity profile. Similarly, substitutions at C_5 position may pay key role in activity profile. Thus, we have synthesized pyrimidine derivatives containing phenyl carbamoyl at C_5 position. Further, thiazolo pyrimidine derivatives phenyl carbamoyl moiety at C_5 position are synthesized and characterized.

Thiazolo pyrimidine and pyrimido thiazine are very important bicyclic system in medicinal chemistry. Various synthetic routes have been reported in literature to synthesize these bicyclic systems. Utility of dihydropyrimidine ring to synthesize such bicyclic system can be used to obtain derivatives with phenyl carbamoyl as side chain on pyrimidine ring of bicyclic system.

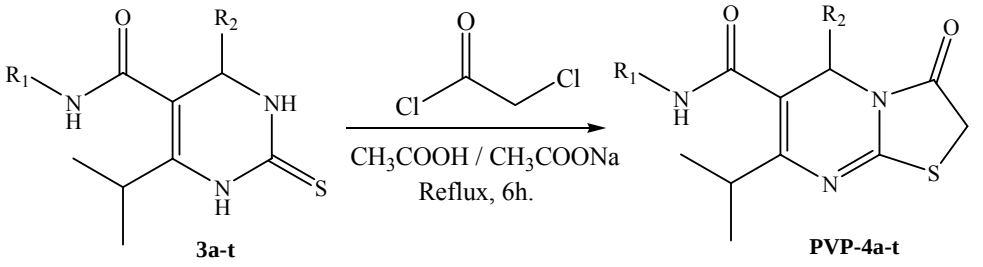
Dihydropyrimidine ring, substituted with phenyl carbamoyl side chain at C_5 position, was synthesized by reacting acetoacetanilide, thiourea and aldehyde. This dihydropyrimidine ring was reacted with dihalo ketone to get fused bicyclic systems. The synthesis of thiazolo pyrimidine derivatives achieved by the reaction of 2-thiodihydropyrimidine with chloro acetyl chloride. Various solvents were utilized as reaction media to get better results. Among them glacial acetic acid with sodium acetate as catalytic amount was succeeded to give better yield with shorten reaction time and easy in isolation of product. All the synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy. All the synthesized compounds were evaluated for their anti microbial activity.

4.5 RESULTS AND DISCUSSION

Scheme:-1 Synthesis of substituted Thiazolopyrimidines.



Scheme:-2



R_1, R_2 = CH_3, F, Cl, Br, OCH_3

Initially, the reaction of 4-methyl-3-oxo-*N*-arylpentanamide **1a-t** with appropriate aldehyde **2a-t** and thiourea in MeOH. Few drops of con. HCl were added to reaction mixture as a catalyst and was refluxed for 8-12 h. **(Scheme 1)** gives 1,2,3,4–tetrahydro-6-isopropyl-*N*,4-diaryl-2-thioxopyrimidine-5-carboxamide **3a-t,** When **3a-t** was reacted with chloro acetyl chloride **(scheme 2)** affords the 3,5-dihydro-7-isopropyl-3-oxo-*N*,5-diaryl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide derivatives **PVP-4a-t** was obtained in excellent yield.

The structures of **4a-t** were established on the basis of their elemental analysis and spectral data (MS, IR, and ^{1}H NMR). The analytical data for **3e** revealed a molecular formula $C_{22}H_{25}N_3OS$ (*m/z* 379). The ^{1}H NMR spectrum revealed a two singlet at δ = 1.45-1.62 ppm assigned to isopropyl-CH_3, a singlet at δ = 2.24 - 2.26 ppm assigned to the – (2xCH_3) protons, a multiplet at δ = 3.85 ppm assigned to the isopropyl-CH protons , a doublet at δ = 4.76 - 4.77 ppm assigned to the -CH protons, a multiplet at δ = 7.10 - 7.48 ppm assigned to the aromatic protons, a doublet at δ = 8.86 - 8.87ppm

assigned to –NH protons, a singlets at δ = 8.93 ppm assigned to –NH protons, and one broad singlets at δ = 9.73 ppm assigned to -CONH groups.

Table 1: Synthesis of substituted Thiazolopyrimidines.

Entry	R_1	R_2	Yield %	M.P.
PVP-4a	3-Cl,4-FC_6H_3	4-ClC_6H_4	90	260-262
PVP-4b	4-ClC_6H_4	4-ClC_6H_4	91	258-261
PVP-4c	4-FC_6H_4	4-ClC_6H_4	84	245-247
PVP-4d	4-$OCH_3C_6H_4$	4-$OCH_3C_6H_4$	90	243-245
PVP-4e	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	86	266-268
PVP-4f	4-$OCH_3C_6H_4$	4-FC_6H_4	90	268-270
PVP-4g	3-Cl,4-FC_6H_4	C_6H_5	90	238-240
PVP-4h	C_6H_5	3,4-di- $OCH_3C_6H_3$	86	248-250
PVP-4i	4- BrC_6H_4	3,4-di- $OCH_3C_6H_3$	90	256-258
PVP-4j	4-ClC_6H_4	4-$OCH_3C_6H_4$	91	236-238
PVP-4k	4-FC_6H_4	4-FC_6H_4	88	226-228
PVP-4l	C_6H_5	C_6H_5	92	256-258
PVP-4m	4-ClC_6H_4	4-$CH_3C_6H_4$	90	264-266
PVP-4n	2,5-di-$CH_3C_6H_3$	4-OHC_6H_4	87	239-241
PVP-4o	3-ClC_6H_4	4-$CH_3C_6H_4$	85	241-243
PVP-4p	4- BrC_6H_4	4-FC_6H_4	93	258-261
PVP-4q	4-$OCH_3C_6H_4$	4-$NO_2C_6H_4$	90	250-252
PVP-4r	4-ClC_6H_4	4-OHC_6H_4	89	256-257
PVP-4s	4-FC_6H_4	2-OHC_6H_4	85	248-250
PVP-4t	3-ClC_6H_4	3-ClC_6H_4	90	246-248

The mechanism for the formation of DHPM involves acid catalyzed formation of an N- acylinium (**4**) ion intermediate from the aldehyde (**1**) and thiourea (**2**) precursors. Interception of the iminium ion by acetoacetanilidie, presumably through it's active methylene produce an open chain uride (**5**) which subsequently cylices to hexahydropyrimidine (**6**). Acid catalyzed elimination of water from ultimately leads to the DHPM product (**7**).

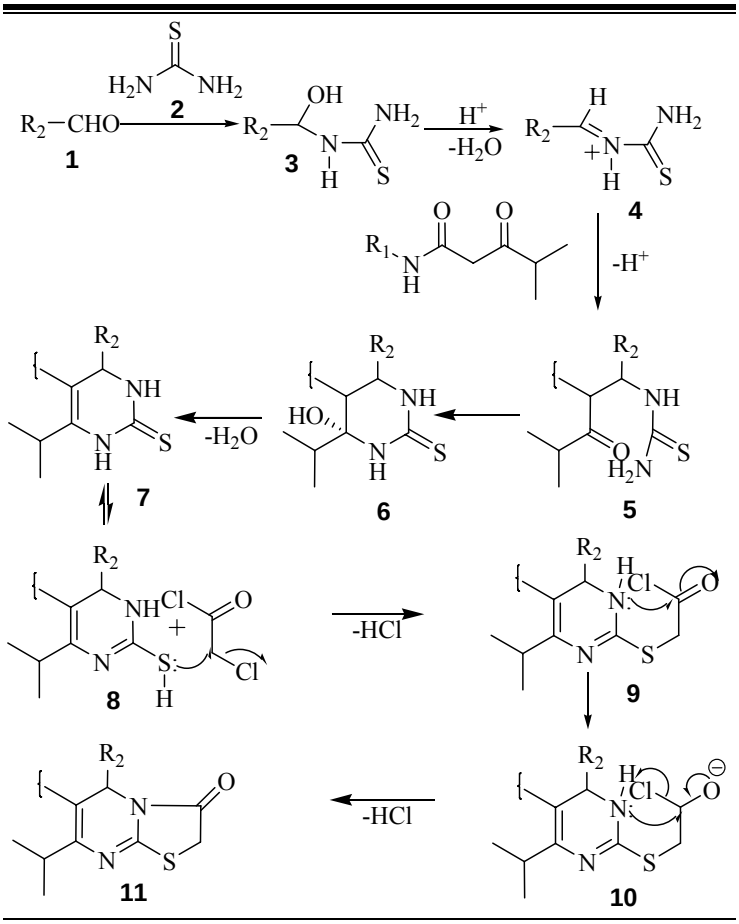


Figure 36: Proposed mechanism for the formation of Thiazolopyrimidine.

The thio atom act as neucleophile and attack on methylene of chloro acetyl chloride and form **(9)** like product, which on acid catalyzed elimination of HCl leads the final compound thiazolopyrimidine **(11).**

4.6 ANTIMICROBIAL SENSITIVITY TESTING

WELL DIFFUSION / AGAR CUP METHOD (Lt. General Raghunath D. 1998, Ashok Rattan, 1998; Patel R., Patel K. 2004,)

In vitro effectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective then many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive then using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Requirements

1. Young broth culture of a standard test organism
2. Sterile Mueller Hinton Agar plate
3. Solution of antimicrobial substance
4. Cup borer
5. Alcohol etc.

Inoculum preparation

Inoculum was prepared by selecting 4-5 colonies from slope of stock culture of the indicator organism and emulsifying them in a suitable broth. The inoculated broth was incubated at 37ºC till it equals turbidity of a 0.5 McFarland standard. This happens in 2-8 h.

Procedure

1. Inoculate test organism on the top of Mueller Hinton Agar plate with help of sterile swab. (it can be inoculated in melted agar also)
2. The swab was dipped in the inoculum and surface of plate was streaked with swab.
3. Streaking was repeated for 3 times and each time the plate was rotated at angle of 60º.
4. Sterilize the cup-borer make four cups of the diameter of 8-10 mm. at equal distance in the plate previously inoculated with seed culture.
5. The depth of well was 2.5-5.0 mm.
6. The wells have been clearly punched so the surrounding medium is not lifted when the plug was removed out.
7. The plates were incubated at 37ºC for 24 h. Then the zone of inhibition measured and the size of zone cited in table.

❖ *Antibiotic Sensitivity Assay*

(Concentration250/500/ 1000 µG/ml)

Sr. No.	CODE No.	*Pseudomonas aeruginosa*			*Proteus vulgaris*			*Escherichia coli*			*Staphylococcus aureus*			*Candida albicans*		
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
1.	**4a**	R	R	R	R	1	1.3	1.2	1.4	2	R	R	R	R	R	R
2.	**4b**	1	1.1	1.2	1.2	1.5	1.7	1.2	1.6	1.9	R	1	1.2	R	1.1	1.4
3.	**4c**	R	R	1.2	1.1	1.5	2	1.4	1.5	2.1	R	1.3	1.5	1.1	1.4	2
4.	**4d**	R	R	R	1	1.2	1.4	1.2	1.4	1.5	R	R	R	1	1.2	1.5
5.	**4e**	1.2	1.3	1.5	R	1.1	1.3	1.3	1.4	1.7	1	1.2	1.5	1.1	1.4	2
6.	**4f**	1.2	1.3	1.6	1.1	1.4	1.7	1.1	1.3	1.5	1	1.2	1.4	1.2	1.5	2
7.	**4g**	1.3	1.5	1.8	R	1.2	1.5	1.2	1.2	1.6	1	1.2	1.3	1.1	1.2	1.6
8.	**4h**	1.3	1.5	1.9	1	1.2	1.7	1.2	1.7	1.9	R	1.4	1.8	1.2	1.5	2
9.	**4i**	1.2	1.4	1.7	1.3	1.5	1.9	1.3	1.6	2	R	1.1	1.2	1.1	1.3	1.8
10.	**4j**	1.1	1.2	1.5	1	1.1	1.3	1.3	1.4	1.6	1.1	1.3	1.5	1.2	1.4	1.8
11.	**4k**	1.9	1.2	1.7	2.1	R	1.1	1.2	2	R	R	R	1.1	1.2	1.4	1.7
12.	**4l**	1.5	1.3	1.4	1.7	1.2	1.4	2	1.4	R	R	R	1.2	1.1	1.3	1.5
13.	**4m**	1.3	1.2	1.3	1.6	1.2	1.3	1.7	1.8	1.1	1.1	1.3	R	R	1.3	1.7
14.	**4n**	1.8	1.2	1.2	1.5	1.1	1.3	1.5	1.7	R	1.6	2	R	1.2	1.5	2
15.	**4o**	2	R	R	R	1.1	1.2	1.4	1.3	1.1	1.1	1.3	R	R	R	R
16.	**4p**	1.4	R	R	R	1.2	1.3	1.6	1.4	1.2	1.4	1.7	R	R	1.2	1.5
17.	**4q**	1.8	1.1	1.1	1.3	1.1	1.2	1.3	1.2	1.1	1.2	1.4	1.1	R	1	1.3
18.	**4r**	1.7	R	1.6	2	1	1.3	1.5	2.1	1.2	1.5	2	1.1	R	R	R
19.	**4s**	1.3	1.1	1.1	1.3	1.1	1.3	1.6	1.6	1.3	1.4	1.9	1.2	1.1	1.2	1.7
20.	**4t**	1.4	1.2	1.4	1.7	1.3	1.5	1.9	1.7	1.1	1.3	1.6	R	1	1.2	1.8
21.	**A**	1.8			1.8			1.9			1.9			-		
22.	**CPD**	2.2			2.1			2.1			2.2			-		
23.	**GF**	1.8			1.9			2.0			2.0			-		
24.	**GRF**	-			-			-			-			2.6		
25.	**FLC**	-			-			-			-			2.8		

Note: Zone of inhibition interpretation is as follows.

1. ZONE SIZE <1.0 C.M.- RESISTENT(R)
2. ZONE SIZE 1.0 To 1.5 – INTERMEDIATE
3. ZONE SIZE >1.5 – SENSITV

STD Antibiotic Sensitivity Assay Concentration 40 µG/ml

A: AMPICILLIN
CPD: CEFPODOXIME
GF: GATIFLOXACIN
GRF: GRESIOFULVIN
FLC: FLUCONAZOLE

4.7 CONCLUSION

In summary, we have described the synthesis substituted thiazolo pyrimidine derivatives in excellent yields. The reaction of various 2-thioxopyrimidine with chloro acetyl chloride **(scheme 2)** affords the 3,5-dihydro-7-isopropyl-3-oxo-*N*,5-diaryl-2*H*-thiazolo[3,2-a] pyrimidine-6-carboxamide derivatives **(PVP-4a-t)** was obtained in excellent yield. All the synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy. All the synthesized compounds were evaluated for their anti microbial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-4a-t** showed moderate to potent activity. The compounds **PVP-4c and 4m** showed comparatively good activity against all the bacterial strains.

4.8 EXPERIMENTAL SECTION

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. Melting points were measured in open capillaries and are uncorrected.

❖ **General synthesis of 2-thioxopyrimidines 3a-t.**

A 100mL conical flask equipped with magnetic stirrer and septum was charged with a solution of 4-methyl-3-oxo-*N*-phenylpentanamide **1a-t**, (0.01M), aldehyde (0.01M) and thiourea (0.015M) was dissolved in minimum quantity of methanol. It was than heated for 5-10 minutes to get the clear solution. Few drops of con. HCl were added to the reaction mixture as a catalyst. The reaction mixture was then refluxed in water bath for 6-12 h. The progress of reaction was monitored by thin layer chromatography. The reaction mixture was allowed to cool at room temperature. The solid separated upon cooling was filtered, washed with hot methanol and dried. Compounds were directly used for the next step.

❖ **General procedure for the synthesis of substituted Thiazolopyrimidines PVP-4a-t.**

To a well stirred mixture of compound **3a-t** (10 mmol) and chloro acetyl chloride (10 mmol) were dissolved in glacial acetic acid with sodium acetate and refluxed for 6 h. The reaction was monitored with thin layer chromatography and after completion of the reaction, the reaction mixture was poured on crushed ice and was extracted with chloroform. The chloroform was removed in vacuum. The residue was dried and recrystallized from ethanol to afford analytically pure products **PVP-4a-t.**

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❖ **Spectral data of the synthesized compounds**

1,2,3,4-tetrahydro-6-isopropyl-2-thioxo-*N*,4-di*p*-tolylpyrimidine-5-carboxamide 3e: White solid; R_f 0.60 (6:4 hexane-EtOAc); IR (KBr): 3373, 3072, 2895, 2828, 1694, 1635, 1482, 1343, 1298 cm^{1}; ^{1}H NMR: δ ppm 1.45-1.62 (d, 6H, 2 x ^{i}prCH$_3$), 2.24 - 2.26 (s, 6H, 2 x CH$_3$), 3.85 (m, 1H, ^{i}prCH), 4.76 - 4.77 (s, 1H, -CH), 7.10 - 7.48 (m, 8H, Ar-H), 8.86 - 8.87 (s, 1H, -NH), 8.93 (s, 1H, -NH), 9.73 (br, s, 1H, -CONH); ^{13}C NMR : 15.08, 19.28, 30.56, 31.27, 43.26, 54.35, 64.96, 116.38, 116.59, 119.13, 119.77, 121.15, 127.50, 128.48, 132.74, 135.67, 137.97, 148.89, 152.11, 154.53, 168.04, 169.67; MS (*m/z*): 379 (M^+); Anal. Calcd for $C_{22}H_{25}N_3OS$: C, 69.62; H, 6.64; N, 11.07; Found: C, 69.58; H, 6.55; N, 11.05.

***N*-(3-chloro-4-fluorophenyl)-5-(4-chlorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4a):** yellow solid; R_f 0.80 (6:4 hexane-EtOAc); IR (KBr): 3443, 3371, 3230, 3058, 1703, 1648, 1586, 1244, 1016 cm^{-1}; ^{1}H NMR: δ 1.43-1.82 (d, 6H, 2 x ^{i}prCH$_3$), 3.94 (m, 1H, ^{i}prCH), 4.12 (s, 2H, CH$_2$), 5.59 (s, 1H, -CH), 7.20 (d, 2H, Ar-H, *j*=9*Hz*), 7.36-7.42 (d, 4H, Ar-H, ,*j*=9*Hz*), 7.90 (s, 1H, Ar-H), 10.18 (s, 1H, CONH); ^{13}C NMR : 18.07, 20.45, 20.65, 30.51, 45.06, 55.59, 114.28, 119.80, 121.69, 125.47, 128.64, 128.76, 132.61, 135.89, 138.42, 167.19, 173.19; MS (*m/z*): 478(M^+); Anal. Calcd for $C_{22}H_{18}Cl_2FN_3O_2S$: C, 55.24; H, 3.79; N, 8.78; Found: C, 55.18; H, 3.75; N, 8.62.

***N*,5-bis(4-chlorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4b):** yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3452, 3307, 3223, 2980, 1653, 1509, 1461, 1051 cm^{-1}; ^{1}H NMR: δ 1.60-1.82 (d, 6H, 2 x ^{i}prCH$_3$), 3.95 (m, 1H, ^{i}prCH), 4.12 (s, 2H, CH$_2$), 5.57 (s, 1H, -CH), 7.18-7.21 (d, 2H, Ar-H, *j*=9*Hz*), 7.33-7.44 (d, 4H, Ar-H, ,*j*=9*Hz*), 7.58-7.60 (d, 2H, Ar-H), 10.15 (s, 1H, CONH) ; MS (*m/z*): 460 (M^+); Anal. Calcd for $C_{22}H_{19}Cl_2N_3O_2S$: C, 57.40; H, 4.16; N, 9.13; Found: C, 57.38; H, 4.12; N, 9.08.

5-(4-chlorophenyl)-*N*-(4-fluorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4c): yellow solid; R_f 0.82 (6:4 hexane-EtOAc); IR (KBr): 3412, 3317, 3253, 2950, 1613, 15039, 1431, 1041 cm^{-1}; ^{1}H NMR: δ 1.60-1.82 (d, 6H, 2 x ^{i}prCH$_3$), 3.94 (m, 1H, ^{i}prCH), 4.11 (s, 2H, CH$_2$), 5.57 (s, 1H, -CH), 7.10-7.13 (d, 2H, Ar-H, ,*j*=9*Hz*), 7.16-7.21 (d, 2H Ar-H, ,*j*=9*Hz*), 7.42-

7.44 (d, 2H, Ar-H, *j*=9*Hz*), 7.53-7.59 (d, 2H, Ar-H, *j*=9*Hz*), 10.05 (s, 1H, CONH) ; MS (*m/z*): 443(M^+); Anal. Calcd for $C_{22}H_{18}ClFN_3O_2S$; C, 59.52; H, 4.31; N, 9.47; Found: C, 59.48; H, 4.25; N, 9.32.

3,5-dihydro-7-isopropyl-*N*,5-bis(4-methoxyphenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4d): yellow solid; R_f 0.80 (6:4 hexane-EtOAc); IR (KBr): 3429, 3307, 3123, 2959, 1658, 1546, 1265, 1041 cm^{-1}; MS (*m/z*): 451 (M^+); Anal. Calcd for $C_{24}H_{25}N_3O_4S$: C, 63.84; H, 5.58; N, 9.31; Found: C, 63.68; H, 5.60; N, 9.42.

3,5-dihydro-7-isopropyl-3-oxo-*N*,5-di*p*-tolyl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4e): yellow solid; R_f 0.84 (6:4 hexane-EtOAc); IR (KBr): 3335, 3026, 2972, 2814, 1712, 1680, 1553, 1462, 1174, 1060 cm^{-1}; MS (*m/z*): 419 (M^+); Anal. Calcd for $C_{24}H_{25}N_3O_2S$: C, 68.71; H, 6.01; N, 10.02; Found: C, 68.81; H, 6.15; N, 10.12.

5-(4-fluorophenyl)-3,5-dihydro-7-isopropyl-*N*-(4-methoxyphenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4f):
yellow solid; R_f 0.79 (6:4 hexane-EtOAc); IR (KBr): 3462, 3307, 3223, 2990, 1653, 1509, 1461, 1061 cm^{-1}; MS (*m/z*): 439 (M^+); Anal. Calcd for $C_{23}H_{22}FN_3O_3S$: C, 62.85; H, 5.05; N, 9.56; Found: C, 62.78; H, 5.10; N, 9.45.

***N*-(3-chloro-4-fluorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-5-phenyl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4g):** yellow solid; R_f 0.79 (6:4 hexane-EtOAc); IR (KBr): 3459, 3327, 3173, 2989, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 443 (M^+); Anal. Calcd for $C_{22}H_{19}ClFN_3O_2S$: C, 59.52; H, 4.31; N, 9.47; Found: C, 59.46; H, 4.40; N, 9.52.

3,5-dihydro-7-isopropyl-5-(3,4-dimethoxyphenyl)-3-oxo-*N*-phenyl-2*H*-thiazolo [3,2-a]pyrimidine-6-carboxamide (PVP-4h): yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3449, 3227, 3193, 2966, 1628, 1522, 1217, 1041 cm^{-1}; MS (*m/z*): 451 (M^+); Anal. Calcd for $C_{24}H_{25}N_3O_4S$: C, 63.84; H, 5.58; N, 9.31; Found: C, 63.74; H, 5.55; N, 9.35.

***N*-(4-fluorophenyl)-3,5-dihydro-7-isopropyl-5-(3,4-methoxyphenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4i):** yellow solid; R_f 0.80 (6:4 hexane-EtOAc); IR (KBr): 3449, 3227, 3173, 2989, 1648, 1586, 1251, 1061 cm^{-1}; MS (*m/z*): 530 (M^+); Anal. Calcd for $C_{24}H_{24}BrN_3O_4S$: C, 54.34; H, 4.56; N, 7.92; Found: C, 54.23; H, 4.65; N, 7.82.

***N*-(4-chlorophenyl)-3,5-dihydro-7-isopropyl-5-(4-methoxyphenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4j):** yellow solid; R_f 0.80 (6:4 hexane-EtOAc); IR (KBr): 3442, 3226, 3143, 2986, 1642, 1566, 1241, 1061 cm^{-1}; MS (*m/z*): 455 (M^+); Anal. Calcd for $C_{23}H_{23}ClN_3O_3S$: C, 60.59; H, 4.86; N, 9.22; Found: C, 60.63; H, 4.94; N, 9.12.

***N*,5-bis(4-fluorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-thiazolo[3,2a]pyrimidine-6-carboxamide (PVP-4k):** yellow solid; R_f 0.82 (6:4 hexane-EtOAc); IR (KBr): 3459, 3252, 3143, 2919, 1648, 1586, 1241, 1051 cm^{-1}; MS (*m/z*): 427 (M^+); Anal. Calcd for $C_{22}H_{19}F_2N_3O_2S$: C, 61.81; H, 4.48; N, 9.83; Found: C, 61.83; H, 4.45; N, 9.72.

3,5-dihydro-7-isopropyl-3-oxo-*N*,5-diphenyl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4l): yellow solid; R_f 0.79 (6:4 hexane-EtOAc); IR (KBr): 3420, 3226, 3143, 2988, 1632, 1546, 1231, 1061 cm^{-1}; MS (*m/z*): 391 (M^+); Anal. Calcd for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73; Found: C, 67.43; H, 5.34; N, 10.62.

***N*-(4-chlorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-5-p-tolyl-2*H*-thiazolo[3,2-a] pyrimidine-6-carboxamide (PVP-4m):**

yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3412, 3317, 3253, 2950, 1613, 1539, 1431, 1061 cm^{-1}; MS (*m/z*): 435 (M^+); Anal. Calcd for $C_{23}H_{22}ClN_3O_3S$: C, 62.79; H, 5.04; N, 9.55; Found: C, 62.68; H, 5.15; N, 9.52.

3,5-dihydro-5-(4-hydroxyphenyl)-7-isopropyl-*N*-(2,5-dimethylphenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4n): yellow solid; R_f 0.82 (6:4 hexane-EtOAc); IR (KBr): 3442, 3327, 3253, 2980, 1623, 1569, 1431, 1051 cm^{-1}; MS (*m/z*): 435 (M^+); Anal. Calcd for $C_{24}H_{25}N_3O_3S$: C, 66.18; H, 5.79; N, 9.65; Found: C, 66.10; H, 5.70; N, 9.52.

***N*-(3-chlorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-5-*p*-tolyl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4o):** yellow solid; R_f 0.83 (6:4 hexane-EtOAc); IR (KBr): 3462, 3327, 3220, 2980, 1623, 1509, 1461, 1051 cm^{-1}; MS (*m/z*): 316 (M^+); Anal. Calcd for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71; Found: C, 60.68; H, 6.55; N, 17.62.

***N*-(4-bromophenyl)-5-(4-fluorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4p):** yellow solid; R_f 0.80 (6:4 hexane-EtOAc); IR (KBr): 3442, 3327, 3173, 2989, 1653, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 488 (M^+); Anal. Calcd for $C_{22}H_{19}BrFN_3O_2S$: C, 54.11; H, 3.92; N, 8.60; Found: C, 54.16; H, 3.85; N, 8.62.

3,5-dihydro-7-isopropyl-*N*-(4-methoxyphenyl)-5-(4-nitrophenyl)-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4q):: yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3459, 3327, 3173, 2989, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 466 (M^+); Anal. Calcd for $C_{15}H_{17}BrN_4O_2$: C, 49.33; H, 4.69; N, 15.34; Found: C, 49.13; H, 4.45; N, 15.22.

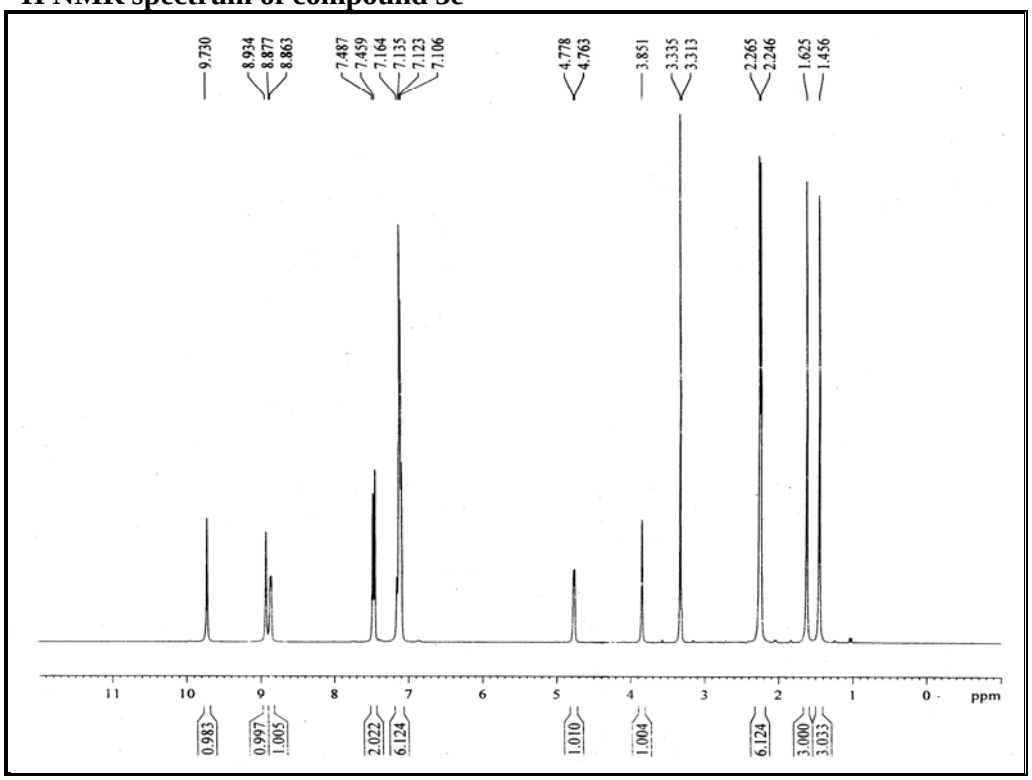
***N*-(4-chlorophenyl)-3,5-dihydro-5-(4-hydroxyphenyl)-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4r):** yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3459, 3327, 3193, 2999, 1648, 1586, 1261, 1061 cm^{-1}; MS (*m/z*): 441 (M^+); Anal. Calcd for $C_{22}H_{20}ClN_4O_3S$: C, 60.59; H, 4.56; N, 9.51; Found: C, 60.52; H, 4.65; N, 9.56.

***N*-(4-fluorophenyl)-3,5-dihydro-5-(2-hydroxyphenyl)-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4s):** yellow solid; R_f 0.83 (6:4 hexane-EtOAc); IR (KBr): 3459, 3252, 3143, 2919, 1648, 1586, 1241, 1051 cm^{-1}; MS (*m/z*): 425 (M^+); Anal. Calcd for $C_{22}H_{20}FN_3O_3S$: C, 62.10; H, 4.74; N, 9.88; Found: C, 62.15; H, 4.65; N, 9.82.

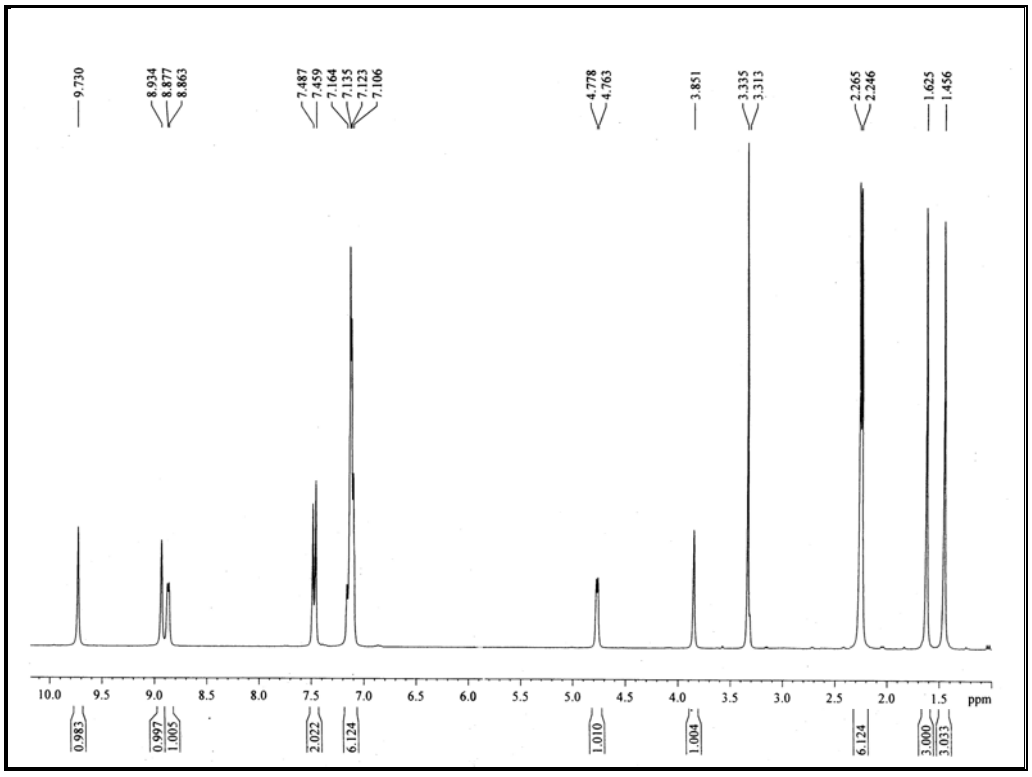
***N*,5-bis(3-chlorophenyl)-3,5-dihydro-7-isopropyl-3-oxo-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide (PVP-4t):** yellow solid; R_f 0.81 (6:4 hexane-EtOAc); IR (KBr): 3449, 3227, 3193, 2966, 1628, 1522, 1217, 1041 cm^{-1}; MS (*m/z*): 460 (M^+); Anal. Calcd for $C_{16}H_{19}ClN_4O_2$: C, 57.40; H, 4.16; N, 9.13; Found: C, 57.44; H, 4.10; N, 9.1

❖ **Spectral representation of synthesized compounds**

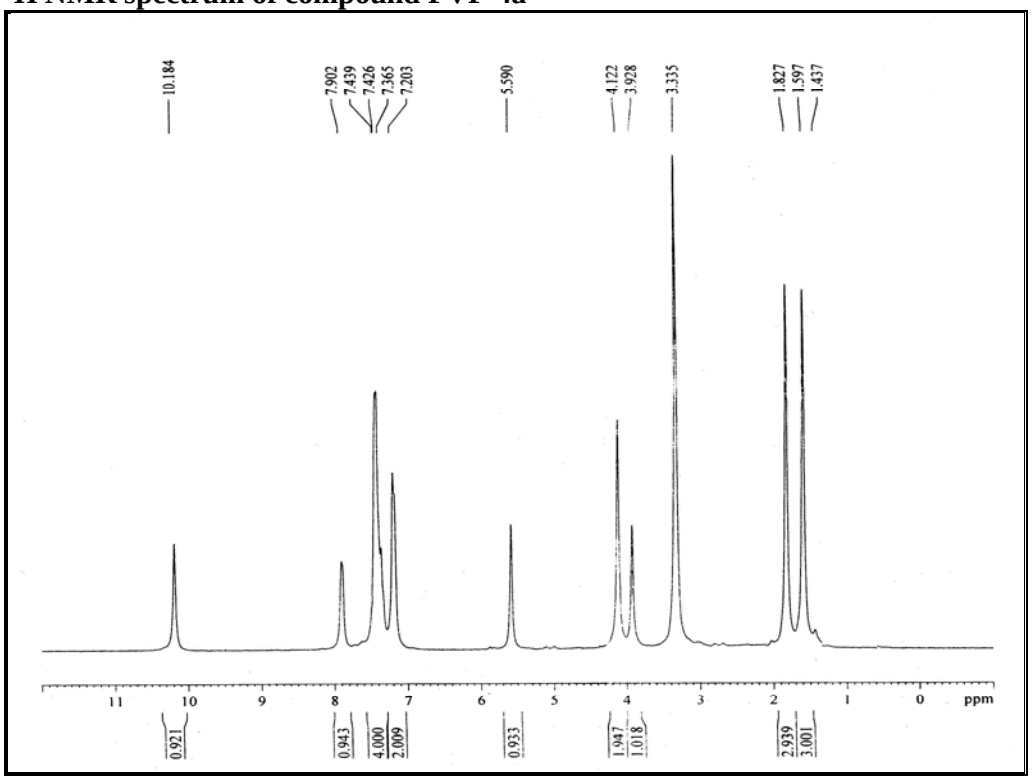
^{1}H NMR spectrum of compound 3e



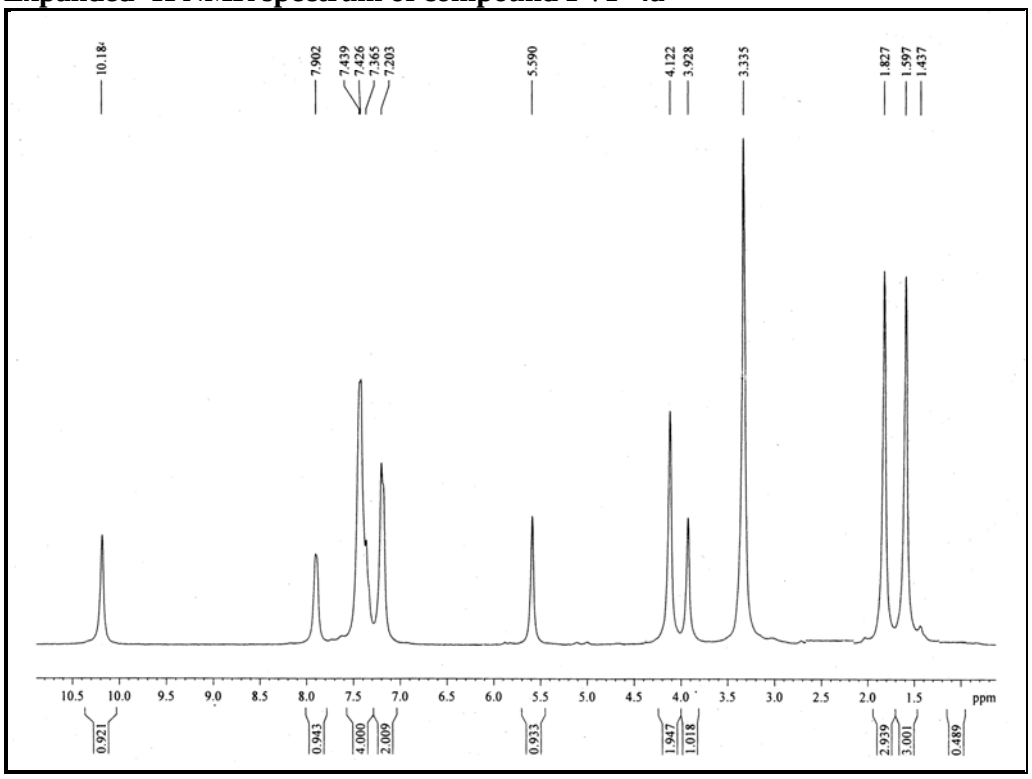
Expanded ^{1}H NMR spectrum of compound 3e



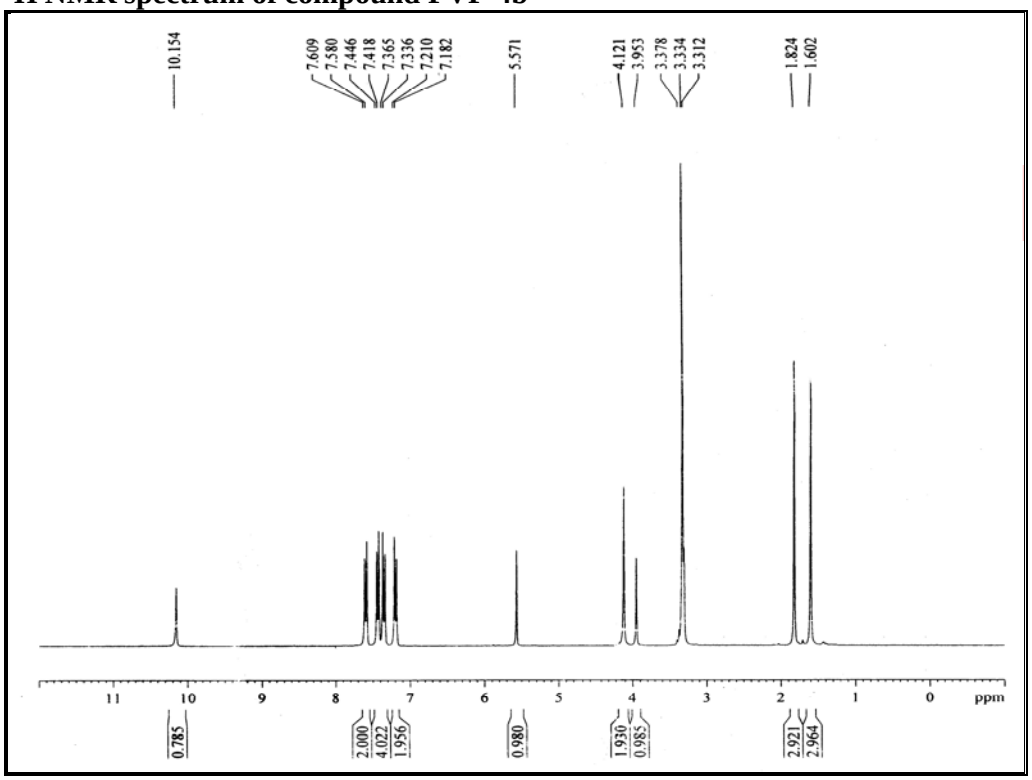
^{1}H NMR spectrum of compound PVP-4a



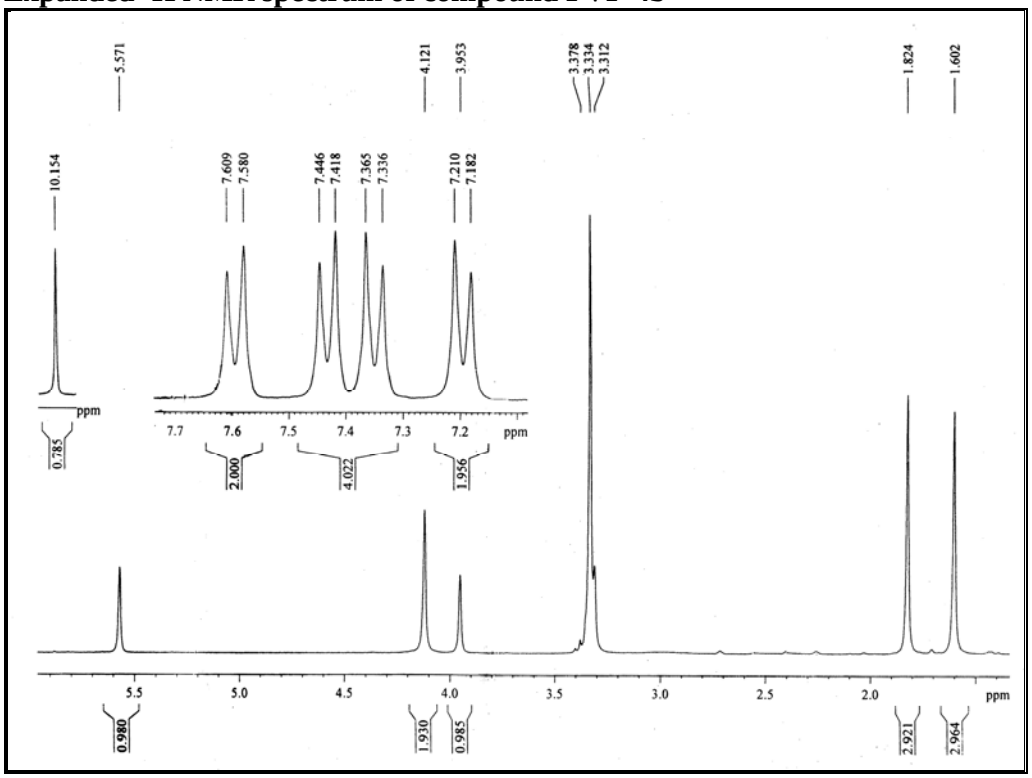
Expanded ^{1}H NMR spectrum of compound PVP-4a



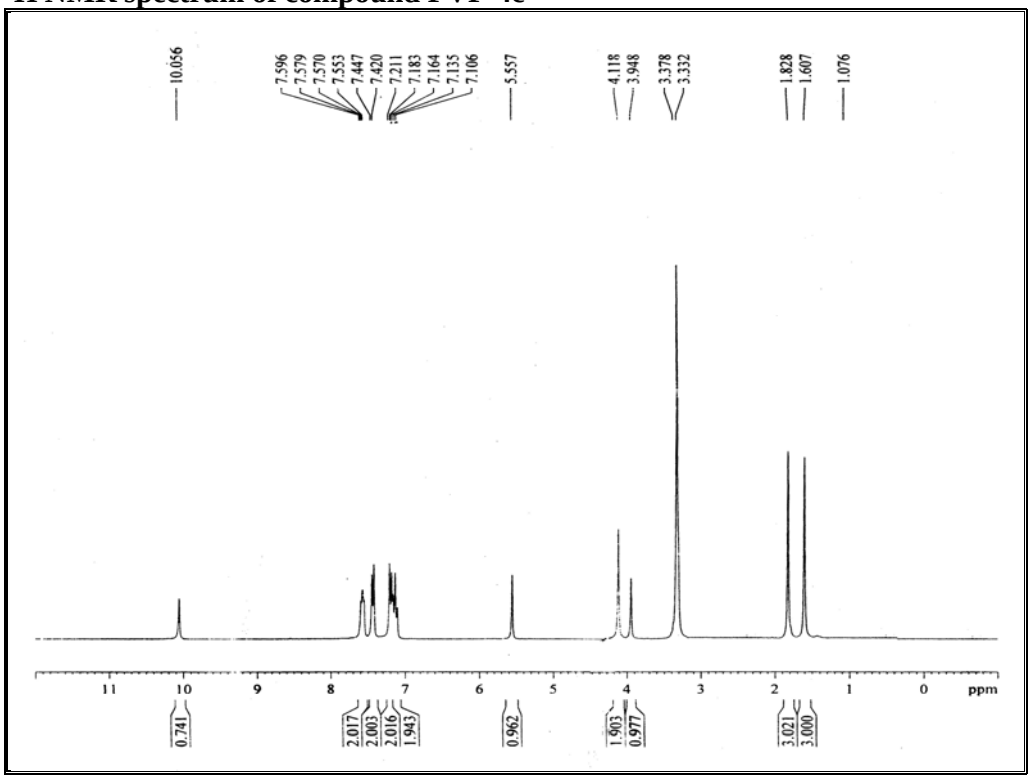
^{1}H NMR spectrum of compound PVP-4b



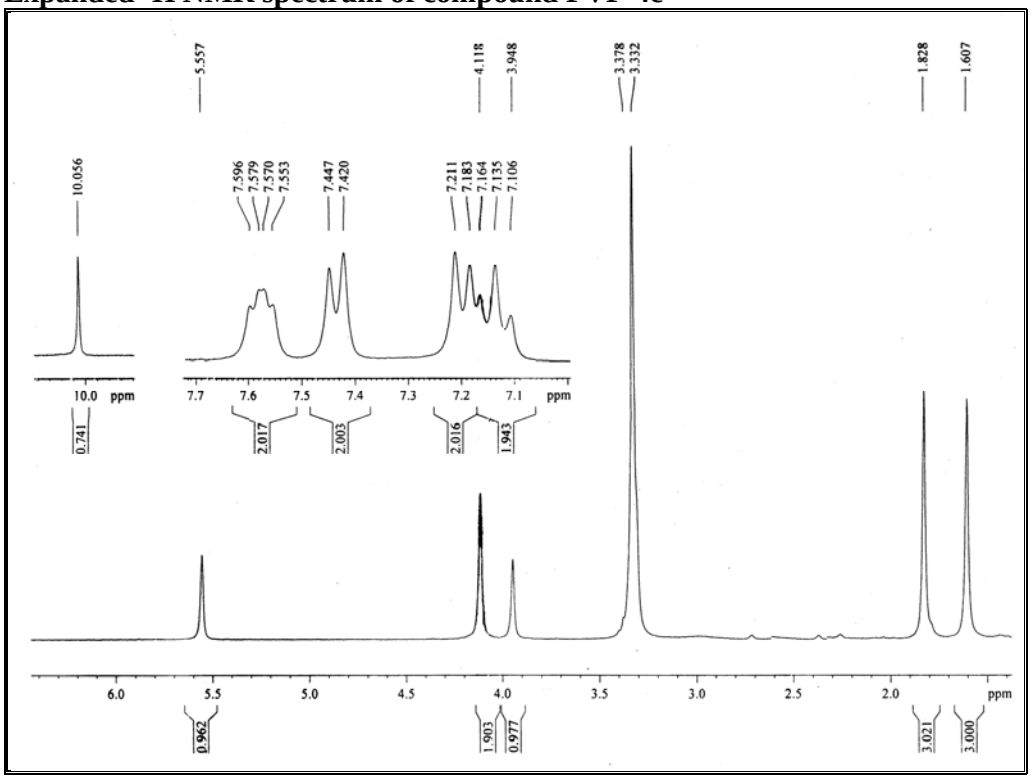
Expanded ^{1}H NMR spectrum of compound PVP-4b



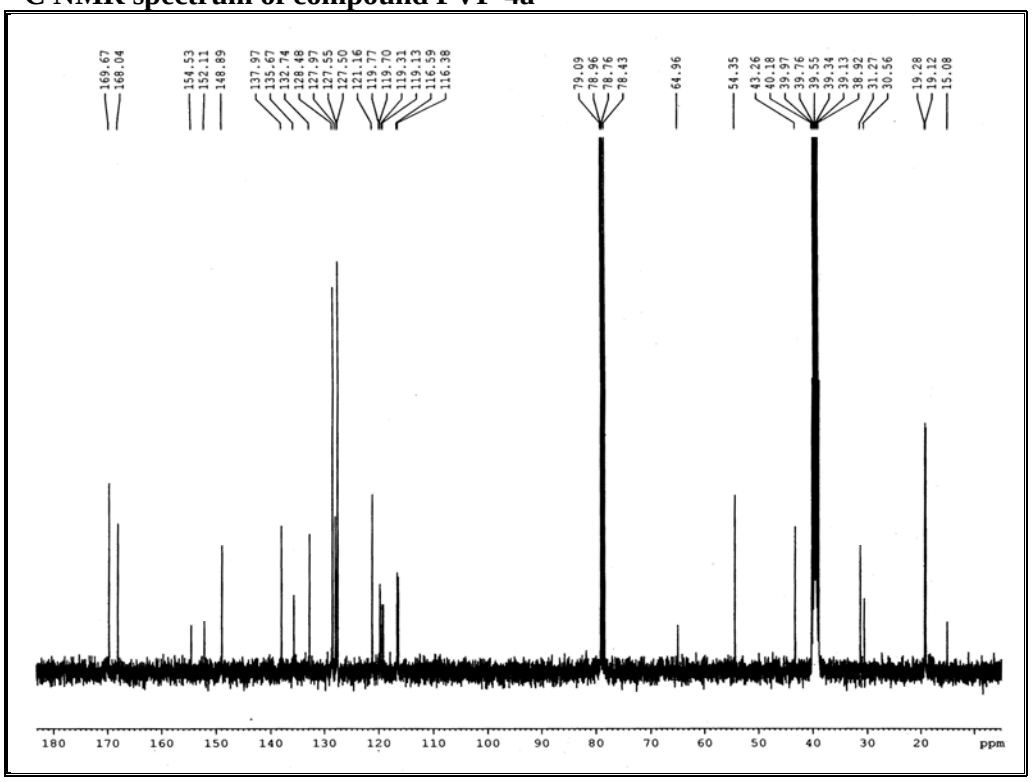
^{1}H NMR spectrum of compound PVP-4c



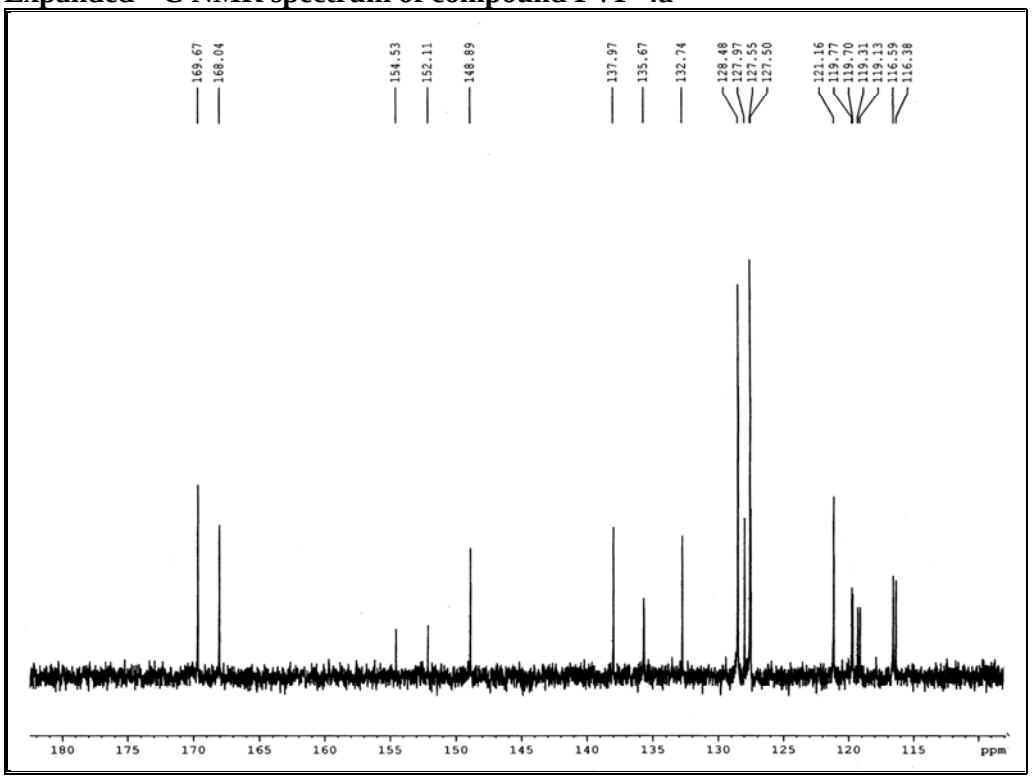
Expanded ^{1}H NMR spectrum of compound PVP-4c



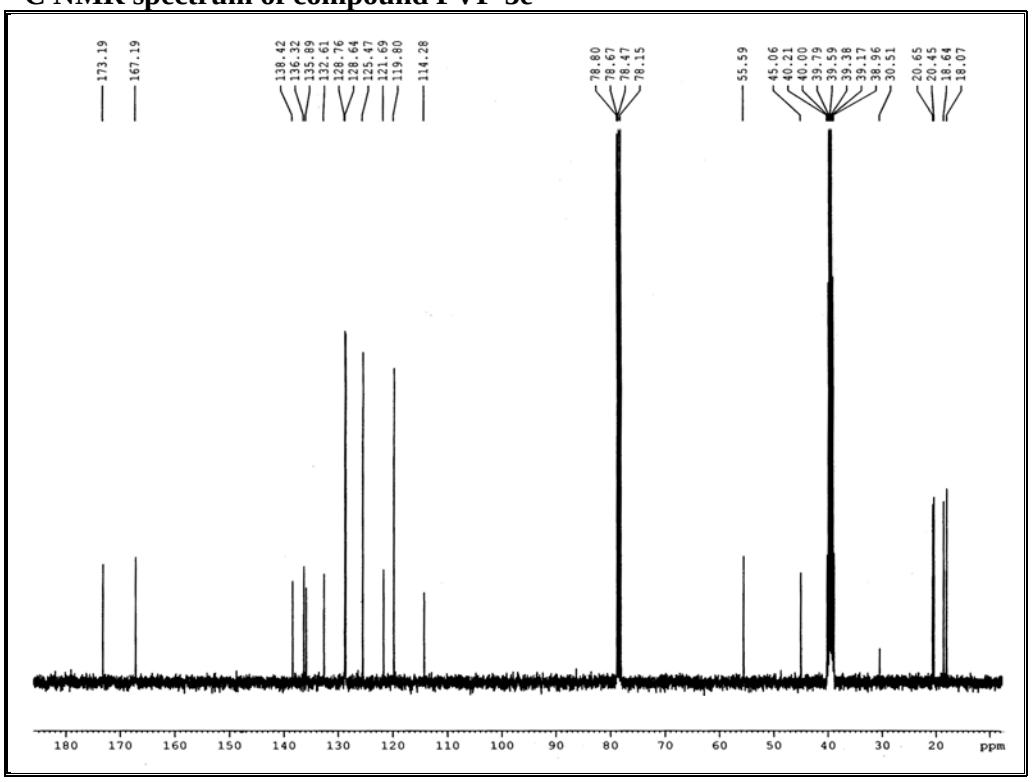
^{13}C NMR spectrum of compound PVP-4a



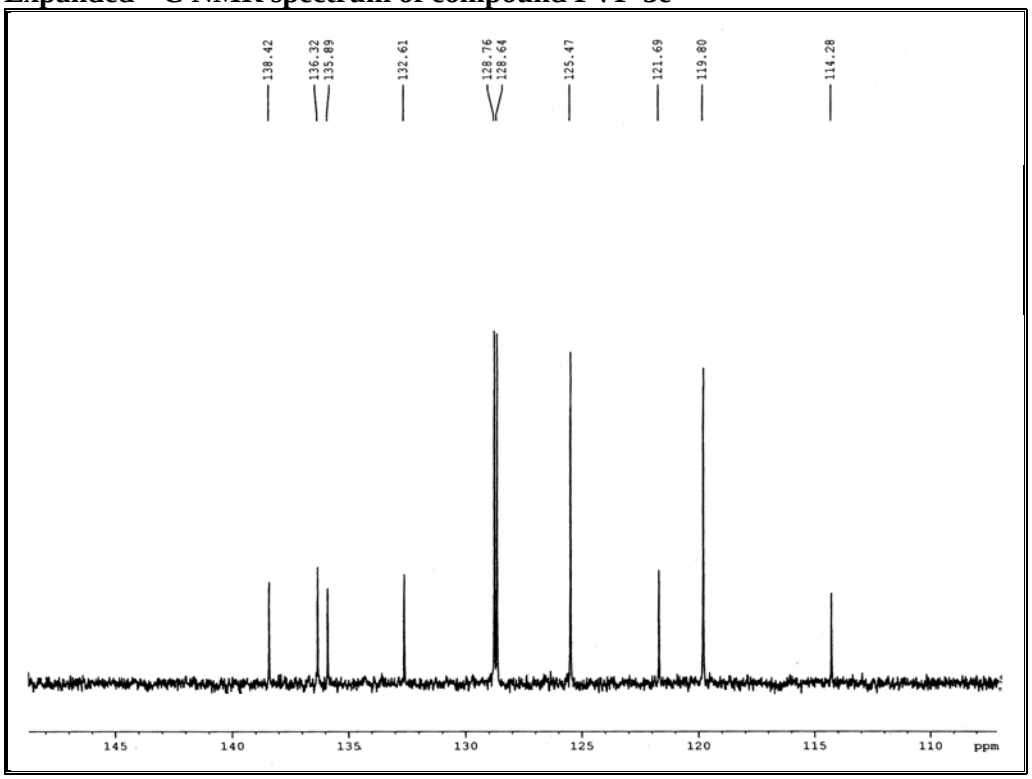
Expanded ^{13}C NMR spectrum of compound PVP-4a



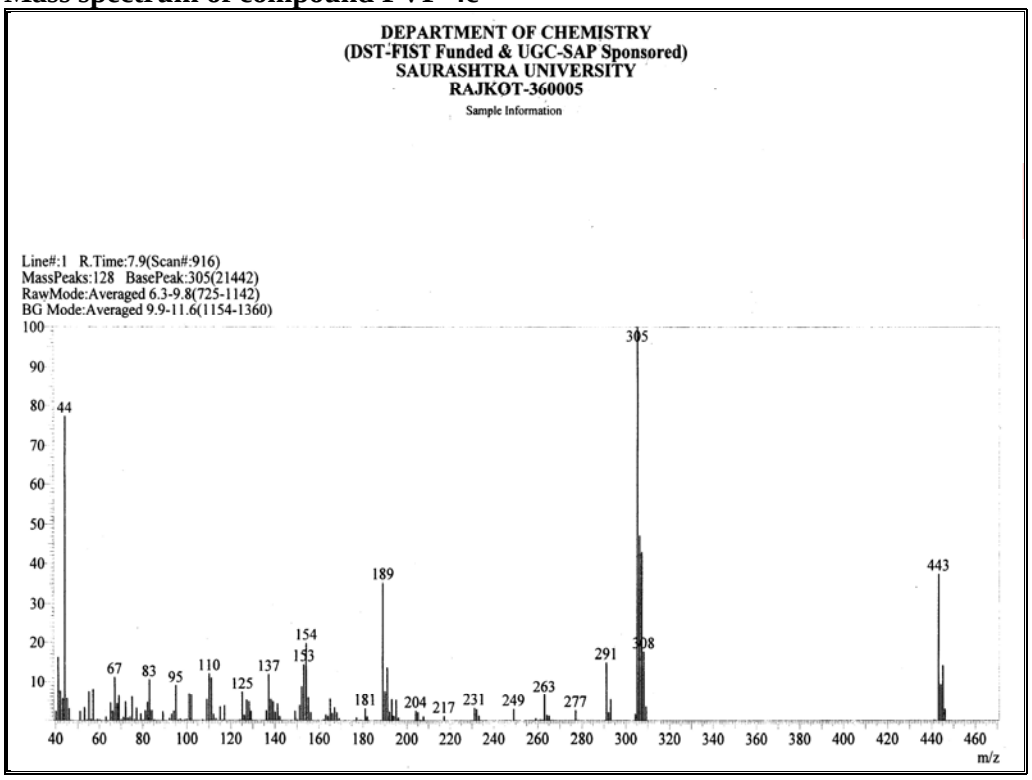
^{13}C NMR spectrum of compound PVP-3e



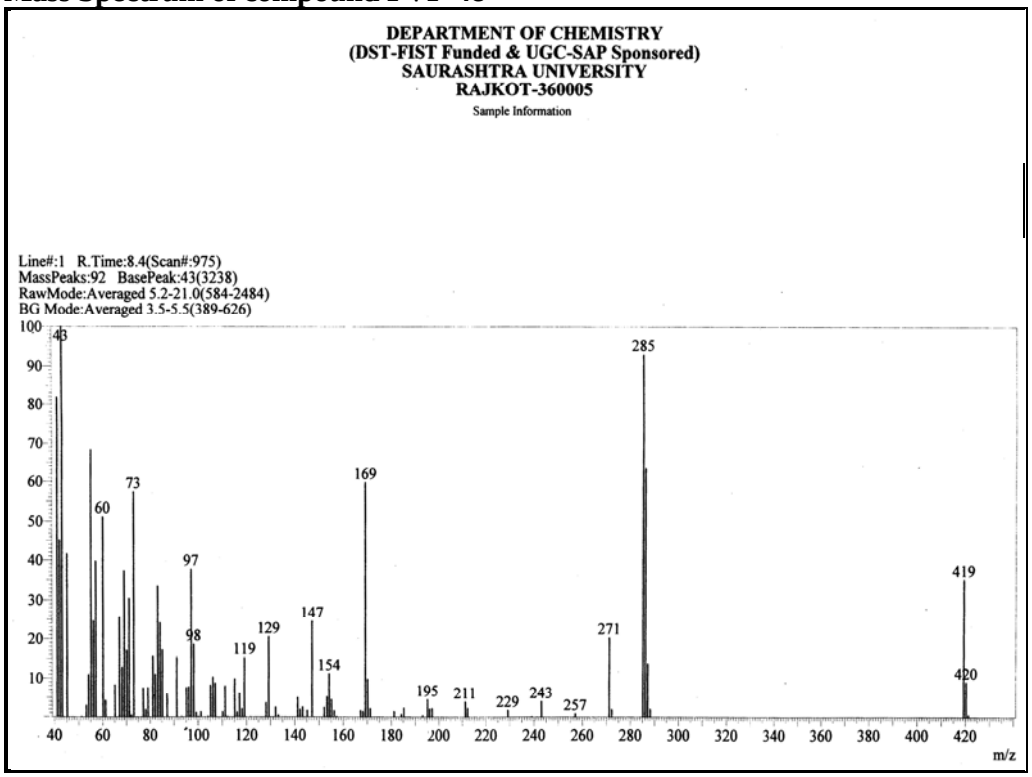
Expanded ^{13}C NMR spectrum of compound PVP-3e



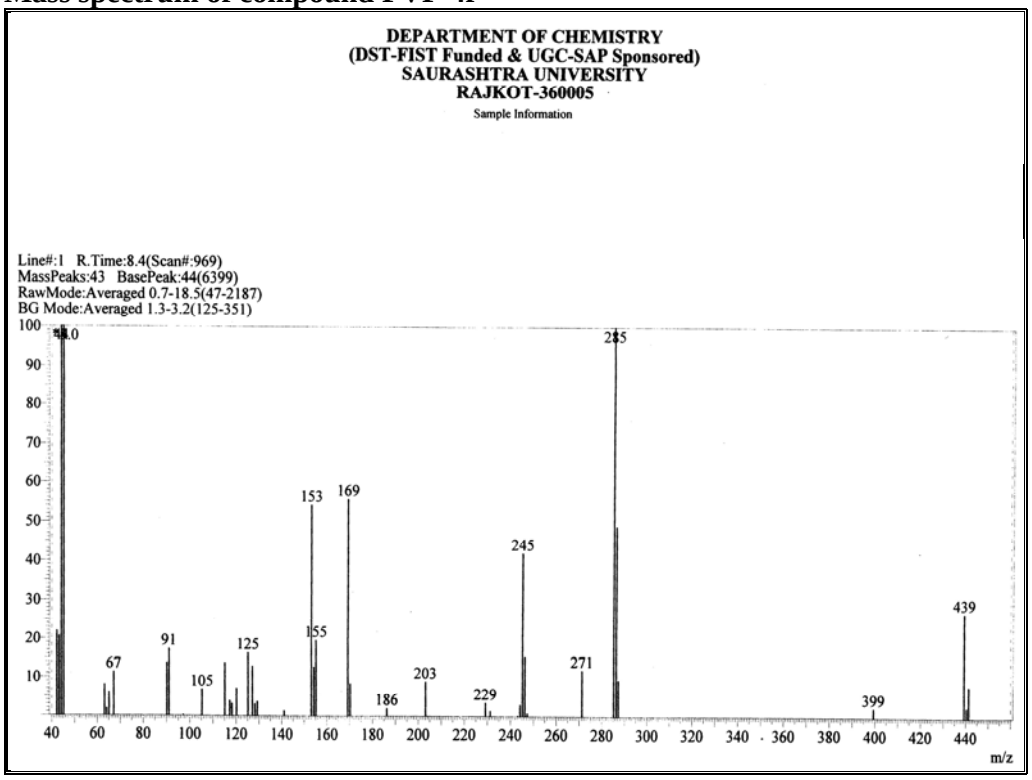
Mass spectrum of compound PVP-4c



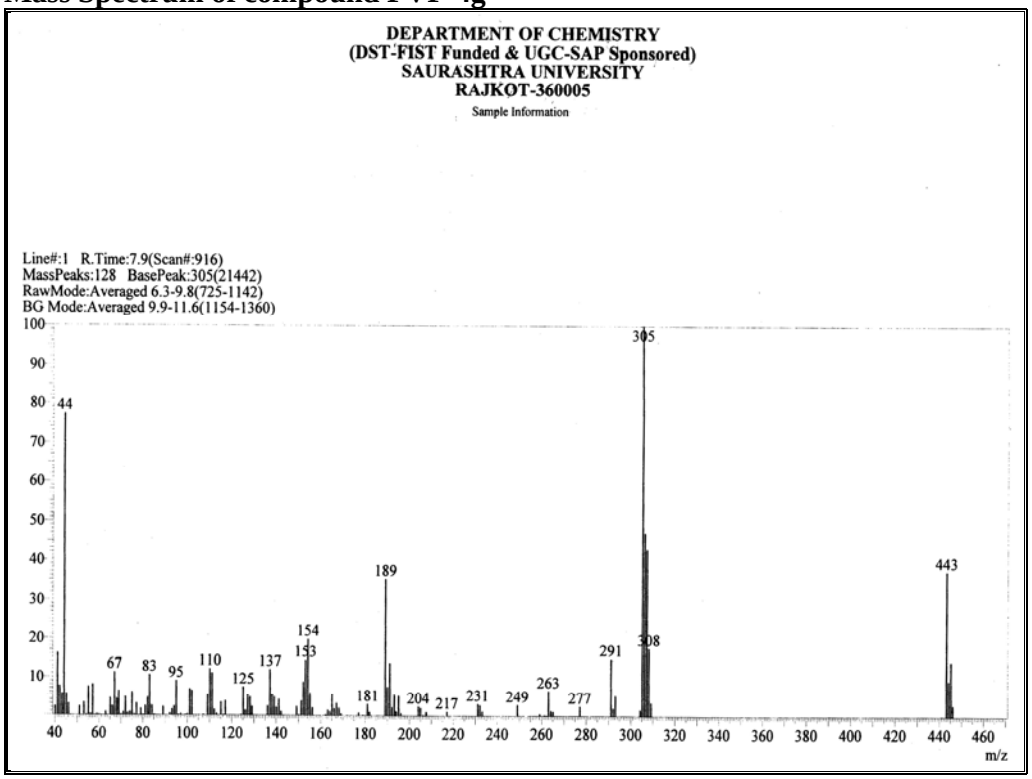
Mass Spectrum of compound PVP-4e



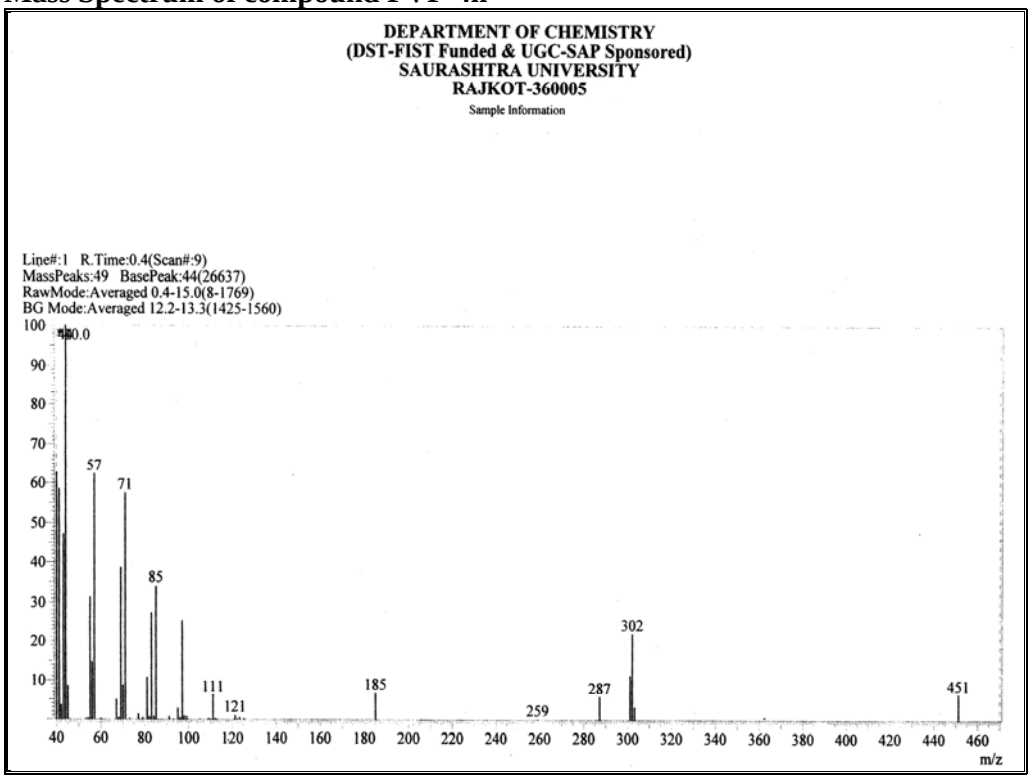
Mass spectrum of compound PVP-4f



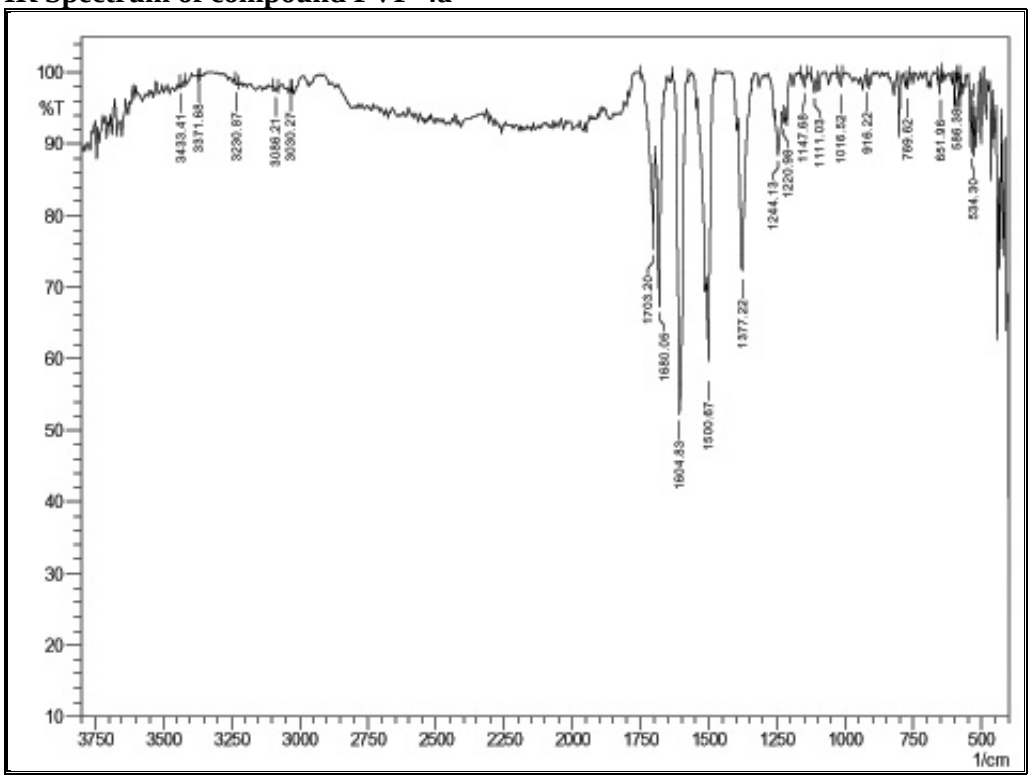
Mass Spectrum of compound PVP-4g



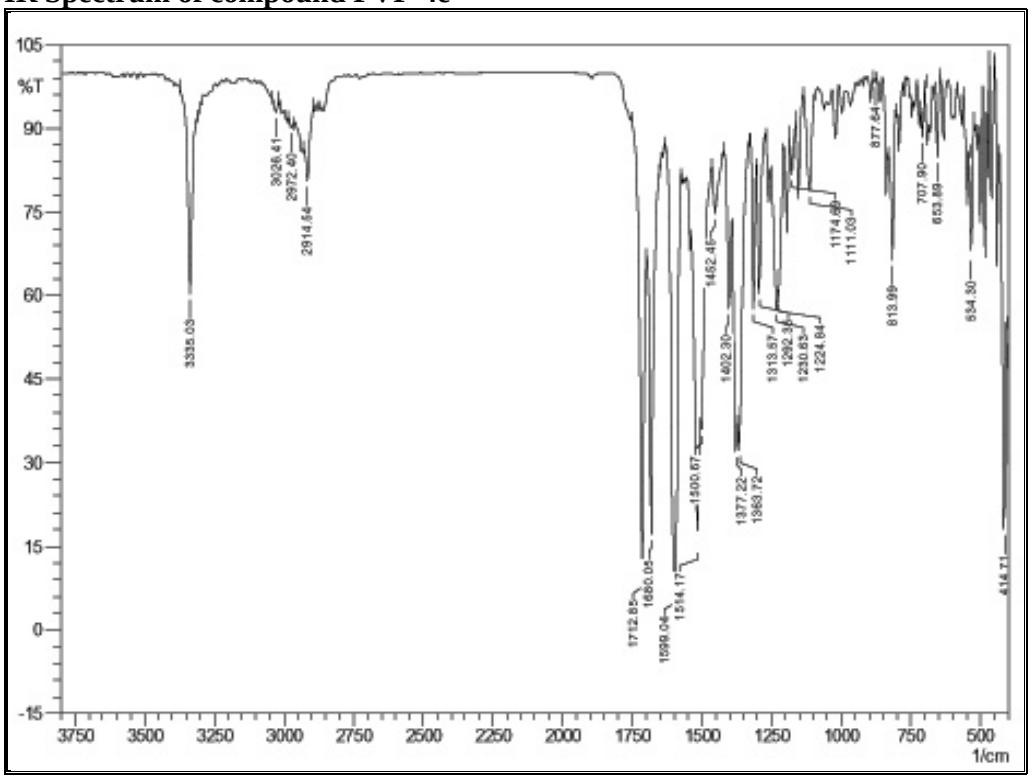
Mass Spectrum of compound PVP-4h



IR Spectrum of compound PVP-4a



IR Spectrum of compound PVP-4e



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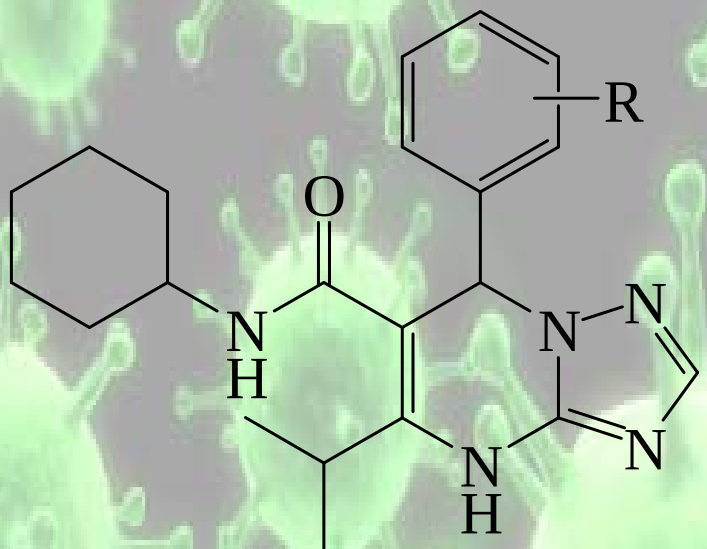
Chapter 5

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF NOVEL SUBSTITUTED TRIAZOLOPYRIDINE DERIVATIVES.



5.1 INTRODUCTION

The condensation of a ring of 1,2,4-triazole and another one of pyrimidine gives rise to the formation of bicyclic heterocycles known as 1,2,4-triazolopyrimidines. Four different possibilities exist for the relative orientation of both rings, so four different isomeric families of compounds are defined: 1,2,4-triazolo[1,5-*a*]pyrimidine **(Figure 1) (1)**, 1,2,4-triazolo[1,5-*c*]pyrimidine **(2)**, 1,2,4-triazolo[4,3-*a*]pyrimidine **(3)** and 1,2,4-triazolo[4,3-*c*]pyrimidine **(4)**.

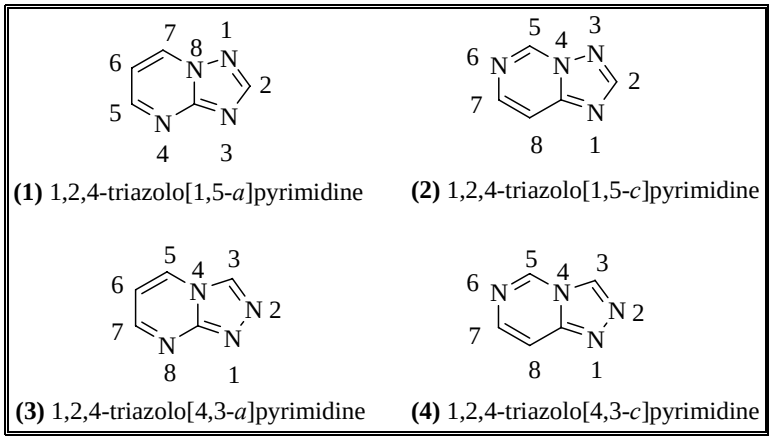


Figure 1

5.2 Pharmacological Profile

Among these isomeric families of compounds, 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives are thermodynamically more stable and, thus, the most studied ones,[1] a few of them being commercially available. Revisions surveying the synthesis, reactivity, spectroscopic characterization and crystallographic studies of 1,2,4-triazolo[1,5-*c*]pyrimidines,[2]1,2,4-triazolo[4,3-*a*]pyrimidines [3] and 1,2,4-triazolo[4,3-*c*]pyrimidines [4] have also been published.

From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Recently, 1,2,4-triazolo[1,5-*a*]pyrimidines have aroused increasing attention from the chemical and biological view points, due to their diverse pharmacological activities, such as antitumor potency [5, 6] inhibition of KDR kinase,[7] antifungal effect [8] and macrophage activation. [9] They have proved to be promising anticancer agents with dual mechanisms of tubulin polymerization promotion[5,6] as well as cyclin dependent

kinases 2 inhibition. [10] Some examples of published derivatives of 1,2,4-triazolo[1,5-*a*]pyrimidine with their biological activities are **(Figure 2)** as following.

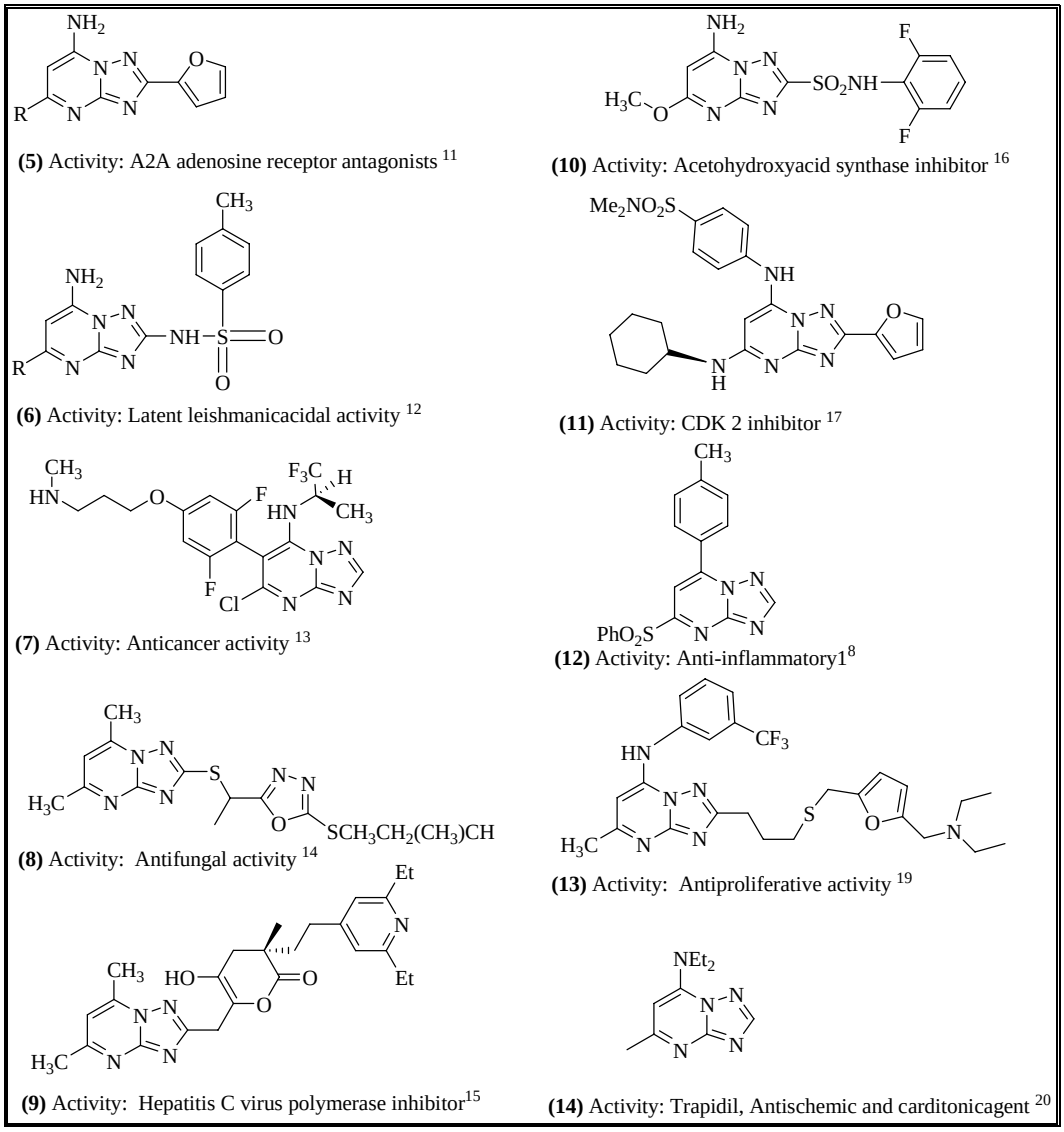


Figure 2

5.3 Reported synthetic strategies

- **Amino-1,2,4-triazole and 1,3-bifunctional synthons**
- **Principle and Conditions**

By far the most triazolo[1,5-*a*]pyrimidine synthesis are condensations of dinucleophilic 5-amino-1,2,4-triazoles with 1,3-bifunctional synthons as shown in the formation of triazolo[1,5-*a*]pyrimidine **(15)**.[21-24] New synthetic conditions recently described involve melting under microwave irradiation, a reaction that is environmental friendly and gives higher yields **(Figure 3)** than conventional heating in solvent.[25] Furthermore, certain lithium 1,3-diketonates have proven to be better synthons than the corresponding diketones.[26]

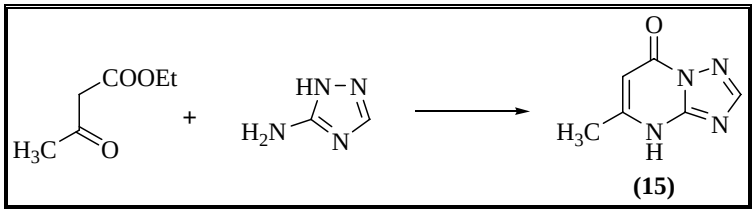


Figure 3

Previous mechanistic conclusions have been confirmed by isolating stable intermediate 5-amino-1,2,4-triazole derivatives such as enamine **(16) (Figure 4)** on reacting 5-amino-1,2,4-triazoles with 3-ketovinyl ethers,[27] 3-ketoenamines,[28] 3-ketoaldehydes,[29] enamine-2-carboxylic esters [30] or ethoxymethylene malonates.[31]

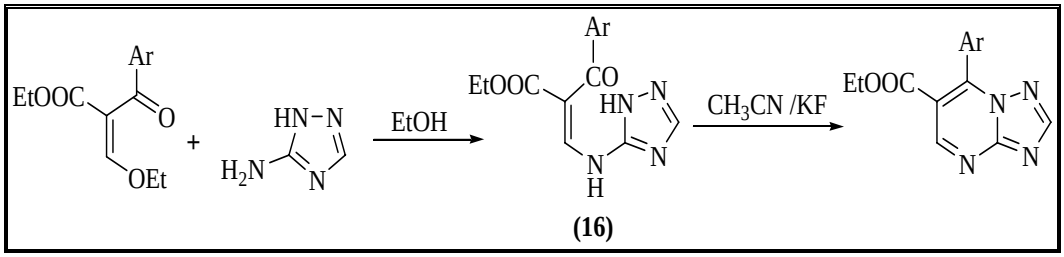


Figure 4

That means, the overall reaction starts with the interaction of the amino-1,2,4-triazoleamino group and the enolic (or analogous) functionality of the three-carbon synthon. In the two-step examples, just mentioned, the first step proceeds under milder conditions (sometimes just in ethanol at room temperature), but the final Cyclization

(or the one-step reaction, if the intermediate is not trapped) requires stronger means (e.g., PPA or boiling acetic acid). Under extreme conditions, triazolylamide **(17)** was subject to flash vacuum pyrolysis between 300 and 450 ºC to give about 50% triazolo[1,5-a]pyrimidine **(18) (Figure 5).**[32] Libraries of fused 3-aminopyrimidin-4-ones **(19)** and other compounds were just recently prepared by the solid-phase and by the solution-phase parallel synthesis. [33] The latter method turned out to be advantageous with respect to yield and purity.

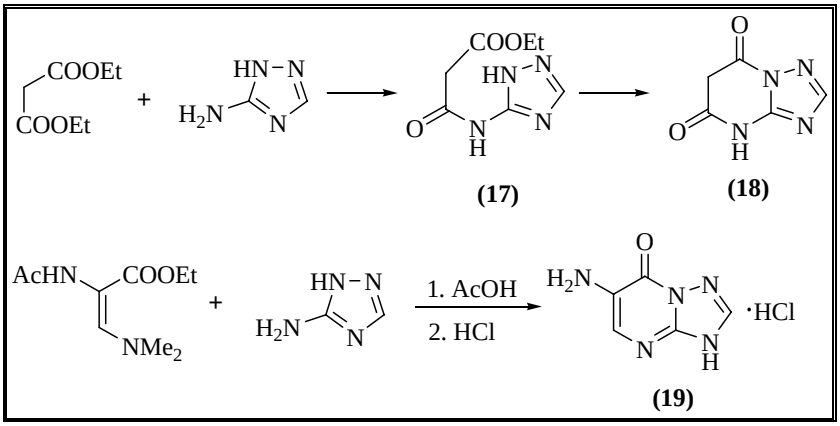


Figure 5

❖ **Use of Modified 5-Amino-1,2,4-triazoles**

Shows two parallel paths of pyrimidine ring annulations **(Figure 6)** the conventional method, route A and a route B using a reactive amino-1,2,4-triazole derivative.[34] Amidine **(22),** formed from 5-amino-1,2,4-triazole and DMF dimethylacetal, can be regarded as the result of incorporating one carbon of the three-carbon synthon **(20)** into the 5-amino-1,2,4-triazole molecule; condensation with a reactive two-carbon component leads to target triazolo[1,5-*a*]pyrimidine **(21)**.

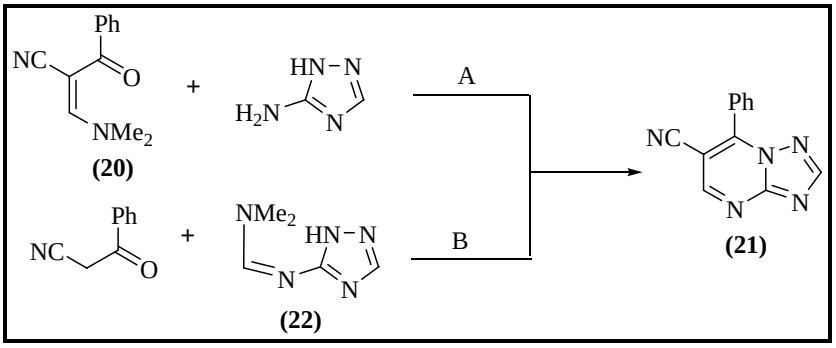


Figure 6

Path B also serves in confirming the structure of product **(21)**. Similar syntheses of 7-aryl and 7-heterocyclyl triazolo[1,5-*a*]pyrimidines have been described, [35-37] for example, that of an antipyrine derivative .[38]

❖ **The diversity of 1,3-bifunctional synthons**

Examples of triazolo[1,5-*a*]pyrimidine **(Figure 7)** synthesis published in the relevant period are listed in Table 1, arranged according to the bifunctional synthons used and to the substituents entering the positions 5 and 7. Triazolo[1,5-*a*]pyrimidines are included in reviews dealing with heterocyclic synthesis by the use of enamines,[39] enamine-2-carboxylic esters [40] and ketene mercaptals.[41]

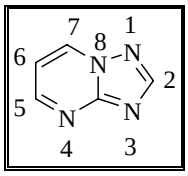


Figure 7

Table 1. Syntheses of triazolo [1, 5-a] pyrimidines from 1, 3-bifunctional synthons and 5-amino-1,2,4-triazoles.

Bifunctional Synthons	R-5[b]	R-7[b]	Bifunctional Synthons	R-5[b]	R-7[b]
1,3-Dialdehyde [42]	H	H	Enamine-2-carboxylate [59]	H	OH
2-Formylacetal [43]	H	H	Acetylenedicarboxylate [60]	CO_2Me	OH
1,3-Diacetal [44]	H	H	3-Ketocarboxylate [61]	R	OH
2-Formylvinyl ether [45]	H	H	3-Alkoxyacrylate [62]	OH	R
2-Formylvinylchloride [46]	H	R	Alkoxyalkylene malonate [63]	R	OH
3-Iminiovinylchloride [47]	H	R	2-Chloroacrylate [64]	OH	R
2-Formylenamine [48]	H	R	Malonic ester [65]	OH	OH
3-Iminioenamine [49]	H	R	Malonyl chloride [66]	OH	OH
3-Ketoaldehyde [50]	R	H	2-Acylketene mercaptal [67]	SR	R’
3-Ketoacetal [51]	R	H	2-Cyanoketene mercaptal [68]	SR	NH_2
3-Ketovinyl ether [52]	H	R	Alkoxyalkylene cyanoacetate [69]	R	NH_2
3-Ketovinyl sulfone [53c]	R	H	Alkoxyalkylene malonitrile[70]	R	NH_2
3-Ketoenamine [54]	H	R	2-Formylnitrile [71]	H	NH_2
1,3-Diketone [55]	R	R’	2-Cyanoenamine [72]	H	NH_2
3-Ketoalkyne [56]	R[d]	H	Malonitrile [73]	NH_2	NH_2
2-Formylcarboxylate [57]	R	OH	2-Thiocarbamylcarboxylate [74]	NHR	OH
2-Alkoxycarbonylacetal [58]	OH	H			

[a]or tautomeric form. [b]Substituents on C-5 and C-7, respectively; R and R’ mean (possibly substituted) alkyl, aryl, heterocyclyl and H; OH means hydroxy or tautomeric oxo form. [c]And regioisomeric 7-R compound. [d]Deoxyaltrose derivative relating C-glycosides.[75]

In recent years, 3-ketoenamines have growing interest as building blocks for 7-aryl triazolo[1,5-*a*]pyrimidines **(Figure 6 , Path A**).[76,77] They also serve to synthesize 7-heterocyclyl triazolo [1,5-*a*]pyrimidines.[78,79] In addition to usual *N*, *N*-dimethyl compounds also analogues having a free amino group can be used as in the synthesis of 7-trifluoromethyl derivatives.[80] Enaminones can be formed in situ, for instance, from dimedone and DMF dimethylacetal. [81]

In the course of the cyclization of the stable tetrafluorobenzoyl derivative **(23) (Figure 8)** fluorine at the *o*-position is involved in the reaction and is replaced to give trifluorobenzo triazolo[1,5-*a*]pyrimidine **(24).**[82] Acetonyl is introduced as substituent into the 7-position by the use of triketone heptan-2,4,6-trione.[83]

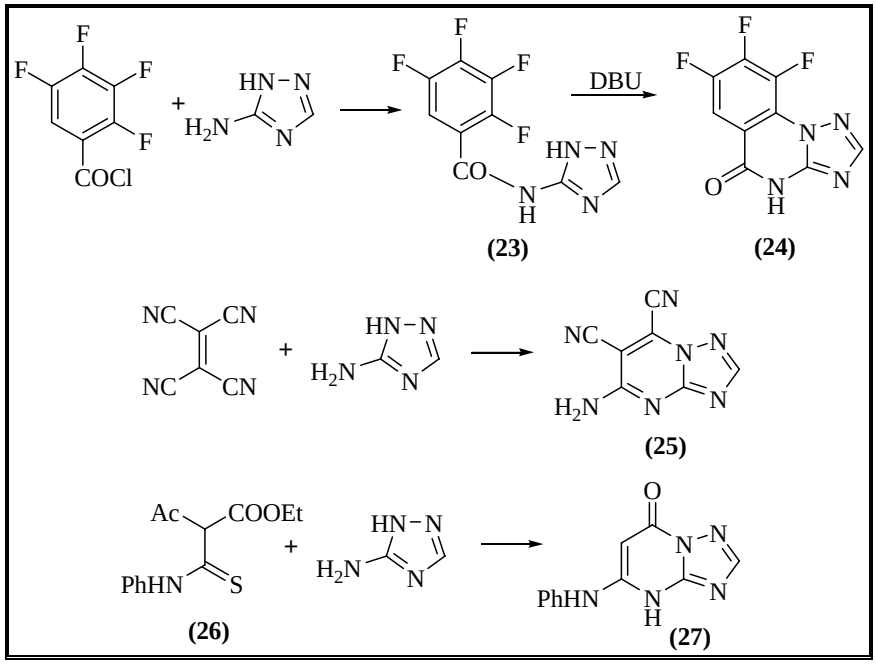


Figure 8

The electron acceptor tetracyanoethylene on interaction with amino-1,2,4-triazole first forms a charge transfer complex that after loss of hydrocyanic acid is transformed into dicyano triazolo[1,5-*a*]pyrimidine **(25)**.[84] Fusion of 1,4-naphthoquinone or indenone onto triazolo[1,5-*a*]pyrimidine can in a similar way be performed by the use of 2,3-dicyano-1,4-naphthoquinone or dicyanomethylene indane-1,3-dione, respectively. Another indeno triazolo[1,5-*a*]pyrimidine is accessible from triketone 2-

acetylindane1,3-dione. [85] On the other hand, acetoacetic ester **(26)** with 5-amino-1,2,4-triazole suffers ester group cleavage to form anilino triazolo[1,5-*a*]pyrimidine **(27)**.[86]

❖ **Other pyrimidine ring synthesis**

The annulation of pyrimidine onto the triazole ring can be accomplished by the use of heterocyclic precursors that can be regarded as masked 1,3-bifunctional reagents. This way, triacetic acid lactone **(27) (Figure 9)** reacts as a masked 1,3-diketone and transforms 5-amino-1,2,4-triazole to triazolo[1,5-*a*]pyrimidine **(28)** together with ring isomer **(29)** and decarboxylation product **(30)**. [87] Oxazolones play a similar part.[88-90] Thus, enol ether **(31)** behaves as a masked 3-ethoxyacrylate and yields, through intermediate **(32)**, benzamido TP **(33)** that, under harsher conditions, directly forms from compound. [30]

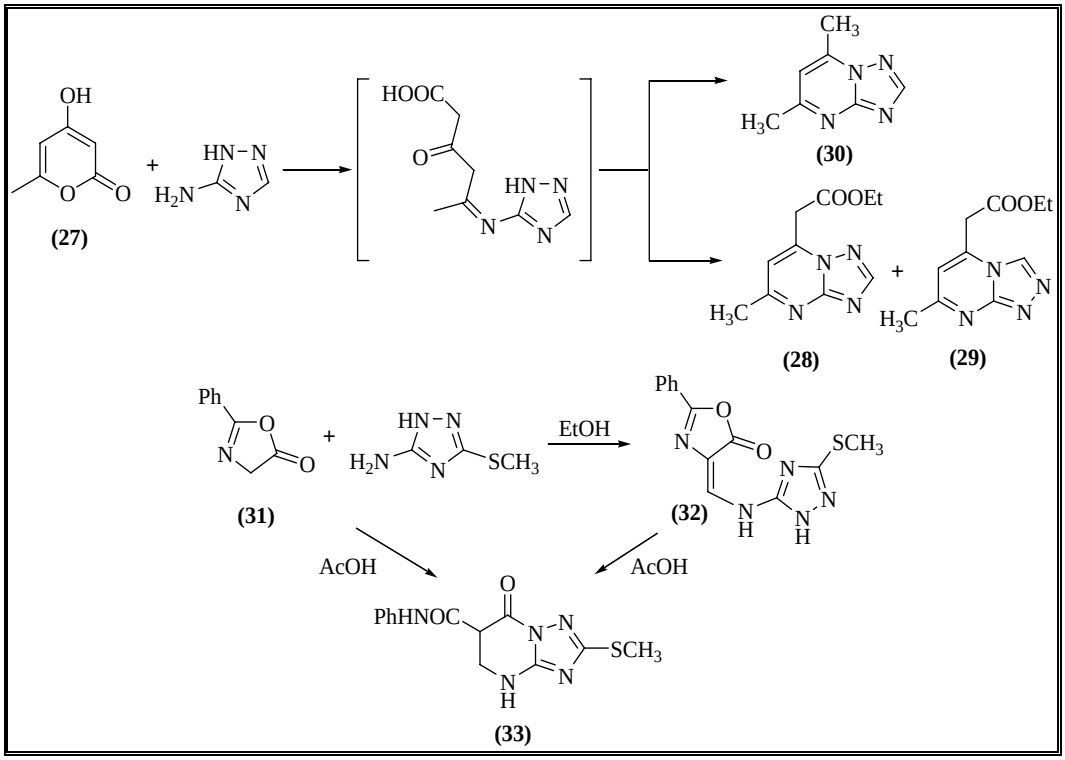


Figure 9

❖ **2-Hydrazinopyrimidines and one-carbon synthons**

A second common triazolo[1,5-*a*]pyrimidine synthesis consists in the condensation of a C_1-synthon with a 2-hydrazinopyrimidine derivative **(e.g., 34, Figure 10)** . A triazolo[4,3-a]-pyrimidine **(35)** initially forms that often can be isolated.[91] Harsher conditions allow it to isomerize to the target triazolo[1,5-*a*]pyrimidine **(36)** by Dimroth rearrangement.

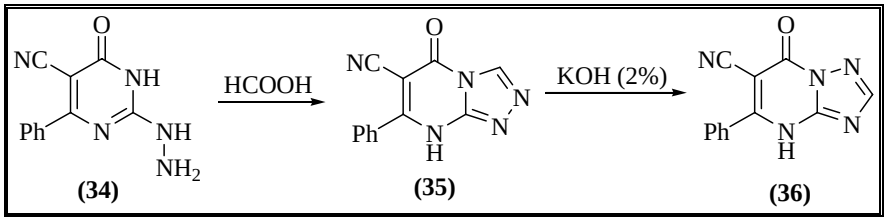


Figure 10

❖ **Other triazole ring synthesis**

Most cyclization of 2,3-diaminopyrimidones **(37)**[92] or corresponding quinazolones proceed with the participation of carboxylic acids or their derivatives (esters, anhydrides, chlorides, or orthoesters) as shown in **(Figure 11)**. Noncyclized or saturated intermediates **(38, 39)** can frequently be found during synthesis of triazolo[1,5-*a*]pyrimidines .

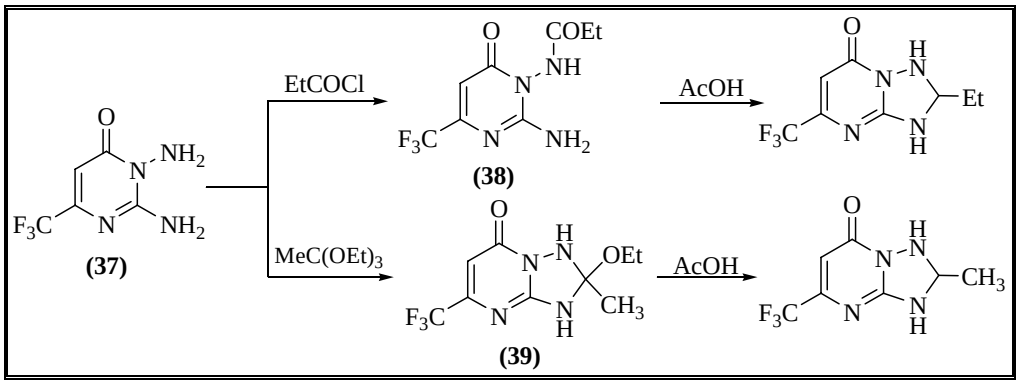


Figure 11

5.4 CURRENT RESEARCH WORK

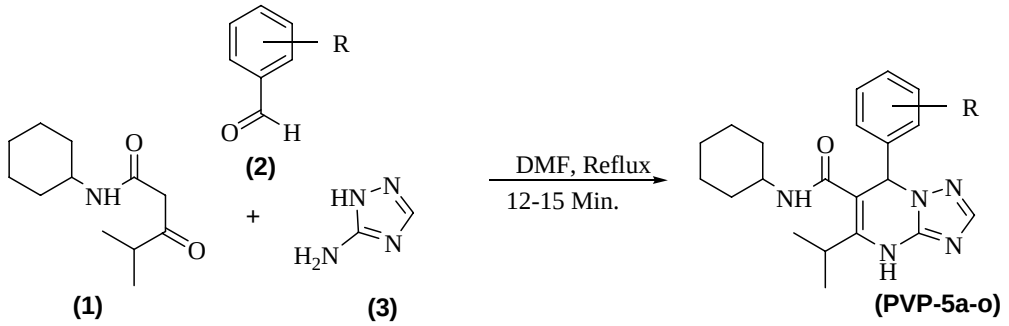
The biological importance of 1,2,4-triazolo[1,5-*a*]pyrimidines is well documented. Over the years, various substituted derivatives of these heterocycles have shown utility against a range of biological targets. For example, they have demonstrated activity against malaria and bronchospasm and shown activity as coronary vasodilators, antihypertensive agents, leishmanicides, antibiotics, adenosine A_{2a} antagonists, immunosuppressants, antitumor agents, fungicides, xanthine oxidase inhibitors, and phosphodiesterase inhibitors.

One of the synthetic pathways to 1,2,4-triazolo[1,5-*a*]pyrimidines is based on the Biginelli like cyclocondensation of aromatic aldehydes and acetoacetic acid derivatives with aminoazoles containing a guanidine fragment. There are literary data about the synthesis of triazolopyrimidines by treatment of 5-amino-1,2,4-triazole or 5-aminotetrazole with aldehydes and ethyl acetoacetate or cyclic *β*-diketones [93]. The cyclocondensations were realized by heating of the starting materials in ethanol with catalytic amounts of hydrochloric acid under reflux conditions [93a-c] or using DMF as solvent. [93d-e] The use of acetoacetamides in these or similar reactions has not been described.

However, the existing methods are suffered with some drawbacks, such as yield, time, product isolation.Recognizing these facts, we have synthesised a new series of *N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-aryl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide starting from, *N*-cyclohexyl-4-methyl-3-oxo-pentanamide.The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their antimicrobial activity.

5.5 RESULTS AND DISCUSSION

Scheme:-1 Synthesis of substituted triazolopyrimidines.



R= CH_3, OCH_3, Cl, F

Initially, the reaction of *N*-cyclohexyl-4-methyl-3-oxo-pentanamide (**1)** with appropriate aldehyde (**2)** and aminoazole **(3)** was refluxed in 0.4 mL of DMF for 12-15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid **(scheme 1)** affords the *N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives **(PVP-5a-o)** was obtained in excellent yield.

The structures of **(PVP-5a-o)** were established on the basis of their elemental analysis and spectral data (MS, IR, and ^{1}H NMR). The analytical data for **5a** revealed a molecular formula $C_{22}H_{29}N_5O$ (*m/z* 379). The ^{1}H NMR spectrum revealed a doublet at δ = 0.95-1.10 ppm assigned to isopropyl-CH_3, a multiplet at δ = 1.17 - 1.58 ppm assigned to the – (5xCH_2) protons, a singlet at δ = 2.23 ppm assigned to the –CH_3 a multiplet at δ = 3.18 - 3.28 ppm assigned to the isopropyl-CH protons , a doublet at δ = 3.34 - 3.40 ppm assigned to the -CH protons, a singlet at δ = 6.25 ppm assigned to the -CH protons, a multiplet at δ = 6.98 - 7.10 ppm assigned to the aromatic protons, a singlet at δ = 7.57 ppm assigned to the -CH protons of triazoloring, a singlet at δ = 7.72 – 7.75 ppm assigned to -NH protons, a singlet at δ = 9.66 ppm assigned to –CONH protons,

Table 1: Synthesis of substituted triazolopyrimidines.

Entry	R	Yield %	M.P.
PVP-5a	4-$CH_3C_6H_4$	92	252-254
PVP-5b	4-ClC_6H_5	91	260-263
PVP-5c	4-FC_6H_5	84	248-250
PVP-5d	4-$OCH_3C_6H_5$	90	245-247
PVP-5e	3-BrC_6H_5	86	255-257
PVP-5f	3,4-di-$OCH_3C_6H_3$	92	259-261
PVP-5g	3-ClC_6H_4	90	265-267
PVP-5h	C_6H_5	86	245-247
PVP-5i	4-OHC_6H_5	93	242-244
PVP-5j	2-ClC_6H_5	91	235-237
PVP-5k	2- $CH_3C_6H_5$	88	255-257
PVP-5l	2-OHC_6H_5	92	257-259
PVP-5m	3-$NO_2C_6H_5$	90	260-262
PVP-5n	4-$NO_2C_6H_5$	87	262-264
PVP-5o	3,5-di-$OCH_3C_6H_3$	85	256-258

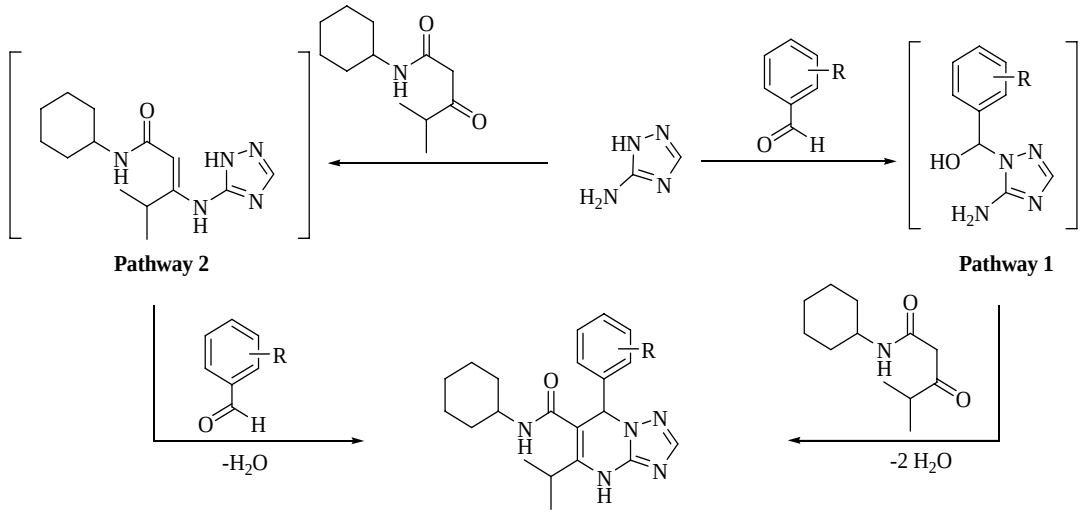


Figure 11: **Proposed mechanism for the formation of Triazolopyrimidine.**

The reaction mechanism of this three-component condensation is probably similar to the described [94] mechanism for the "classical" Biginelli reaction **(Pathway 1)**. The first step is a nucleophilic addition of N_2 of the aminoazole to a carbonyl carbon of aldehyde, followed by subsequent cyclization with acetoacetamide to form the dihydropyrimidine ring. An alternate sequence is also possible and cannot be excluded [95] **(Pathway 2)**, which is the initial formation of an enamine by reaction of aminoazole with the acetoacetamide followed by cyclocondensation. **(Figure 11)** The third alternative involving the formation of 2-benzylidene-*N*-cyclohexyl-4-methyl-3-oxo-pentanamide derivatives as intermediates requires the presence of a strong base [96] and is most likely not possible for the case described herein.

5.6 ANTIMICROBIAL SENSITIVITY TESTING

WELL DIFFUSION / AGAR CUP METHOD (Lt. General Raghunath D. 1998, Ashok Rattan, 1998; Patel R., Patel K. 2004,)

In vitro effectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective then many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive then using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Requirements

1. Young broth culture of a standard test organism
2. Sterile Mueller Hinton Agar plate
3. Solution of antimicrobial substance
4. Cup borer
5. Alcohol etc.

Inoculum preparation

Inoculum was prepared by selecting 4-5 colonies from slope of stock culture of the indicator organism and emulsifying them in a suitable broth. The inoculated broth was incubated at 37ºC till it equals turbidity of a 0.5 McFarland standard. This happens in 2-8 h.

Procedure

1. Inoculate test organism on the top of Mueller Hinton Agar plate with help of sterile swab. (it can be inoculated in melted agar also)
2. The swab was dipped in the inoculum and surface of plate was streaked with swab.
3. Streaking was repeated for 3 times and each time the plate was rotated at angle of 60º.
4. Sterilize the cup-borer make four cups of the diameter of 8-10 mm. at equal distance in the plate previously inoculated with seed culture.
5. The depth of well was 2.5-5.0 mm.
6. The wells have been clearly punched so the surrounding medium is not lifted when the plug was removed out.
7. The plates were incubated at 37ºC for 24 h. Then the zone of inhibition measured and the size of zone cited in table.

❖ *Antibiotic Sensitivity Assay*

(Concentration250/500/ 1000 µG/ml)

Sr. No.	COD E No.	*Pseudomonas aeruginosa*			*Proteus vulgaris*			*Escherichia coli*			*Staphylococcu saureus*			*Candida albicans*		
		250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
1.	**5a**	1.2	1.4	2	1.1	1.3	1.6	R	R	R	R	1	1.2	R	1.2	1.5
2.	**5b**	1.2	1.3	1.7	1.1	1.4	1.6	R	R	R	1.2	1.3	1.6	1	1.3	1.8
3.	**5c**	1.5	1.3	1.5	R	1.1	1.4	1.1	1.2	1.3	R	1	1.2	1.1	1.5	2
4.	**5d**	1.6	1.2	1.4	1	1.3	1.6	R	R	R	1.3	1.4	1.6	1.1	1.4	1.8
5.	**5e**	1.4	1.3	1.6	R	1.2	1.4	R	R	R	1.2	1.4	1.6	1	1.3	1.7
6.	**5f**	1.3	1.5	1.9	1	1.2	1.3	1.3	1.4	1.7	1.1	1.4	1.5	1.1	1.4	1.8
7.	**5g**	1.9	1.5	1.8	1.1	1.4	1.7	1.2	1.4	1.8	1.4	1.5	2	1.2	1.4	1.7
8.	**5h**	1.4	1.7	2	1.1	1.3	1.5	1.1	1.1	1.3	1.4	1.6	2	1.1	1.3	1.5
9.	**5i**	1.2	1.3	1.5	R	R	R	R	R	R	1.3	1.4	1.7	R	1.3	1.7
10.	**5j**	1.7	1.9	2	1.8	1.8	2	1.1	1.8	1.8	1.5	1.7	1.9	1.8	1.8	2
11.	**5k**	1.1	1.2	1.3	R	1	1.2	1.1	1.2	1.4	1.1	1.2	1.5	1.1	1.5	1.9
12.	**5l**	1.3	1.4	1.9	1.3	1.7	2.1	1.2	1.5	2	1.1	1.5	1.9	1.1	1.4	1.6
13.	**5m**	1.2	2	1.5	1.1	1.4	1.9	1.3	1.4	1.9	1.2	1.6	2	1.2	1.5	2
14.	**5n**	R	R	R	1.1	1.3	1.7	1.1	1.3	1.6	R	R	R	1.1	1.4	1.8
15.	**5o**	1.4	1.6	2	1	1.2	1.4	R	R	R	1.1	1.2	1.5	1.2	1.5	2
16.	**A**	1.8			1.8			1.9			1.9			-		
17.	**CPD**	2.2			2.1			2.1			2.2			-		
18.	**GF**	1.8			1.9			2.0			2.0			-		
19.	**GRF**	-			-			-			-			2.6		
20.	**FLC**	-			-			-			-			2.8		

Note: Zone of inhibition interpretation is as follows.

1. ZONE SIZE <1.0 C.M.- RESISTENT(R)
2. ZONE SIZE 1.0 To 1.5 – INTERMEDIATE
3. ZONE SIZE >1.5 - SENSITIVE

STD Antibiotic Sensitivity Assay Concentration 40 µG/ml

A: AMPICILLIN
CPD: CEFPODOXIME
GF: GATIFLOXACIN
GRF: GRESIOFULVIN
FLC: FLUCONAZOLE

5.7 CONCLUSION

In summary, we have described the synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidines derivatives in excellent yields. The reaction of *N*-cyclohexyl-4-methyl-3-oxo-pentanamide (**1)** with appropriate aldehyde (**2)** and aminoazole **(3)** was refluxed in DMF affords the *N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives was obtained in excellent yield. All the synthesized compounds are evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-5a-o** showed moderate to potent activity. The compounds **PVP- 5j** and **5l** showed comparatively good activity against all the bacterial strains.

5.8 EXPERIMENTAL SECTION

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. Melting points were measured in open capillaries and are uncorrected.

- **General procedure for the synthesis of substituted Triazolopyrimidines 5a-o.**

A mixture of the aminoazole (0.01 mol), *N*-cyclohexyl-4-methyl-3-oxo-pentanamide (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) was refluxed in 0.4 mL of DMF for 12-15 min. After cooling, methanol (~10 mL) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid triazolopyrimidine products **PVP- 5a-o**, which were crystallized from ethanol and subsequently dried in air.

- **Spectral data of the synthesized compounds**

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-*p*-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide(PVP-5a):** White solid; R_f 0.64 (9:1Chloroform: Methanol); IR (KBr): 3327, 3093, 2939, 1648, 1586, 1492, 1261, 1069 cm^{-1}; ^1H NMR: δ 0.95-1.10 (d, 6H, 2 x $^{i}prCH_3$), 1.17-1.58 (m, 10H, 5 x CH_2), 2.23 (s, 1H, CH_3) 3.18-3.28 (m, 1H, $^{i}prCH$), 3.34-3.40 (s, 1H, CH), 6.25 (s, 1H, CH), 6.98-7.01 (d, 2H, Ar-H) 7.07-7.10 (d, 2H, Ar-H), 7.57 (s, 1H, CH) 7.72-7.75 (s, 1H, NH) 9.66 (s, 1H, CONH); ^{13}C NMR: δ 19.49, 19.74, 20.69, 24.60, 28.44, 30.52, 32.19, 47.51, 60.49, 102.81, 126.92, 128.64, 137.01, 137.63, 141.85, 148.42, 149.30, 165.30 ; MS (*m/z*): 379 (M^+); Anal. Calcd for $C_{22}H_{29}N_5O$: C, 69.63; H, 7.70; N, 18.45; Found: C, 69.58; H, 7.65; N, 18.52.

7-(4-chlorophenyl)-*N*-cyclohexyl-4,7-dihdro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5b): White solid; R_f 0.62 (9:1Chloroform: Methanol); IR (KBr): 3271, 3215, 3093, 3051, 2933, 2654, 1662, 1593, 1437, 1247, 1076 cm^{-1}; ^{13}C NMR: δ 19.43, 19.74, 24.60, 28.54, 30.59, 32.15, 47.59, 60.09, 99.49, 102.25, 128.09, 128.83, 132.75, 139.17, 142.17, 148.43, 149.68, 165.11, 177.08 ; MS (*m/z*): 399 (M^+); Anal. Calcd for $C_{21}H_{26}ClN_5O$: C, 63.07; H, 6.55; N, 17.51; Found: C, 63.10; H, 6.45; N, 17.42.

***N*-cyclohexyl-7-(4-fluorophenyl)-4,7-dihydro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5c):** White solid; R_f 0.60 (9:1Chloroform: Methanol); IR (KBr): 3300, 3215, 3093, 3051, 2933, 2674, 1662, 1593, 1437, 1297, 1076 cm^{-1}; ^1H NMR: δ 1.009-1.114 (d, 6H, 2 x $^{i}prCH_3$), 1.19-1.56 (m, 10H, 5 x CH_2), 3.17-3.26 (m, 1H, $^{i}prCH$), 3.31-3.42 (m, 1H, CH), 6.31 (s, 1H, CH), 7.09-721 (m, 4H, Ar-H), 7.59 (s, 1H, CH), 7.70-7.73 (s, 1H, NH), 9.73 (s, 1H, CONH); MS (*m/z*): 383 (M^+); Anal. Calcd for $C_{21}H_{26}FN_5O$: C, 65.78; H, 6.83; N, 18.26; Found: C, 65.68; H, 6.75; N, 18.32.

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-(4-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5d):** White solid; R_f 0.65 (9:1Chloroform: Methanol);IR (KBr): 3281, 3115, 3093, 3051, 2923, 2654, 1662, 1593, 1437, 1257, 1061 cm^{-1}; MS (*m/z*): 396 (M^+); Anal. Calcd for $C_{22}H_{29}N_5O_2$: C, 66.81; H, 7.39; N, 17.71; Found: C, 66.78; H, 7.30; N, 17.62.

7-(3-bromophenyl)-*N*-cyclohexyl-4,7-dihdro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5e): White solid; R_f 0.61 (9:1Chloroform: Methanol); IR (KBr): 3291, 3215, 3093, 3021, 2933, 2654, 1662, 1593, 1437, 1247, 1048 cm^{-1}; MS (*m/z*): 444 (M^+); Anal. Calcd for $C_{21}H_{26}BrN_5O$: C, 56.76; H, 5.90; N, 17.98; Found: C, 56.68; H, 5.85; N, 17.82.

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-(3,4-di-methoxyphenyl)-1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (PVP-5f):**White solid; R_f 0.64 (9:1Chloroform: Methanol); IR (KBr): 3276, 3265, 3083, 3059, 2933, 2654, 1662, 1593, 1247, 1076 cm^{-1}; MS (*m/z*): 425 (M^+); Anal. Calcd for $C_{23}H_{31}N_5O_3$: C, 64.92; H, 7.34; N, 16.46; Found: C, 64.88; H, 7.25; N, 16.32.

7-(3-chlorophenyl)-*N*-cyclohexyl-4,7-dihdro-5-isopropyl-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (PVP-5g): yellow solid; R_f 0.64 (9:1Chloroform: Methanol); IR (KBr): 3271, 3215, 3093, 3045, 2933, 2854, 1662, 1518, 1492, 1244, 1089 cm^{-1}; MS (*m/z*): 399 (M^+); Anal. Calcd for $C_{21}H_{26}ClN_5O$: C, 63.07; H, 6.55; N, 17.51; Found: C, 63.13; H, 6.45; N, 17.52.

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5h):** White solid; R_f 0.62 (9:1Chloroform: Methanol);IR (KBr): 3300, 3215, 3093, 3051, 2933, 2654, 1662, 1593, 1437, 1247, 1056 cm^{-1}; MS (*m/z*): 365 (M^+); Anal. Calcd for $C_{21}H_{27}N_5O$: C, 69.01; H, 7.45; N, 19.16; Found: C, 69.10; H, 7.55; N, 19.03.

***N*-cyclohexyl-4,7-dihdro-7-(4-hydroxyphenyl)-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5i):** White solid; R_f 0.62 (9:1Chloroform: Methanol); IR (KBr): 3261, 3215, 3073, 3051, 2933, 2634, 1672, 1593, 1437, 1247, 1086 cm^{-1}; MS (*m/z*): 381 (M^+); Anal. Calcd for $C_{21}H_{27}N_5O_2$: C, 66.12; H, 7.13; N, 18.36; Found: C, 66.13; H, 7.15; N, 18.42.

7-(2-chlorophenyl)-*N*-cyclohexyl-4,7-dihdro-5-isopropyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (PVP-5j): White solid; R_f 0.60 (9:1Chloroform: Methanol); IR (KBr): 3241, 3215, 3193, 3051, 2933, 2654, 1662, 1553, 1437, 1247,

1047 cm^{-1}; MS (*m/z*): 399 (M^+); Anal. Calcd for $C_{21}H_{26}ClN_5O$: C, 63.07; H, 6.55; N, 17.51; Found: C, 63.13; H, 6.48; N, 17.56.

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-*o*-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide(PVP-5k):** White solid; R_f 0.64 (9:1Chloroform: Methanol);IR (KBr): 3271, 3215, 3093, 3051, 2933, 2654, 1662, 1593, 1437, 1247, 1076 cm^{-1}; MS (*m/z*): 379 (M^+); Anal. Calcd for $C_{22}H_{29}N_5O$: C, 69.63; H, 7.70; N, 18.45; Found: C, 69.68; H, 7.75; N, 18.54.

***N*-cyclohexyl-4,7-dihdro-7-(2-hydroxyphenyl)-5-isopropyl-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (PVP-5l):** White solid; R_f 0.62 (9:1Chloroform: Methanol); IR (KBr): 3301, 3215, 3073, 3041, 2933, 2664, 1682, 1593, 1437, 1247, 1076 cm^{-1}; MS (*m/z*): 381 (M^+); Anal. Calcd for $C_{21}H_{27}N_5O_2$: C, 66.12; H, 7.13; N, 18.36; Found: C, 66.07; H, 7.16; N, 18.24.

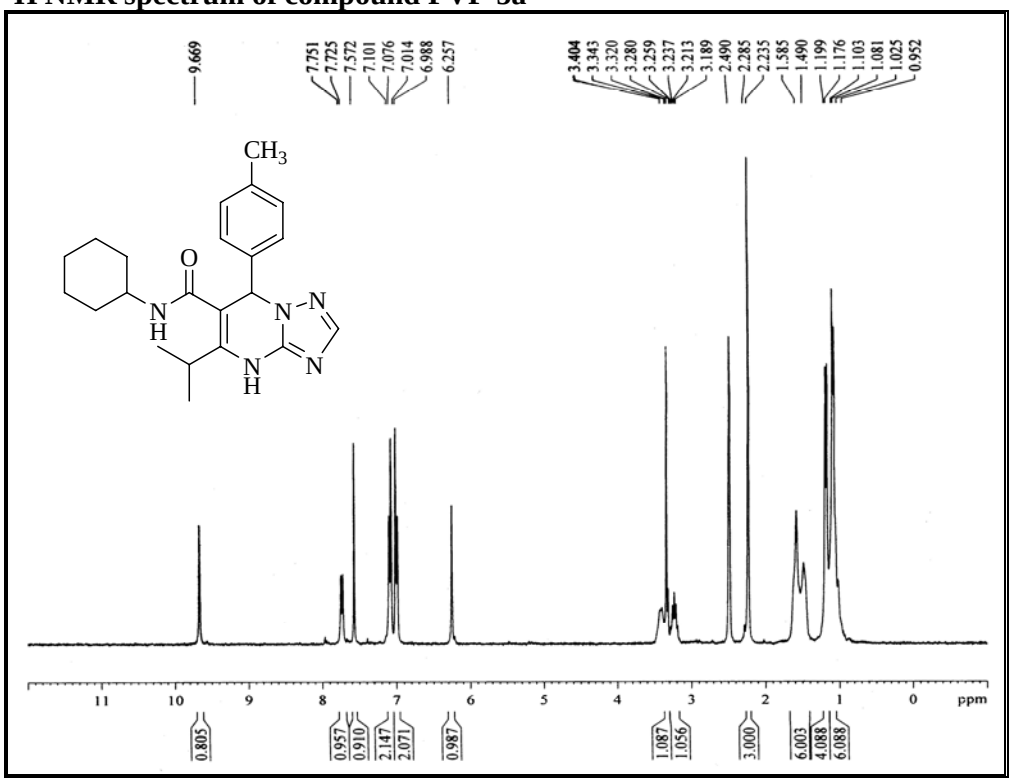
***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-(4-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (PVP-5m):** White solid; R_f 0.60 (9:1Chloroform: Methanol); IR (KBr): 3271, 3093, 3051, 2933, 1662, 1593, 1437, 1247, 1057 cm^{-1}; MS (*m/z*): 410 (M^+); Anal. Calcd for $C_{21}H_{26}N_6O_3$: C, 61.45; H, 6.38; N, 20.47; Found: C, 61.38; H, 6.35; N, 20.52.

***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-(3-nitrophenyl)-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide (PVP-5n):** White solid; R_f 0.65 (9:1Chloroform: Methanol); IR (KBr): 3300, 3215, 3193, 3051, 2833, 2654, 1622, 1593, 1247, 1076 cm^{-1}; MS (*m/z*): 410 (M^+); Anal. Calcd for $C_{21}H_{26}N_6O_3$: C, 61.45; H, 6.38; N, 20.47; Found: C, 61.49; H, 6.28; N, 20.42.

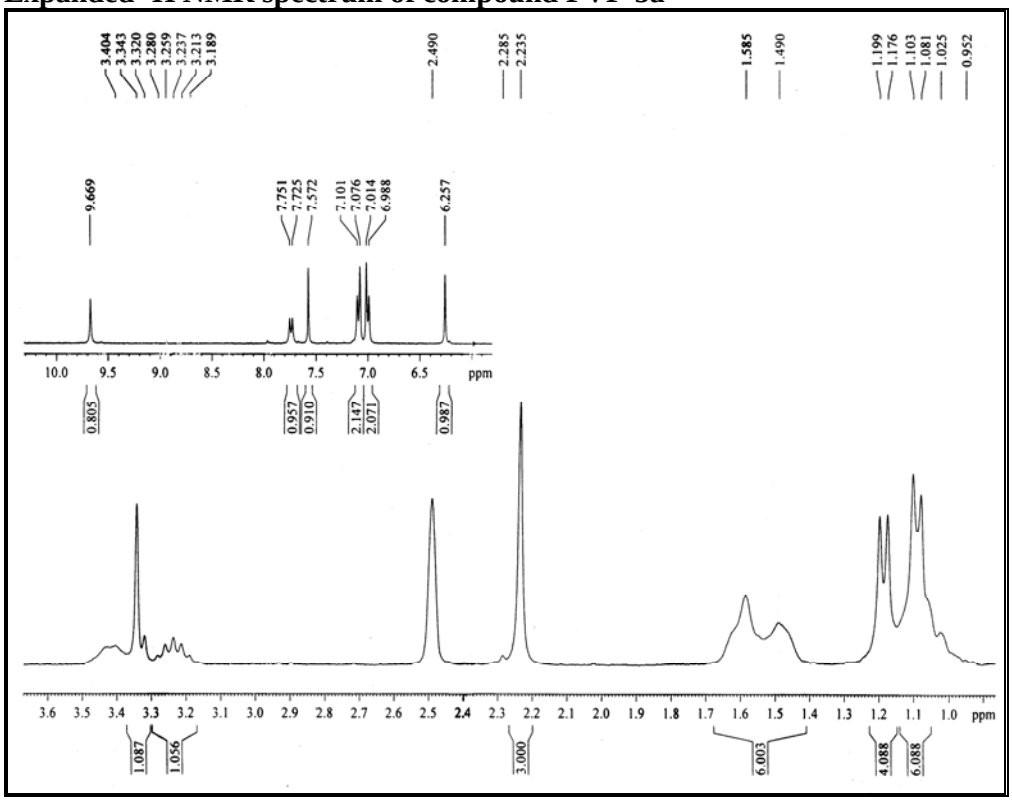
***N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-(3,4-di-methoxyphenyl)-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxamide (PVP-5o):**White solid; R_f 0.63 (9:1Chloroform: Methanol); IR (KBr): 3271, 3215, 3183, 3051, 2910, 2654, 1642, 1437, 1247, 1067 cm^{-1}; MS (*m/z*): 425 (M^+); Anal. Calcd for $C_{23}H_{31}N_5O_3$: C, 64.92; H, 7.34; N, 16.46; Found: C, 64.83; H, 7.28; N, 16.42.

❖ **Spectral representation of synthesized compounds**

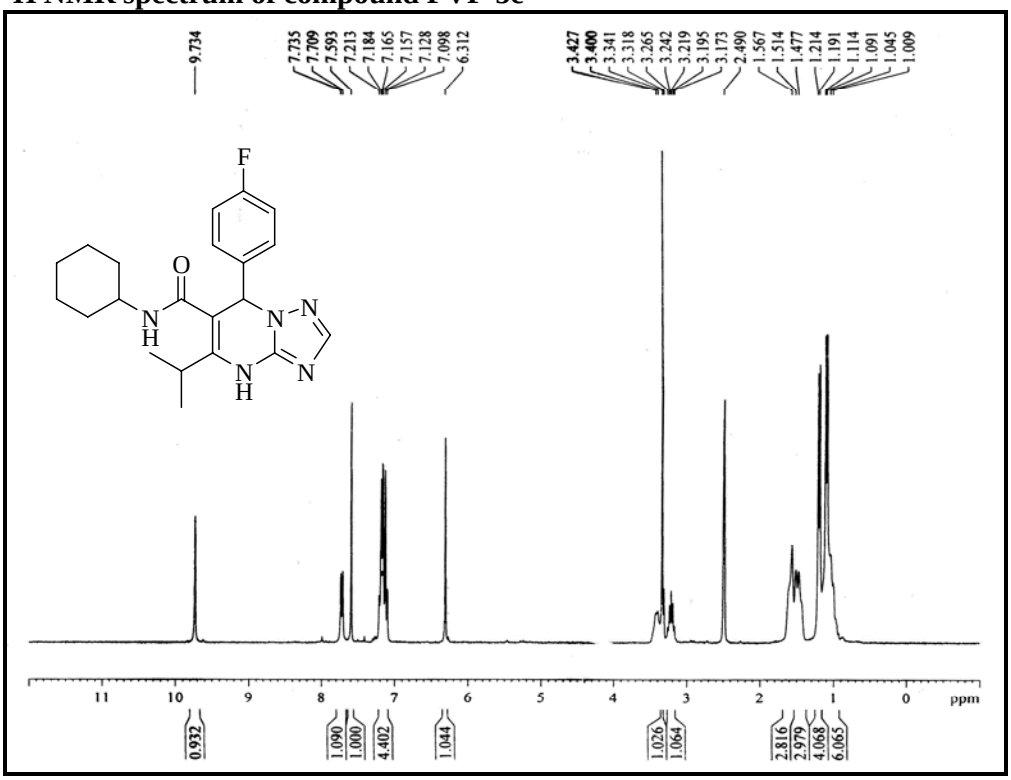
^{1}H NMR spectrum of compound PVP-5a



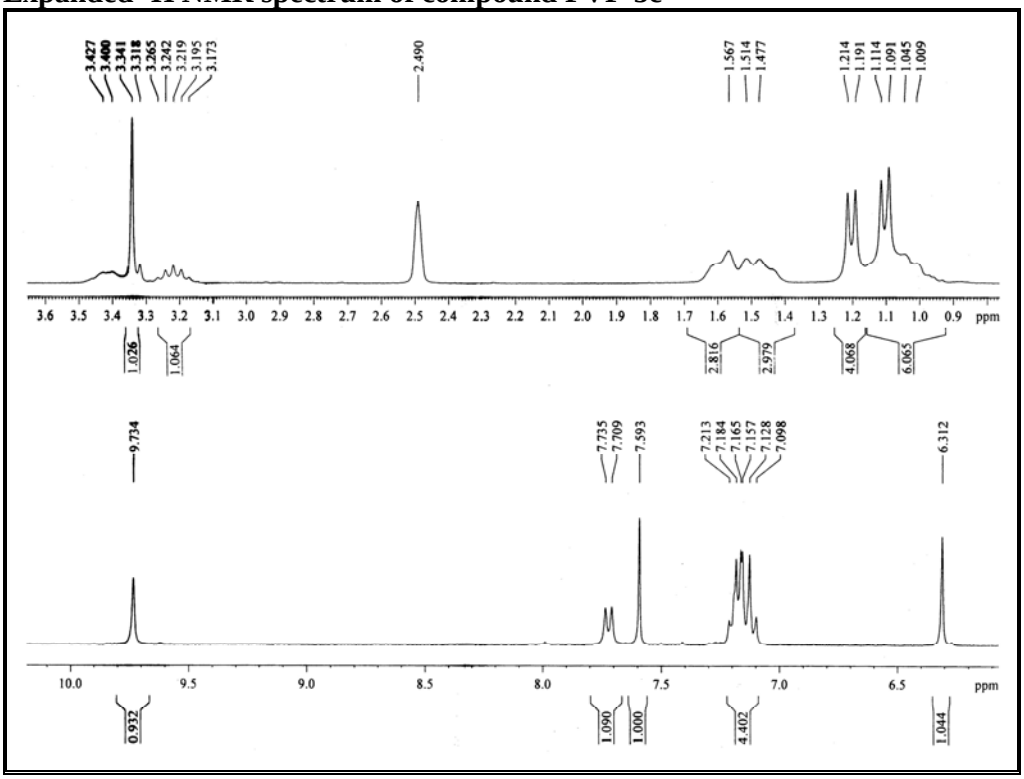
Expanded ^{1}H NMR spectrum of compound PVP-5a



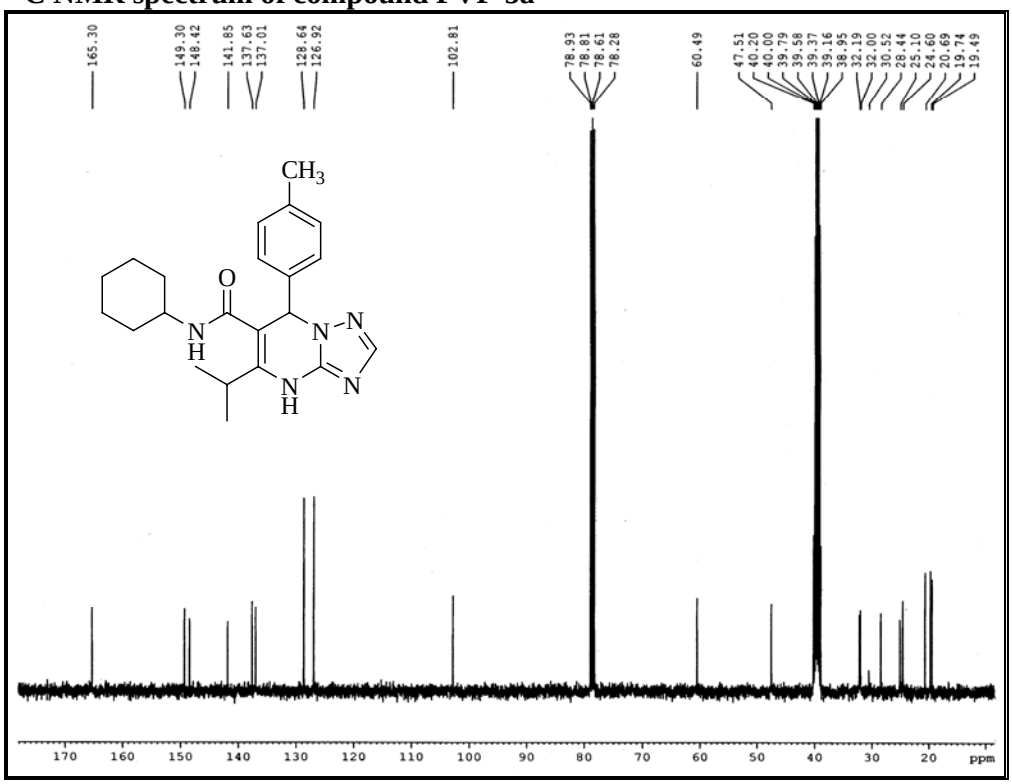
^{1}H NMR spectrum of compound PVP-5c



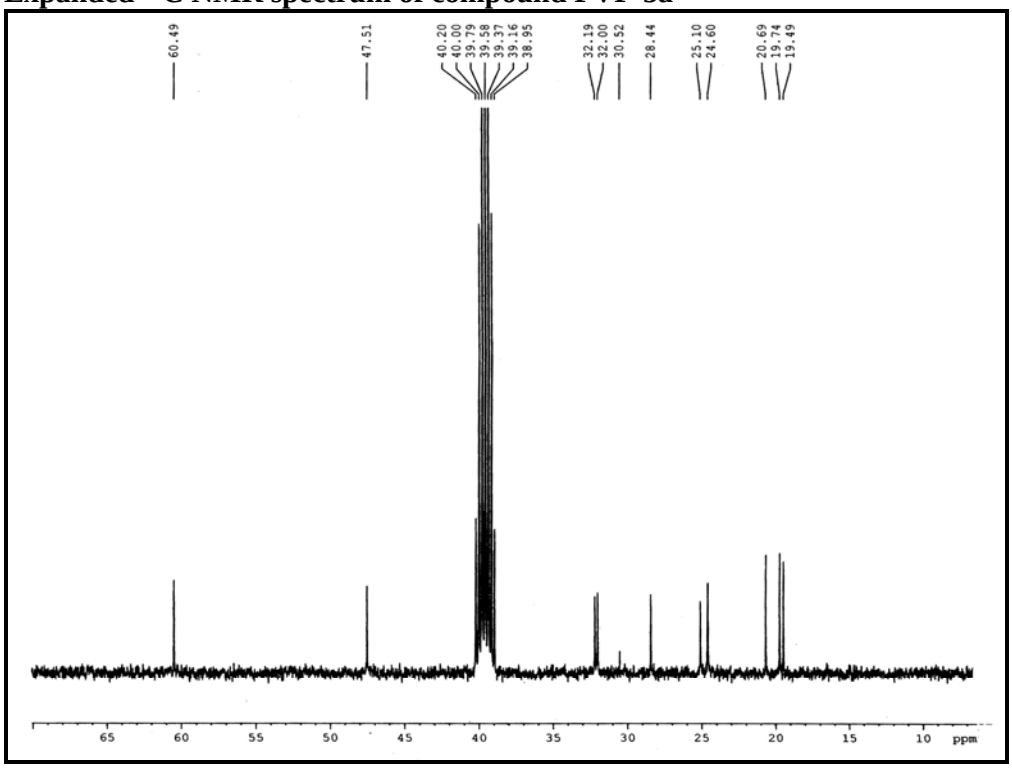
Expanded ^{1}H NMR spectrum of compound PVP-5c



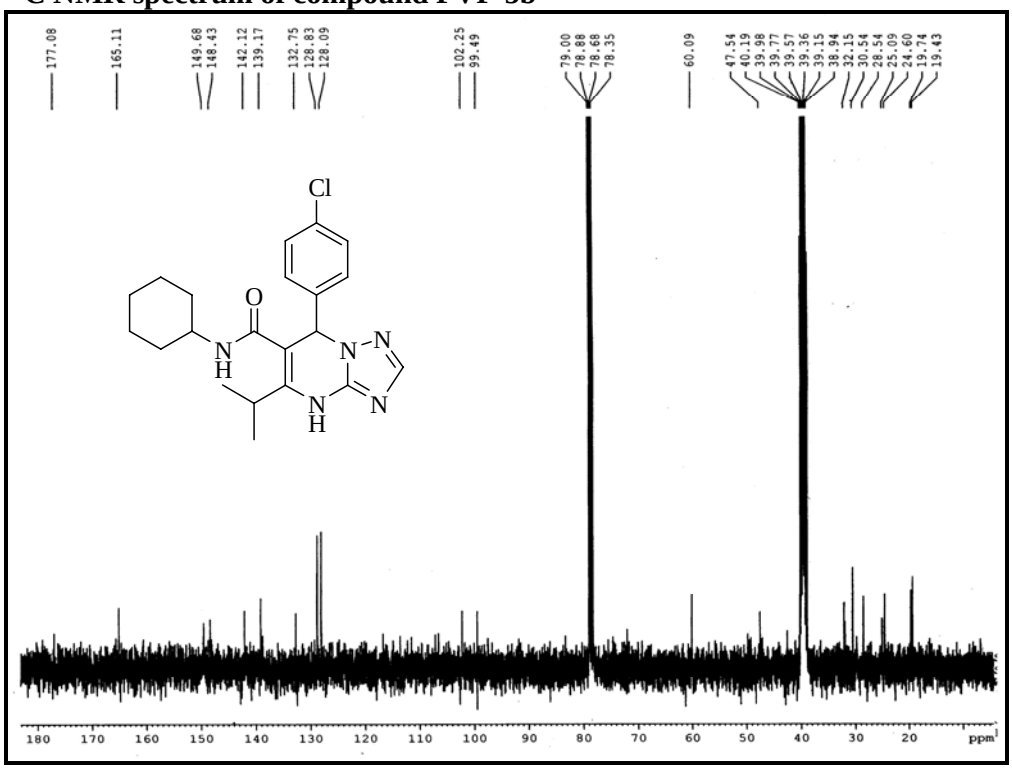
^{13}C NMR spectrum of compound PVP-5a



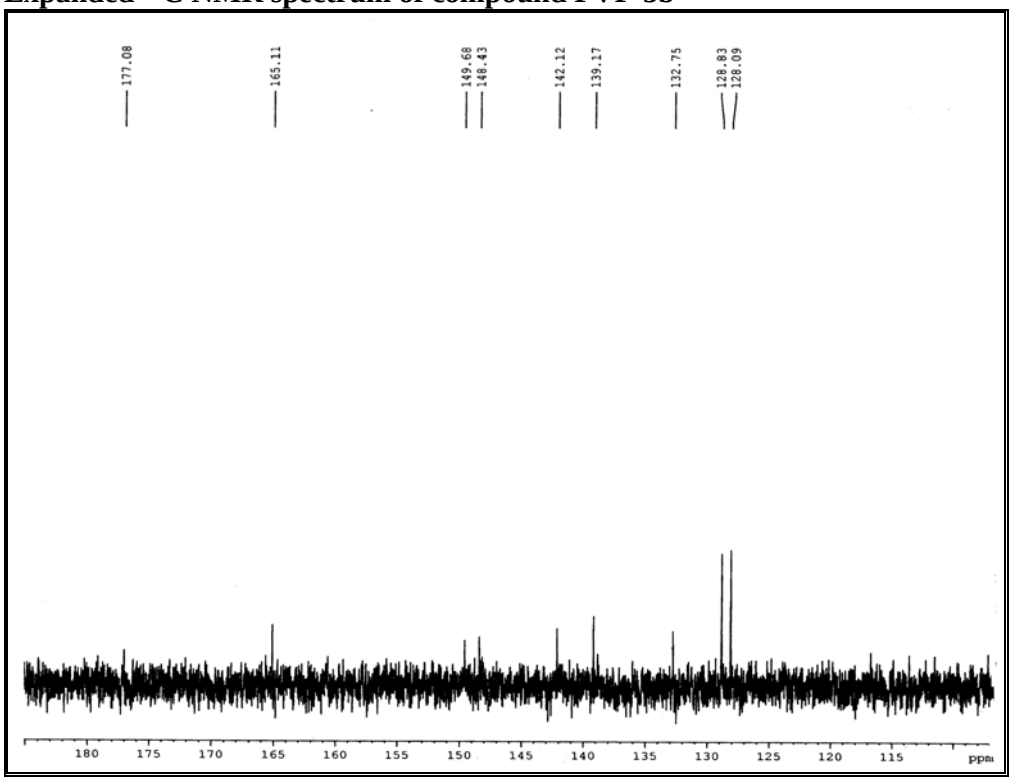
Expanded ^{13}C NMR spectrum of compound PVP-5a



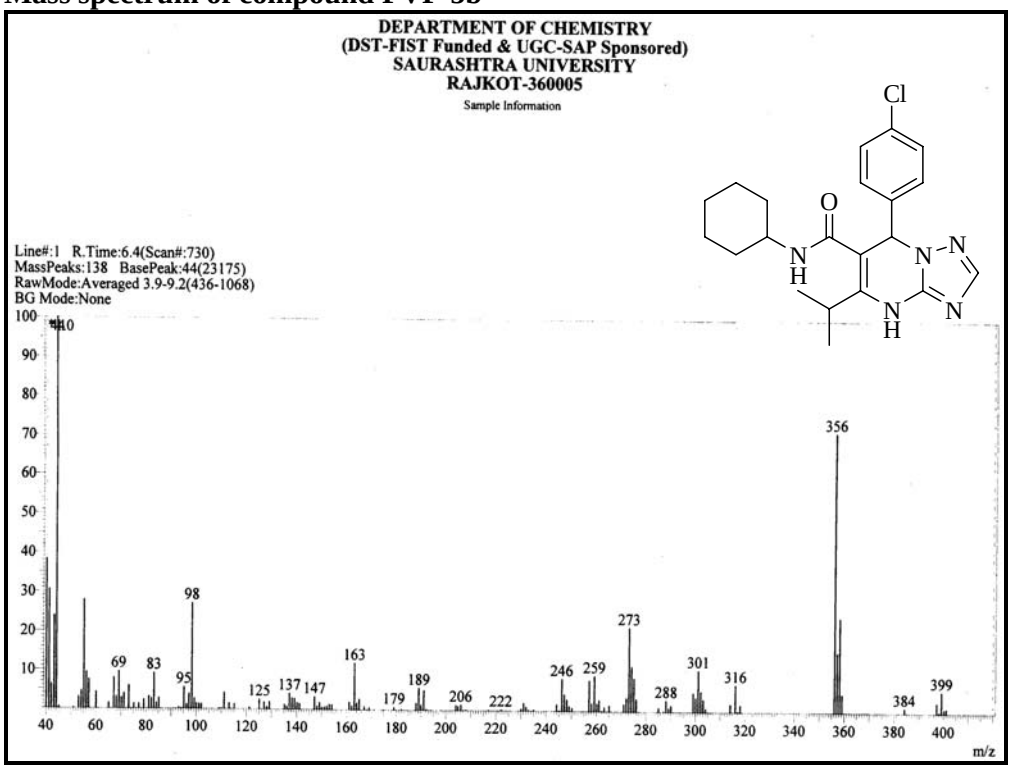
^{13}C NMR spectrum of compound PVP-5b



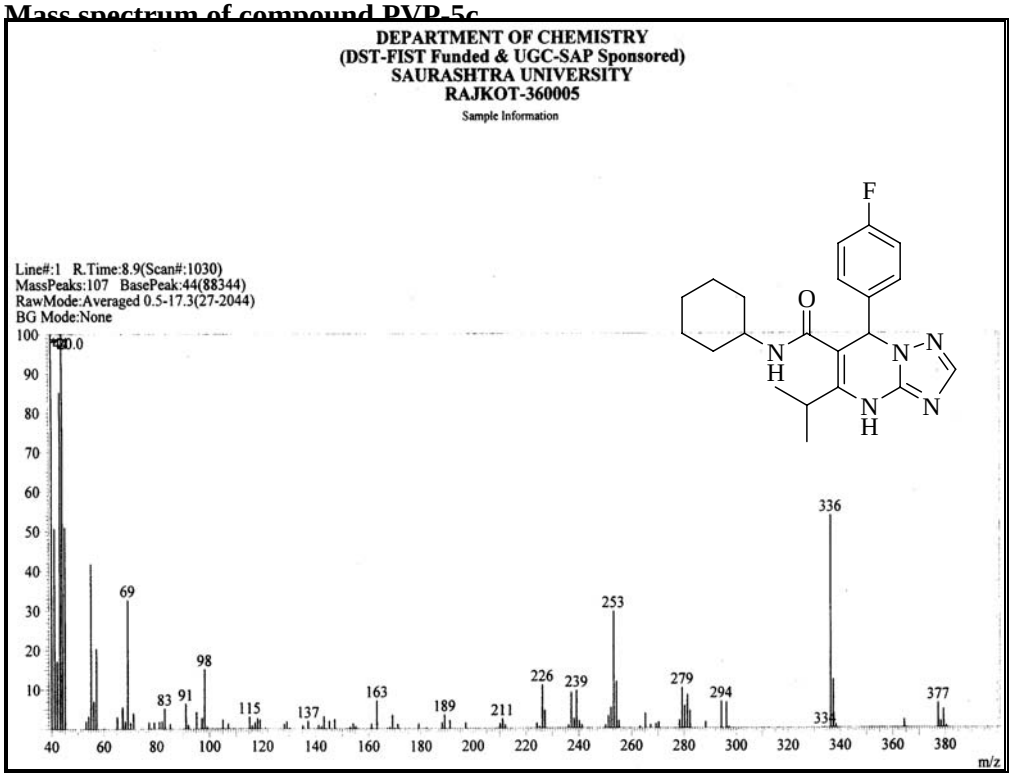
Expanded ^{13}C NMR spectrum of compound PVP-5b



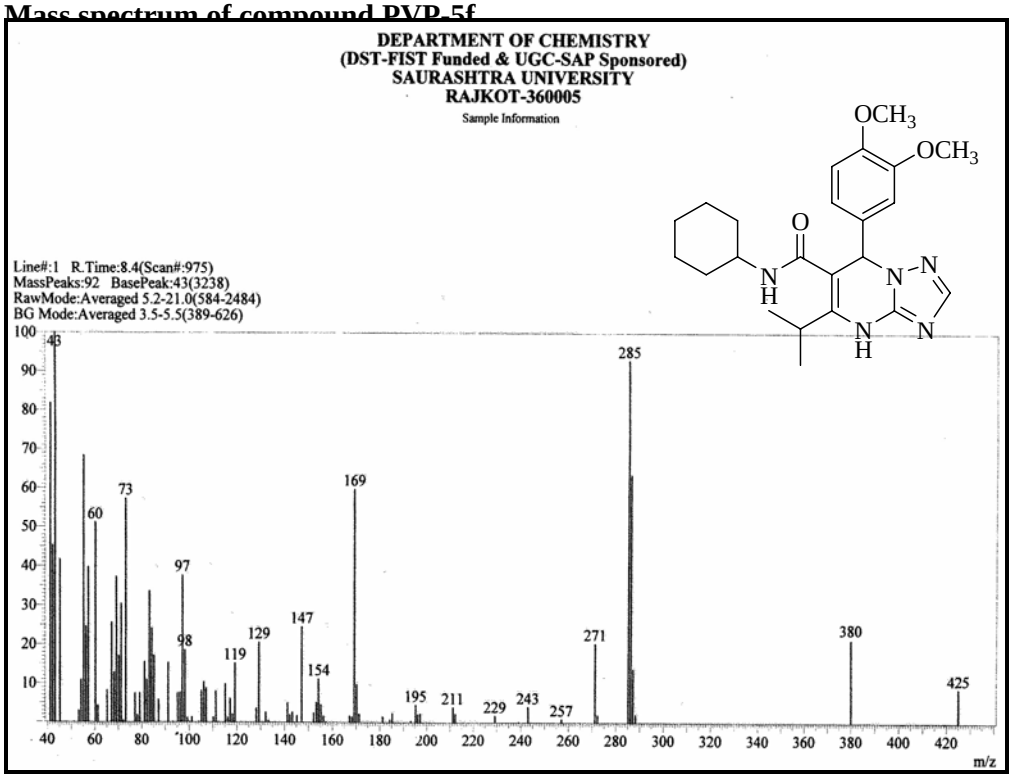
Mass spectrum of compound PVP-5b



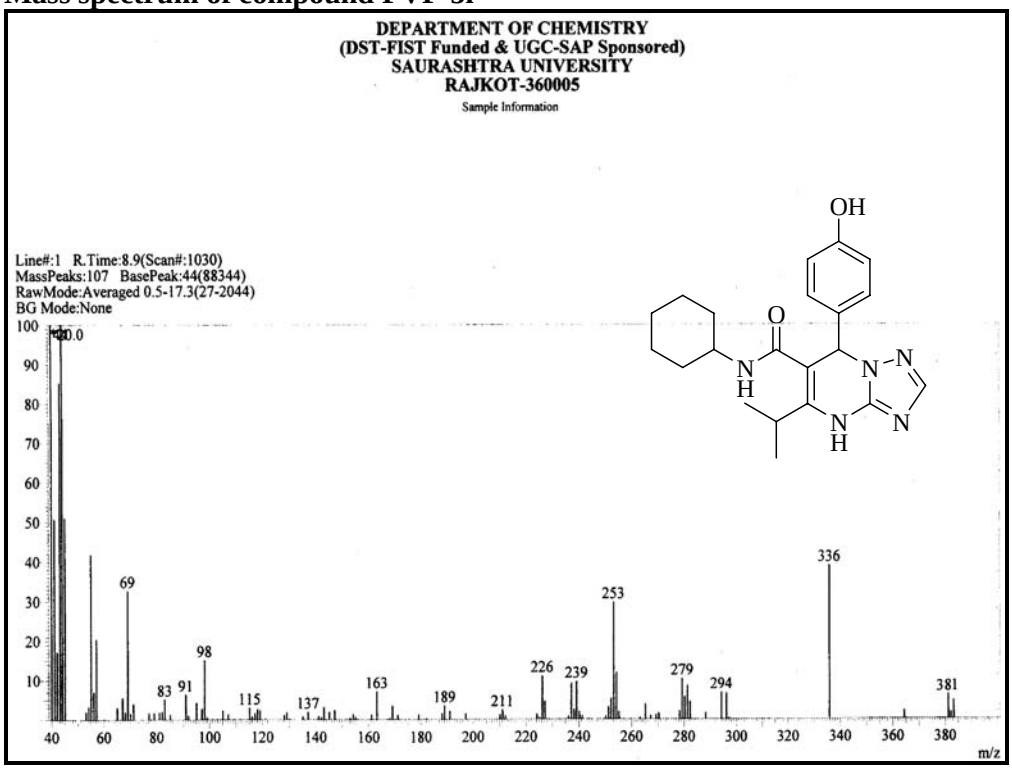
Mass spectrum of compound PVP-5c



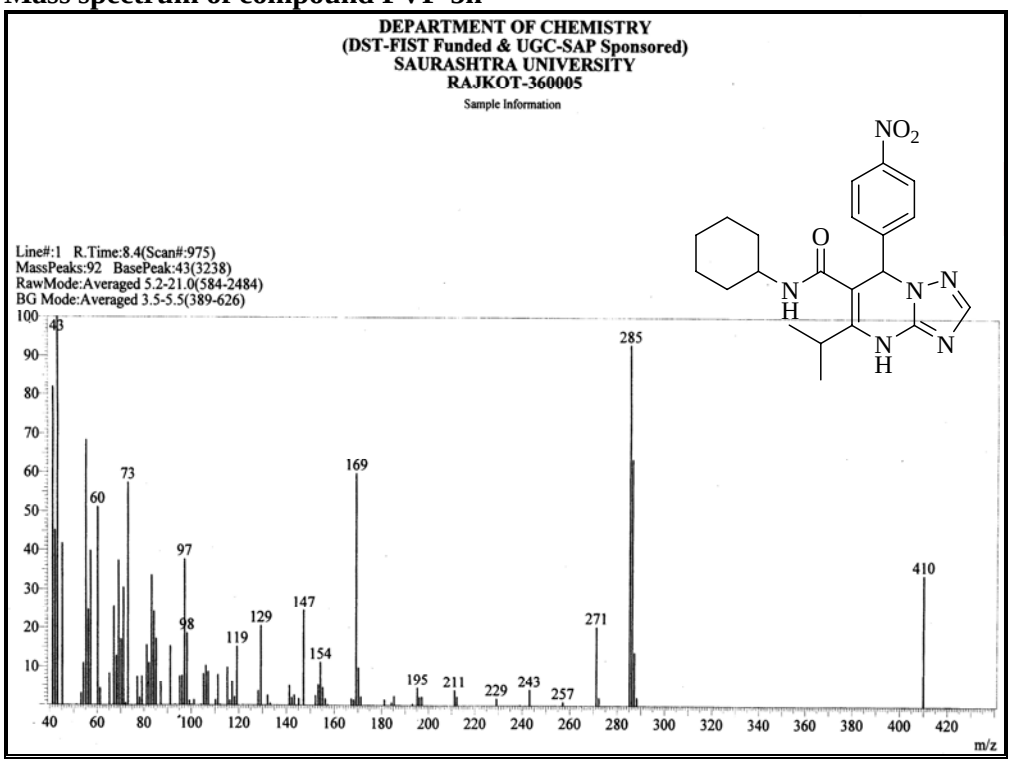
Mass spectrum of compound PVP-5f



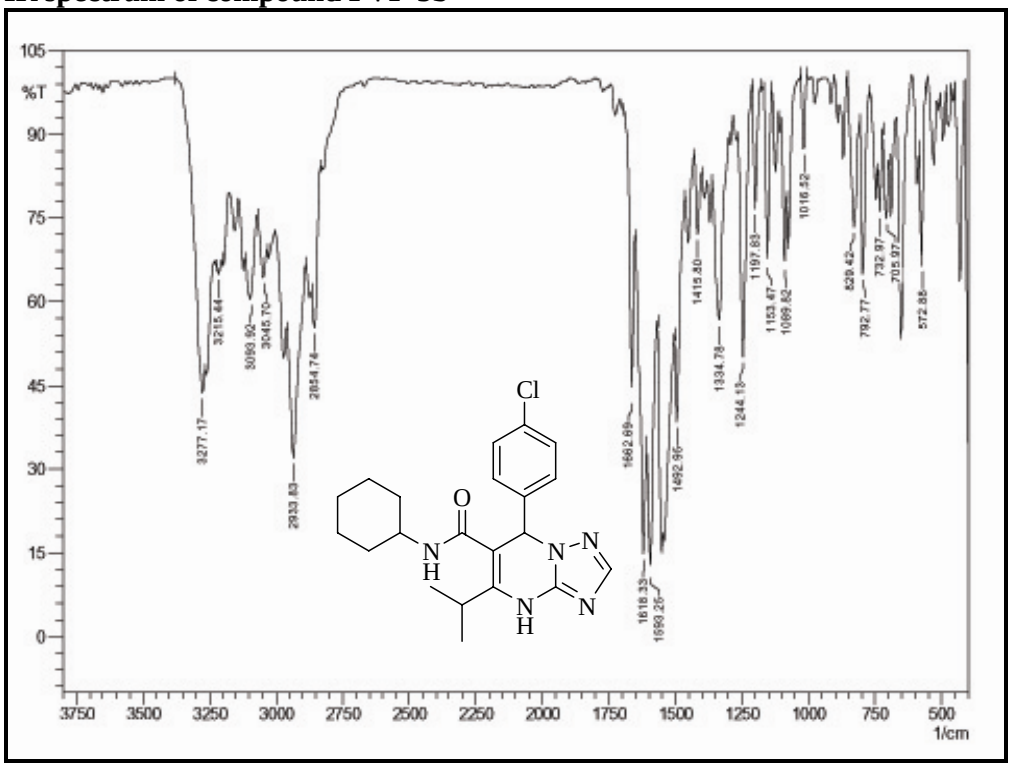
Mass spectrum of compound PVP-5i



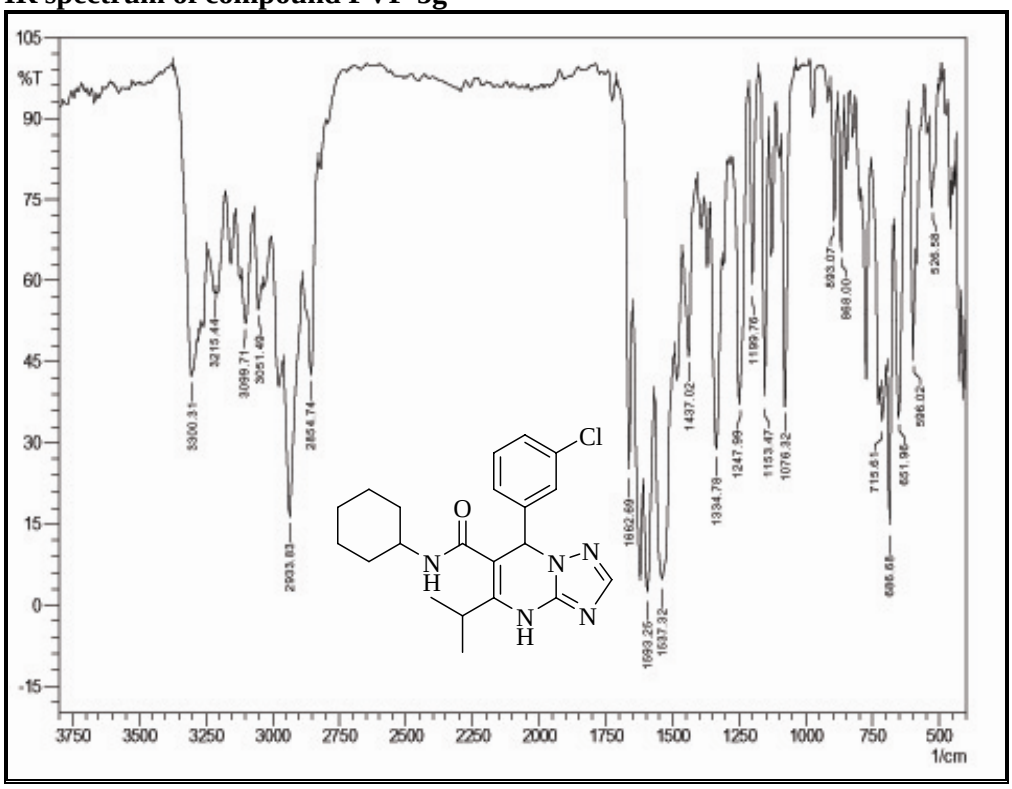
Mass spectrum of compound PVP-5n



IR spectrum of compound PVP-5b



IR spectrum of compound PVP-5g



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Research Summary

The work presented in the Thesis entitled "**Studies on Pharmacologically active Heterocycles**" can be summarized as below.

Chapter 1, we have described the synthesis of substituted pyrimidine derivatives in excellent yields. The reaction of various ketene dithioacetals with guanidine nitrate in refluxing methanolic sodium methoxide or ethanolic sodium ethoxide affords the 2-amino-4-isopropyl-6-alkoxy-*N*-arylpyrimidine-5-carboxamide derivatives with good yields. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-1a-t** showed moderate to potent activity. The compounds **PVP -1i, 1k** and **1l** showed comparatively good activity against all the bacterial strains.

Chapter 2, we have demonstrated 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. All the synthesized compounds were evaluated for their anti microbial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-2a-t** showed moderate to potent activity. The compounds **PVP- 2h** and **2g** showed comparatively good activity against all the bacterial strains.

Chapter 3, we have explained the synthesis of novel indazole bearing oxadiazole derivatives and triazole derivatives. The reaction of hydrazide of *2H*-indazole with substituted carboxylic acid in the presences of $POCl_3$ afforded desired oxadiazole derivatives (**3A**). However the reaction of hydrazide of *2H*-indazole with carbon disulfide and base afforded the potassium salt of hydrazide which on reaction with hydrazine hydrate and followed by aldehyde afforded desired triazole derivatives (**3B**) in excellent yields. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening

data revealed that all the tested compounds **PVP-3Aa-o** and **3Ba-o** showed moderate to potent activity. The compounds **PVP-3Ab, 3Be** and **3Bf** showed comparatively good activity against all the bacterial strains.

Chapter 4, we have described the synthesis substituted thiazolo pyrimidine derivatives in excellent yields. The reaction of various 2-thioxopyrimidine with chloro acetyl chloride **(scheme 2)** affords the 3,5-dihydro-7-isopropyl-3-oxo-*N*,5-diaryl-2*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide derivatives **(PVP-4a-t)** was obtained in excellent yield. All the synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy. All the synthesized compounds were evaluated for their anti microbial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-4a-t** showed moderate to potent activity. The compounds **PVP-4c and 4m** showed comparatively good activity against all the bacterial strains.

Chapter 5, we have described the synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidines derivatives in excellent yields. The reaction of *N*-cyclohexyl-4-methyl-3-oxo-pentanamide (**1)** with appropriate aldehyde (**2)** and aminoazole **(3)** was refluxed in DMF affords the *N*-cyclohexyl-4,7-dihydro-5-isopropyl-7-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives was obtained in excellent yield. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **PVP-5a-o** showed moderate to potent activity. The compounds **PVP- 5j** and **5l** showed comparatively good activity against all the bacterial strains.

List of Publications

1. 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*. ***Journal of Combinatorial Chemistry***, **2010**, *12*, 176-180.

2. Synthesis of some novel trifluoromethylated tetrahydropyrimidines using etidronic acid and evaluation for antimicrobial activity.

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.

3. Tetraethylammoniumbromide mediated Knoevenagel condensation in water: Synthesis of 4-arylmethylene-3-methyl-5-pyrazolone.

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)

4. Fuller's earth catalyzed a rapid synthesis of Bis(indolyl)methanes under solvent free condition.

Naval Kapuriya, Rajesh Kakadiya, Mahesh M. Savant, Akshay M. Pansuriya, Chirag V. Bhuva, Anil S. Patel, **Piyush V. Pipaliya**, Vipul B. Audichya, Sarala Gangadharaiah, Sridhar M. Anandalwar, Javaregowda S. Prasad, Anamik Shah, Yogesh T. Naliapara* ***Indian Journal of Chemistry B,*** Under Review.

Conferences participated

1. "15th International conference on Bridging gaps in discovery and development: chemical and biological sciences for affordable health, wellness and sustainability" **on 4-7th Feb. 2011 at Department of Chemistry, Saurashtra University, Rajkot-360005. (Best Poster Presentation Award)**

2. "National seminar on emerging Trends in polymer science and Technology" **on 8-10th Oct. 2009 (poly-2009) at Department of Chemistry, Saurashtra University, Rajkot-360005.**

3. "Two Days National workshop on Patents & IPR related updates" **on 19-20th Sep. 2009 at Department of Chemistry, Saurashtra University, Rajkot-360005.**

4. "International seminar on recent Development in structure and ligand based Drug Design" **on 23rd Dec. 2009 at Department of Chemistry, Saurashtra University, Rajkot-360005.**