Chapter 1

STUDIES ON TETRAHYDROPYRIMIDINES BY CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD
INTRODUCTION

Pyrimidine is a well known heterocyclic compound, which has been subjected to a large variety of structural modification in order to synthesize derivatives with different biological properties. Pyrimidine derivatives have been reported to possess a broad spectrum of pharmacological properties.

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms. Purines, uric acid, barbituric acid and some antimalarial and anti-bacterial agents also contain the pyrimidine ring. The chemistry of pyrimidine has been widely studied. Pyrimidine was first isolated by Gabriel and Colman in 1899. Despite the importance of dihydroazines (particularly those containing the 1,4-dihydropyrimidine and dihydropyridine moiety) for clarifying a wide range of theoretical, medicinal and biological problems, the chemistry of this group of compounds is still extremely spotty.

From the theoretical point of view, it is essential to predict the structure, binding properties, chemical reactivity, etc. of dihydro compounds from the number and positioning of nitrogen atoms in the ring, as well as from the disposition of double bonds. Such quantum mechanical calculations also enable an evaluation of the degree of aromatic character in potential “homoaromatic” and “antiaromatic” isomers. Availability of novel model compounds for verifying these predictions would open up new horizons in theoretical heterocyclic chemistry, particularly in clarifying the structures leading to spontaneous isomerization of a derivative or in verifying its redox properties.

From the biochemical point of view, dihydroazines are of intense interest because of presence of this group at the active site of the hydrogen transferring coenzyme (nicotinamide adenine dinucleotied hydrogenase-NADH or reduced nicotinamide adenine dinucleotide). This nucleotide, a central participant in metabolic processes in living organisms, participates in the reduction of various unsaturated functionalities.

In drug development, dihydroazines show great promise, particularly since the 4-aryldihydropyridines exhibit powerful vasodilation activity via modifying the calcium ion membrane channel. Additionally, dihydropyridines have been found to actively transport medication across biological membranes.

Until recently, most of the information available on dihydroazines centered around dihydropyridines, with very little data extending to the related dihydropyrimidines.

This lacuna has motivated our deep involvement in developing dihydropyrimidine chemistry, particularly dihydropyrimidines without any substituents on the ring.
nitrogen. These molecules have long been considered unstable for oxidation, polymerization or disproportionation reactions.

Figure below present the five possible isomeric structures of dihydropyrimidines, exhibiting different dispositions of the double bonds.

However, these structures are not easy to synthesize and, as a result, most of the known dihydropyrimidines have either 1,2- or the tautomeric 1,4- and 1,6- geometry. On the basis of data available in the literature, the dihydropyrimidines can be conveniently divided into two groups, within each of which interconversion between isomers is possible under thermal conditions, namely, the 1,4-, 1,6-, and 4,5- isomers, and the 1,2- and 2,5- isomers. It is worthwhile to note that, while thermal interconversion between the two groups is not observed, photochemical rearrangement of 1,4- (or 1,6-) dihydropyrimidines to 1,2-isomers has been reported.

It should be stressed that dihydroazines take part in various isomerization processes, usually characterized by reversible or irreversible migrations within the ring, the study of which is still in its infancy. Hydrogen migration, for example, is classified either as rearrangement or tautomerism depending on its kinetic and thermodynamic parameters, the former term is reserved for irreversible processes, while the latter is used to describe fast reversible exchanges. A study of isomerization in dihydropyrimidines provides an excellent opportunity for clarifying the factors regulating these processes.

After successfully developing versatile synthetic techniques for obtaining a variety of 1,4- and 1,6-dihydropyrimidines, as well as the observation of amidinic tautomerism between the two, A. L. Weis et al. examining the possibility of preparative synthesis of similarly 1,2-dihydro derivatives and studying their properties. Particularly important goals of this study were the possible observation of the formally allowed hydrogen shift, of homoaromaticity or of imine-enamine tautomerism in these compounds, behaviors of which have been seen in other systems.

Recently few reports on the formation of 1,2-dihydropyrimidines exist in the literature, and in those cases where a product could be isolated and characterized, the material was either an N-substituted derivative or else it contained geminal disubstitution...
at position 2, situations that prevent the molecule from oxidizing to the corresponding pyrimidine.

Pyrimidine ring carrying various substituents may be built up from two or three small fragments by the principle synthesis or by a variety of other synthesis, which are complimentary rather than alternative to it. A second type of synthesis is the isomerisation or break down of another heterocycles such as hydration of purine but such roots are frequently used.

SYNTHETIC ASPECT

Biginelli Reaction

In 1893, Italian chemist Pietro Biginelli\(^\text{27}\) reported an acid catalyzed cyclocondensation reaction of ethyl acetoacetate, benzaldehyde, and urea. The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1H)-one.

\[
\begin{align*}
\text{EtO}_2\text{C} & \text{Me} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{C} \\
& + \quad \text{EtOH} \\
\text{NH}_2 \quad \text{H}_2 \text{N} & \quad \text{NH}_2 \quad \text{EtO}_2\text{C} \quad \text{Me} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{C}
\end{align*}
\]

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

Various modifications have been applied to Biginelli reaction to get better yield and to synthesize biologically active analogs. Different catalysts have been reported to increase the yield of the reaction. Microwave synthesis strategies have also 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\(^\text{28}\) have synthesized the different DHPMs by using different inorganic salts as a catalyst. 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 hr
to 20-30 min. This report discloses 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.

\[
\begin{align*}
R^1 O & \quad + \quad R^2 CO \quad + \quad R^3 NH_2 \quad + \quad Yb(OTf)_3 \quad \xrightarrow{100 \, ^\circ C} \\
\quad & \quad \quad R^1 NH \quad R^2 COO
\end{align*}
\]

Indium(III) chloride was emerged as a powerful lewis catalyst imparting high region and chemo selectivity in various chemical transformations. B. C. Ranu and coworkers\textsuperscript{29} reported indium(III) chloride (InCl$_3$) as an efficient catalyst for synthesis of 3,4-dihydropyrimidin-2(1H)-ones. 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(1H)-thiones.

\[
\begin{align*}
R^1 O & \quad + \quad R^2 CO \quad + \quad NH_2 \quad + \quad InCl_3 \quad \xrightarrow{InCl_3, \text{THF}} \\
\quad & \quad \quad R^1 NH \quad R^2 COO
\end{align*}
\]

Where $X = O$ or $S$

Majid M. Heravi et al. have reported a simple, efficient and cost-effective method for the synthesis of 3,4-dihydropyrimidin-2(1H)-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\textsuperscript{30} and 12-molybdophosphoric acid\textsuperscript{31} as a recyclable catalyst.

\[
\begin{align*}
R^1 O & \quad + \quad R^2 CO \quad + \quad NH_2 \quad + \quad \text{12-tungstophosphoric acid/12-molybdophosphoric acid} \quad \xrightarrow{AcOH/\text{reflux}} \\
\quad & \quad \quad R^1 NH \quad R^2 COO
\end{align*}
\]

Where $X = O$ or $S$

A novel covalently anchored sulfonic acid onto the surface of silica was prepared and investigated for the Biginelli reaction by Satya Paul and co-workers\textsuperscript{32}. The catalyst is
highly stable, completely heterogeneous and recyclable for several times. The workup procedure is very simple and products were obtained in good to excellent yields.

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\textsuperscript{33}.

Very recently, many researchers\textsuperscript{34-40} have investigated an efficient Biginelli reaction under solvent-free conditions for one-pot synthesis of 3,4-dihydropyrimidin-2-ones/thiones using various catalyst.

**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\textsuperscript{41,42}. Multi-component reactions (MCRs) leading to heterocycles are particularly useful for the creation of diverse chemical libraries, since the combination of \(n\) (where \(n \geq 3\)) building blocks in a single operation leads to high combinatorial efficiency\textsuperscript{41-43}. Therefore, solid phase modifications of MCRs are rapidly become the cornerstone of combinatorial synthesis of small-molecule libraries\textsuperscript{41-47}.

The first solid-phase modification of the Biginelli condensation was reported by Wipf and Cunningham\textsuperscript{48} in 1995. In this sequence, \(\gamma\)-aminobutyric acid derived urea was attached to Wang resin using standard procedures. The resulting polymer-bound urea was condensed with excess \(\beta\)-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.

Weiwei Li and Yulin Lam\textsuperscript{49} described the synthesis of 3,4-dihydropyrimidin-2-(1H)ones/thiones using sodium benzenesulfinic acid as a traceless linker. The key steps involved in the solid-phase synthetic procedure include sulfinic acidification, condensation of urea or thiourea with aldehydes and sulfenic 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.

Recently, Gross et al.\textsuperscript{50} developed a protocol based on immobilized α-ketoamides to increase the diversity of DHPM. The resulting synthetic protocol proved to be suitable for the preparation of a small library using different building blocks. They found that the expected DHPM derivatives were formed in high purity and yield, if aromatic aldehyde and α-ketoamide building blocks were used. The usage of an aliphatic aldehyde leads to an isomeric DHPM mixture. Purities and yields were not affected if thiourea was used instead of urea.
**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. Recently, organic synthesis of small molecular compounds on soluble polymers, i.e. liquid phase chemistry has increasingly become attractive field. 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 Zuliang et al. reported cheap and reusable TSILs for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones via one-pot three component Biginelli reaction.

Ionic liquid-phase bound acetoacetate react with thiourea and various aldehydes with a cheap catalyst to afford ionic liquid-phase supported 3,4-dihydropyrimidin-2(1H)-thiones by Jean Pierre Bazureau and co-workers. 3,4-dihydropyrimidinones was synthesized in one-pot of aldehydes, β-dicarbonyl compounds and urea, catalyzing by non-toxic ionic liquid 1-n-butyl-3-methylimidazolium saccharinate (BMImSac) at room temperature.
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 times involving reflux temperatures, are the moderate yields frequently observed when using more complex building blocks. Microwave irradiation (MWI) has become an recognized tool in organic synthesis, because the rate enhancement, higher yields and often, improved selectivity with respect to conventional reaction conditions. The publication by Anshu Dandia et al. described microwave-enhanced solution-phase Biginelli reactions employing ethyl acetoacetate, thiourea and a wide variety of aromatic aldehydes as building blocks. 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 markedly improved compared to those reported earlier using conventional conditions.

In recent years, solvent free reactions using either organic or inorganic solid supports have received increasing attention. There are several advantages to performing synthesis in dry media: (i) short reaction times, (ii) increased safety, (iii) economic advantages due to the absence of solvent. In addition, solvent free MWI processes are also clean and efficient. Activated fly ash, an industrial waste (pollutant) is an efficient and novel catalyst for some selected organic reactions in solvent free conditions under microwave irradiation. M. Gopalakrishnan and co-workers have reported Biginelli reaction under microwave irradiation in solvent-free conditions using activated fly ash as a catalyst.
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. Ultrasound irradiated and amidosulfonic acid (NH2SO3H) catalyzed synthesis of 3,4-dihydropyrimidi-2-(1H)ones have reported by Ji-Taai Li and co-workers using aldehydes, β-ketoester and urea.

Chenjiang Liu et al. have synthesized a novel series of 4-substituted pyrazolyl-3,4-dihydropyrimidin-2(1H)-thiones under ultrasound irradiation using magnesium perchlorate [Mg(ClO4)2] as catalyst, 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.

N
N
CHO
Ph
R
1
X
N
H
2
N
H
2
O
O
R
2
N
N
Ph
R
1
N
H
X
O
R
2
R
3
R
1
H
O
N
H
2
N
H
2
O
O
N
H
COOEt
H
O
N
H
COOEt

Where X = O/S

Sonication of aromatic aldehydes, urea and ethyl acetoacetate in presence of solvent (ethanol) or solvent-less dry media (bentonite clay) by supporting-zirconium chloride (ZrCl4) 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 reported by Harish Kumar et al.
REACTION MECHANISM

In 1893 Biginelli\textsuperscript{65} reported the first synthesis of dihydropyrimidines by a simple one-pot condensation reaction of ethyl acetoacetate, benzaldehyde and urea.

\[
\begin{align*}
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\text{Me} & \quad \text{H} \\
\text{O} & \quad \text{H} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{O} \\
(1) & \quad (2) \\
\text{H}_2\text{N} & \quad \text{H}_2\text{N} \\
\text{O} & \quad \text{O} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{Ph} & \quad \text{Ph} \\
(3) & \quad (5) \\
\end{align*}
\]

Despite the importance and current interest in dihydropyrimidines of the Biginelli type, the mechanism of the classical three-component Biginelli condensation has not been elucidated with certainty\textsuperscript{66}. Since the 1930s several mechanistic pathways have been proposed for the Biginelli reaction. In 1933, Folkers and Johnson\textsuperscript{67} reported that one of three intermediates was likely to be present in this reaction.

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{H} \\
\text{H}_2\text{N} & \quad \text{NH}_2 \\
\text{Ph} & \quad \text{Ph} \\
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{H} \\
\text{Ph} & \quad \text{Ph} \\
\text{O} & \quad \text{O} \\
\text{EtOOC} & \quad \text{COOEt} \\
(6) & \quad (7) \\
\end{align*}
\]

Fourty years after the initial proposal, In 1973 Sweet and Fissekis\textsuperscript{68} proposed more detailed pathway involving carbenium ion spices. The mechanism was then reexamined 25 years later in 1997 by Kappe, C. O. Kappe\textsuperscript{69} used \textsuperscript{1}H and \textsuperscript{13}C-NMR spectroscopy to support the argument that the key intermediate in the Biginelli reaction was iminium species (9). In the event, benzaldehyde (2) reacted with urea (3) to form an intermediate “hemiaminal” (8) which subsequently dehydrated to deliver N-acyl imminium spices (9). Iminium cation (9) then reacted with ethyl acetoacetate (1) to give uride (11), which underwent facile cyclodehydration to give (4). Kappe also noted that in the absence of (1), bisureide (10) was afforded as a consequence of nucleophilic attack of (9) by urea (3). This discovery confirmed the conclusion of Folkers and Johnson\textsuperscript{67} in 1933. As far as the proposal from 25 years earlier by Sweet and Fisselus, Kappe saw no evidence by \textsuperscript{1}H and \textsuperscript{13}C-NMR spectroscopy that a carbenium ion was a required species.
in the Biginelli reaction. The reaction mechanism can therefore be classified as α-amidoalkylation, or more specifically as α-uridoalkylation\(^70\).

\[
\begin{align*}
\text{(2)} & \quad \text{Ph} - \text{CHO} \\
\text{(3)} & \quad \text{H}_2\text{N} - \text{CONH}_2 \\
\text{(4)} & \quad \text{EtOOC} - \text{N} - \text{H} \\
\text{(8)} & \quad \text{Ph} - \text{N} - \text{CONH}_2 \\
\text{(9)} & \quad \text{Ph} - \text{H} - \text{N} - \text{COOEt} \\
\text{(11)} & \quad \text{EtOOC} - \text{N} - \text{H} \\
\text{(10)} & \quad \text{Ph} - \text{N} - \text{CONH}_2 \\
\end{align*}
\]

**THERAPEUTIC IMPORTANCE**

4-Aryl-1,4-dihydropyridines (DHPs) of the nifedipine type e.g. nifedipine are the most studied class of organic calcium channel modulators. More than 30 years after the introduction of nifedipine (12), many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market e.g. nitrendipine, nicardipine and amlodipine\(^71\). The aza-analogs such as dihydropyrimidines (13) which show a very similar pharmacological profile to classical dihydropyridine calcium channel modulators\(^72-76\). Over the past several lead-compounds were developed e.g. (13) SQ 32926 and (14) SQ 32574\(^73,75\) that are superior in potency and duration of antihypertensive activity to classical dihydropyridine drugs and compare favorable with second-generation analogs such as amlodipine and nicardipine\(^73\).

\[
\begin{align*}
\text{(12)} & \quad \text{MeOOC} - \text{CONH}_2 \\
\text{(13) SQ 32926} & \quad \text{i-Pr} - \text{O} - \text{CONH}_2 \\
\text{(14) SQ 32574} & \quad \text{i-Pr} - \text{O} - \text{CONH}_2 \\
\end{align*}
\]

Calcium ion plays a vital role in a large number of cellular processes, including excitation-contraction and stimulus-secretion\(^77,78\). The regulation of the intracellular concentration of this ion makes possible the control of such Ca\(^{2+}\) dependent processes. One means of accomplishing this is by the use of agents known as calcium channel
antagonists, which inhibit the movement of calcium through certain membrane channel\textsuperscript{79-81}.

K. S. Atwal\textsuperscript{82} prepared the 2-heterosubstituted-4-aryl-1,4-dihydro-6-methyl-5 pyrimidinecarboxylic acid esters (15), which lack the potential C\textsubscript{3} symmetry of dihydropyridine calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit artery and radioligand binding techniques showed that some of these compounds are potent mimics of dihydropyridine calcium channel blockers. The combination of a branched ester e.g. isopropyl, sec-butyl and thioalkyl group e.g. –SMe group was found to be optimal for biological activity. Dihydropyrimidines (15) were found to be 30 fold less active than dihydropyridines. The solid-state structure of dihydropyrimidine analogue (16) shows that these compounds can adopt a molecular conformation which is similar to the reported conformation of dihydropyridine calcium channel blockers.

$\text{N}^{+} \text{H} \text{N}^{+} \text{R}^{2}$ \text{X} \text{COOR}^{3}$

(15)

$\text{N}^{+} \text{H} \text{N}^{+} \text{COOEt}$

(16)

K. Atwal et al.\textsuperscript{83} synthesized the 3-substituted 1,4-dihydropyrimidine (17) and documented that vasorelaxant activity was critically dependent on the size of the C\textsubscript{5} ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, alkyl) were tolerated at third position. The dihydropyrimidines (17) are significantly more potent than corresponding 2-heteroalkyl-1,4-dihydropyrimidines. Dihydropyridine enantiomer usually show 10-15 fold difference in activity, while the enantiomers of dihydropyrimidine (18) show more than a 1000 fold difference in activity. These results strengthen the requirement of an enamino ester for binding to the dihydropyridine receptor and indicate a nonspecific role for the substituent present on the third position.
George C. Rovnyak et al.\textsuperscript{84} examined a series of novel dihydropyrimidine calcium channel blockers that contain a basic group attached to either C\textsubscript{5} or N\textsubscript{3} of the heterocyclic ring. One of these compounds was identified as a lead, and the individual enantiomers (19a) (R) and (19b) (S) were synthesized. Dihydropyrimidine (19a) is equipotent to nifedipine and amlodipine in vitro. In the spontaneously hypertensive rat, dihydropyrimidine (19a) is more potent and longer acting than nifedipine and compares most favorably with the long-acting dihydropyridine derivative amlodipine. Dihydropyrimidine (19a) has the potential advantage of being a single enantiomer.

Selma Sarac and co-workers\textsuperscript{85,86} have synthesized 4-aryl-3,4-dihydropyrimidin-2(1H)-one/thione derivatives. The calcium channel blocker activities of all compounds performed on isolated rat ileum. Product (20), 2-nitrophenyl derivative and (21), 2-bromophenyl derivative have potent antispasmodic activity on BaCl\textsubscript{2} stimulated rat ileum.

N. Dhanapalan and co-workers\textsuperscript{87} have synthesized dihydropyrimidinones and describe compound (22) have a high binding affinity ($Ki = 0.2\text{nM}$) for $\alpha_{1a}$ receptor and greater than 1500 fold selectivity over $\alpha_{1b}$ and $\alpha_{1d}$ adreno receptors. Modification of the
linker in (22) gave compounds (23) and (24)\textsuperscript{88} viz \(\mu\)-opioid receptor. Both these compounds showed good \(\alpha_{1A}\) binding affinity (\(Ki = 0.2\text{nM}\)) and selectivity (>800-fold over \(\alpha_{1B}\) and \(\alpha_{1D}\)), also showed good selectivity over several other recombinant human \(G\)-protein coupled receptors. They have also identified that compound (25)\textsuperscript{89} was a lead compound with a binding and functional profile comparable to that of (22). (25) have negligible affinity for the \(\mu\)-opioid receptor.

![Chemical structures](images)

The synthesis and differential antiproliferative activity of monastrol (26a), oxomonastrol (26b) and eight oxygenated derivatives (28a,b)-(31a,b) on seven human cancer cell lines are described by Dennis Russowsky\textsuperscript{90}. For all evaluated cell lines, monastrol (26a) was shown to be more active than its oxo-analogue, except for HT-29 cell line, suggesting the importance of the sulfur atom for the antiproliferative activity. Monastrol (26a) and the thio derivatives (28a), (29a) and (31a) displayed relevant antiproliferative properties with 3,4-methylenedioxy derivative (31a) being approximately more than 30 times more potent than monastrol (26a) against colon cancer (HT-29) cell line.
Y. Mizutani and co-workers\textsuperscript{91,92} identify that dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the pathway of uracil and thymine metabolism. DPD is also the principle enzyme involved in the degradation of 5-fluorouracil and anticancer chemotherapeutic agent that is used clinically to treatment of bladder cancer and renal cell carcinoma.

**CATALYTIC STUDY OF ETIDRONIC ACID THROUGH MICROWAVE IRRADIATION TECHNIQUE**

More than 100 year ago the first 1,4-DHPMs were synthesized by Pietro Biginelli\textsuperscript{93}. The classical method involves a simple one-pot condensation reaction of ethylacetoacetate, aryl aldehyde and urea/thiourea under strong acidic condition\textsuperscript{94}. The major limitations of biginelli reaction are lower yield and longer reaction time. Moreover, product separation and work up also cause problem and needed special techniques. Thus, main disadvantages in biginelli reaction are lower yield, longer reaction time and tedious work up.

Due to these disadvantages of biginelli method, there are several efficient methods developed for the synthesis of 1,4-DHPMs, which comprise the use of Silica triflate\textsuperscript{95}, Iodotrimethylsilane in acetonitrile\textsuperscript{96}, Strontium(II)nitrate\textsuperscript{97}, PEG-4000\textsuperscript{98}, chloroacetic acid\textsuperscript{99}, InBr\textsubscript{3}\textsuperscript{100}, microwave\textsuperscript{101,102}, KSF montmorillonite\textsuperscript{103} etc as catalysts. However, the use of high temperatures, expensive metal precursors and longer reaction times are limits of these methods.

**CURRENT WORK**

To avoid these unconvenency, the development of an efficient and versatile method has been made for the synthesis of 1,4-DHPMs, The work of such heterocycle is an interesting research area and there is a scope for further improvement towards
conventional reaction conditions and to improve the reaction yield and decrease the reaction time.

In extension of our work, the tetrahydropyrimidine derivatives have been synthesized through green chemistry approach by utilizing Etidronic acid through microwave irradiation technique.

$$\begin{align*}
\text{Etidronic acid} & \quad \text{[(1-hydroxyethylidene)bisphosphonic acid]} \\
\text{Etidronic acid} & \quad \text{[(1-hydroxyethylidene)bisphosphonic acid]} \\
\text{Etidronic acid} & \quad \text{Etidronic acid} \\
\text{Etidronic acid} & \quad \text{Etidronic acid} \\
\end{align*}$$

Etidronic acid [(1-hydroxyethylidene)bisphosphonic acid]

Etidronic acid [(1-hydroxyethylidene)bisphosphonic acid] is a phosphonic acid and is also known as a bisphosphonate having a molecular formula $C_2H_8O_7P_2$. The two $\text{PO}_3$ (phosphonate) groups are covalently linked to a single carbon atom. By using this method we found increased yield about 20-25% more than conventional method.

**COMPARISION OF CONVENTIONAL BIGINELLI METHOD AND MICROWAVE ASSISTED METHOD**

The microwave assisted organic synthesis (MAOS) are attracting the interest of organic chemists and other researchers due to their significant potential for converting reactant into respective product in short reaction time with quantitative yields. Several procedures have been reported in the literature for the synthesis of DHPMs under microwave irradiations with different conditions and different catalyst.

In this chapter we have synthesised tetrahydropyrimidine derivatives of type-I and type-II by conventional beginelli method and also by microwave assisted method, the results obtained from the both method are noted. Comparision of the catalyst used, reaction time and yield in both methods are given below in tabular form.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventional beginelli method</th>
<th>Microwave assisted method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst</td>
<td>Con. HCl</td>
<td>Etidroni acid</td>
</tr>
<tr>
<td>Reaction time</td>
<td>10 to 20 hours</td>
<td>6 to 18 minutes</td>
</tr>
<tr>
<td>Yield</td>
<td>40 to 60 %</td>
<td>60 to 90 %</td>
</tr>
</tbody>
</table>
Thus, by using microwave assisted improved method, it get optimum yields and also reaction hours are reduced to a great extent (from hours to minutes) in comparison with conventional biginelli method as mentioned in literature.

WORK DONE FROM OUR LABORATORY

Synthesis anticancer, antitubercular and antimicrobial activity of some new pyrimidine derivatives have been reported by K. S. Nimavat\textsuperscript{105}, some new thiopyrimidine and oxopyrimidine heterocycles bearing 4-(methylsulfonyl)phenyl nucleus as potent antitubercular and antimicrobial agents was developed and reported by D. J. Paghdar\textsuperscript{106}. M. R. Patel\textsuperscript{107} have reported synthesis and evaluation of pharmacological activity of some new aminopyrimidine and thiopyrimidine derivatives.

J. D. Akbari and coworkers reported synthesis of some new 1,2,3,4-tetrahydropyrimidine-2-ones and their thiazolo[3,2-\(a\)]pyrimidine derivatives as a potential biological agents\textsuperscript{108}, synthesis of some new pyrazolo[3,4-\(d\)]pyrimidines and thiazolo[4,5-\(d\)]pyrimidines and evaluation of their antimicrobial activities\textsuperscript{109}, green chemistry approach to synthesis of some new trifluoromethyl containing tetrahydropyrimidines under solvent free conditions\textsuperscript{110}, synthesis and antimicrobial activities of some new pyrazolo[3,4-\(d\)]pyrimidines and thiazolo[4,5-\(d\)]pyrimidines\textsuperscript{111}.

SECTION-I: SYNTHESIS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES USING CONVENTIONAL METHOD & MICRO-WAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

SECTION-II: SYNTHESIS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.
SECTION-I
SYNTHESIS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

Much interest has been focused around tetrahydropyrimidine derivatives because of their wide variety of pharmacological properties and industrial applications. In view of these reports, we have synthesized 4-aryl-6-isopropyl-N-(5-methyl-1,3-thiazol-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide of Type-(I) by the cyclocondensation reaction of 4-methyl-N-(5-methylthiazol-2-yl)-3-oxopentanamide, aromatic aldehyde and urea in ethanol.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & \(^1\)H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their in vitro biological assay like antibacterial activity towards Gram positive and Gram negative bacterial strains and antifungal activity towards A. niger, C. Albicans and A. Clavatus at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME
IR SPECTRUM OF 6-ISOPROPYL-N-(5-METHYL-1,3-Thiazol-2-yl)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range: 4000-400 cm$^{-1}$ (KBr disc.)

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<th>Type</th>
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<th>Frequency cm$^{-1}$</th>
<th>Ref.</th>
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<td>Reported</td>
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<td>C-H i.p. def.</td>
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<td>Amide</td>
<td>-NH str.</td>
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1H-NMR SPECTRUM OF 6-ISOPROPYL- N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Internal Standard: TMS; Solvent: DMSO-D$_6$ Instrument: BRUKER Spectrometer (300MHz)

<table>
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<th>Chemical Shift In δppm</th>
<th>Relative No. of Protons</th>
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<th>Inference</th>
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<td>doublet</td>
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</tbody>
</table>
Studies on Biologically Active...

Chapter-1

Studies on Tetrahydropyrimidines...
Studies on Biologically Active...  

Chapter-1  Studies on Tetrahydropyrimidines...  

MASS SPECTRUM OF 6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Mol. Wt. = 356.4  
m/z = 357.3 (M+1)

MASS SPECTRUM OF 4-(3-CHLOROPHENYL)-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Mol. Wt. = 390.5  
m/z = 391.2 (M+1), 393.1 (M+3)
EXPERIMENTAL

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F<sub>254</sub> coated aluminum sheet (Merck prepared plates) as stationary phase. Ethyl acetate:hexane (3:7) was used as a mobile phase.

[A] PREPARATION OF 4-METHYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-3-OXOPENTANAMIDE.

A suspension of methylisobutryl acetate (2.88 gm, 0.02 mol) and 5-methyl-1,3-thiazole-2-yl (1.14 gm, 0.01 mol) in toluene (20 ml) containing catalytic amount of NaOH solution (0.05 ml, 40 %) was reflux on oil bath for 12 hr. The progress of reaction was monitored by TLC, after completion of the reaction solvent was removed under reduced pressure to give residue, the residue was crystallized from mixture of hexane and ethyl acetate. Yield 51 %.

[B] PREPARATION OF 6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-4-PHENYL-1,2,3,4-TETRAHYDROPYRIDIMINE-5-CARBOXAMIDE

(1) Conventional method

The warm mixture of 4-methyl-N-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), benzaldehyde (1.06 gm, 0.01 mol), urea (0.9 gm, 0.015 mol) and ethanol (15 ml), in the presence of catalytic of concentrated HCl (2-3 drops) was stirred at reflux temperature for 12 hr. The progress of reaction was monitored by TLC, after completion of the reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 62 %.

(2) Microwave assisted method

A well stirred mixture of 4-methyl-N-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), benzaldehyde (1.06 gm, 0.01 mol), urea (0.9 gm, 0.015 mol) and ethanol (15 ml) in the presence of etidronic acid (100 mg/0.01 mol) was irradiated under microwave oven for 8 min at 300W. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was allowed to stand at room
temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 89%.

Similarly, other 6-isopropyl-N-(5-methyl-1,3-thiazol-2-yl)-4-aryl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1a-j) were prepared. The physical constants are recorded in Table-1a, page no. 39.

[C] BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

➢ DETERMINATION OF MINIMAL INHIBITION CONCENTRATIONS BY BROTH DILUTION METHOD

All the synthesized compounds (1a-j) were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method\textsuperscript{113-115} with two Gram-positive bacteria Staphylococcus aureus MTCC-96 and Streptococcus pyogenes MTCC 442, two Gram-negative bacteria Escherichia coli MTCC 443 and Pseudomonas aeruginosa MTCC 1688 and three fungal strains Candida albicans MTCC 227, Aspergillus Niger MTCC 282 and Aspergillus clavatus MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all synthesized compounds (1a-j), defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards\textsuperscript{113}.

Minimal Inhibition Concentration [MIC]

The main advantage of the Broth Dilution Method for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.

4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.

5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

**Methods used for primary and secondary screening**

Each sample was diluted obtaining 2000 μg mL⁻¹ concentration, as a stock solution. Inoculum size for test strain was adjusted to 10⁸ cfu (colony forming unit) per milliliter by comparing the turbidity.

*Primary screen:* In primary screening 1000 μg mL⁻¹, 500 μg mL⁻¹ and 250 μg mL⁻¹ concentrations of the synthesized compounds were taken. The active samples found in this primary screening were further tested in a second set of dilution against all microorganisms.

*Secondary screen:* The samples found active in primary screening were similarly diluted to obtain 200 μg mL⁻¹, 100 μg mL⁻¹, 62.5 μg mL⁻¹, 50 μg mL⁻¹, 25 μg mL⁻¹, 12.5 μg mL⁻¹ and 6.25 μg mL⁻¹ concentrations.

*Reading Result:* The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 10⁸ organism/mL.

The results obtained from antimicrobial testing are recorded in *Table-1b*, page no. 40.
### TABLE-1a: PHYSICAL CONSTANTS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

![Chemical structure]

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<th>Sr. No.</th>
<th>R</th>
<th>Molecular Formula/Molecular Weight</th>
<th>MP °C</th>
<th>Classical method</th>
<th>Microwave Method</th>
<th>% Composition Calcd./Found</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yield %</td>
<td>Time (hr.)</td>
<td>Yield %</td>
<td>Time (min.)</td>
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<tr>
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TABLE-1b: BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

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<th>Sr. No.</th>
<th>Code</th>
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<th>Minimal bactericidal concentration μg/ml</th>
<th>Antifungal activity</th>
<th>Minimal fungicidal concentration μg/ml</th>
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<td>Gram –ve Bacteria</td>
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MINIMAL INHIBITION CONCENTRATION

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<th>S.aureus (microgramme/ml)</th>
<th>S.pyogenus (microgramme/ml)</th>
<th>E.coli (microgramme/ml)</th>
<th>P.aeruginosa (microgramme/ml)</th>
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<tbody>
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<td>Ciprofloxacin</td>
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MINIMAL FUNGICIDAL CONCENTRATION

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<tr>
<th>Standard Drugs</th>
<th>C.Albicans (microgramme/ml)</th>
<th>A.Niger (microgramme/ml)</th>
<th>A.Clavatus (microgramme/ml)</th>
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<tbody>
<tr>
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<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Greseofulvin</td>
<td>500</td>
<td>100</td>
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</table>
ANTIBACTERIAL ACTIVITY:

From screening results, substituted Pyrimidines 1c (R= 4-F) & 1h (R= 4-NO₂) against S.aureus and 1b (R= 4-OMe) against E-coli possess excellent activity as compare to ampicillin. While 1c (R= 4-F) against S.pyogenus, 1d (R= 3-Cl) against E-coli and 1g (R= 2,5-di OMe) against P.aeruginosa, possess good activity as compare with ampicillin. The remaining compounds possess moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted Pyrimidine 1b (R= 4-OMe) show highly promising activity against C.albicans & A.niger compare with greseofulvin. While 1e (R = 2,3-di Cl) against C.albicans, 1a (R = -H) against A.niger and 1a (R = -H) & 1b (R= 4-OMe) against A.clavatus, possess good activity compare to standard drug. The remaining compounds exhibit moderate to poor activity.
SECTION-II

SYNTHESIS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE USING CONVENTIONAL METHOD & MICROWAVE ASSISTED METHOD AND BIOLOGICAL SCREENING.

Compounds containing pyrimidine moiety are widely distributed in nature. Many of these derivatives are reported to possess different biological activities. In view of these reports, we have synthesized 4-aryl-6-isopropyl-N-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide of Type-(II) by the cyclocondensation reaction of 4-methyl-N-(5-methylthiazol-2-yl)-3-oxopentanamide, aromatic aldehyde and thiourea in ethanol.

The constitution of the synthesized products have been characterized by using elemental analysis, IR & \(^1\)H-NMR spectroscopy and further supported by mass spectroscopy.

All the compounds have been evaluated for their in vitro biological assay like antibacterial activity towards Gram positive and Gram negative bacterial strains and antifungal activity towards A. niger, C. Albicans and A. Clavatus at a different concentration. The biological activities of synthesized compounds were compared with standard drugs.

REACTION SCHEME

![Reaction Scheme Diagram]
Studies on Biologically Active...

Chapter 1

Studies on Tetrahydropyrimidines...

IR SPECTRUM OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)-N-(5-METHYL-1,3-
THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDRO-PYRIMIDINE-5-CARBOXAMIDE

Instrument: SHIMADZU FTIR 8400 Spectrophotometer; Frequency range:
4000–400 cm⁻¹ (KBr disc.)

<table>
<thead>
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<th>Type</th>
<th>Vibration Mode</th>
<th>Frequency cm⁻¹</th>
<th>Ref.</th>
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<td>Observed</td>
<td>Reported</td>
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<td>Alkane</td>
<td>C-H str. (asym.)</td>
<td>2968</td>
<td>2975-2920</td>
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<tr>
<td></td>
<td>C-H str. (sym.)</td>
<td>2914</td>
<td>2880-2860</td>
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<td></td>
<td>C-H def. (asym.)</td>
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<td></td>
<td>C-H def. (sym.)</td>
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<td>1395-1370</td>
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<td>C-H str.</td>
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<td>3100-3000</td>
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<td>C=C</td>
<td>1570</td>
<td>1585-1480</td>
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<td>C-H i.p. def.</td>
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<td>1125-1090</td>
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<tr>
<td></td>
<td>C-H o.o.p. def.</td>
<td>831</td>
<td>860-810</td>
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<td>Carbonyl</td>
<td>C=O</td>
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<td>1700-1650</td>
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<td>Amide</td>
<td>-NH str.</td>
<td>3433</td>
<td>3400-3200</td>
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Studies on Biologically Active...

Chapter -1

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1H-NMR SPECTRUM OF 4-(4-FLUOROPHENYL)-6-ISOPROPYL-N-(5-METHYL-1,3-
THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

Internal Standard: TMS; Solvent: DMSO-D6 Instrument: BRUKER Spectrometer
(300MHz)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Chemical Shift In δppm</th>
<th>Relative No. of Protons</th>
<th>Multiplicity</th>
<th>Inference</th>
<th>J Value in HZ</th>
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<td>1</td>
<td>1.08-1.10</td>
<td>3H</td>
<td>doublet</td>
<td>-CH(CH3)2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.19-1.22</td>
<td>3H</td>
<td>doublet</td>
<td>-CH(CH3)2</td>
<td>-</td>
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<tr>
<td>3</td>
<td>2.26</td>
<td>3H</td>
<td>singlet</td>
<td>-CH3</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>3.35</td>
<td>1H</td>
<td>multiplet</td>
<td>-CH(CH3)2</td>
<td>-</td>
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<tr>
<td>6</td>
<td>5.49-5.50</td>
<td>1H</td>
<td>doublet</td>
<td>Chiral-Hb</td>
<td>3.3</td>
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<td>7</td>
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<td>doublet</td>
<td>-CO-NH-</td>
<td>1.2</td>
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<td>8</td>
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<td>Ar-H+Hc</td>
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<td>9</td>
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<td>doublet</td>
<td>-CO-NH-</td>
<td>1.8</td>
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<td>10</td>
<td>9.70</td>
<td>1H</td>
<td>singlet</td>
<td>-CO-NH-</td>
<td>-</td>
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</table>
Chapter-1  Studies on Tetrahydropyrimidines...
MASS SPECTRUM OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)-N-(5-METHYL-1,3-
THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

MASS SPECTRUM OF 4-(4-FLUOROPHENYL)-6-ISOPROPYL-N-(5-METHYL-1,3-
THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE
**EXPERIMENTAL**

Melting points of all the synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. TLC was carried out on silica gel-G F$_{254}$ coated aluminum sheet (Merck prepared plates) as stationary phase. Ethyl acetate:hexane (3:7) was used as a mobile phase.

[A] **PREPARATION OF 4-METHYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-3-OXOPENTANAMIDE.**

See Chapter-1, Section-I, Experimental [A], Page no. 36.

[B] **PREPARATION OF 6-ISOPROPYL-4-(4-METHOXYPHENYL)-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE**

(1) **Conventional method**

The warm mixture of 4-methyl-N-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), 4-methoxy benzaldehyde (1.36 gm, 0.01 mol) and thiourea (1.14 gm, 0.015 mol) in ethanol (15 ml), containing 3-4 drops of concentrated HCl was stirred under reflux for 13 hr. The progress of reaction was monitored by TLC, after completion of the reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 52 %.

(2) **Microwave assisted method**

A well stirred mixture of 4-methyl-N-(5-methyl-1,3-thiazole-2-yl)-3-oxopentanamide (2.26 gm, 0.01 mole), 4-methoxy benzaldehyde (1.36 gm, 0.01 mol) and thiourea (1.14 gm, 0.015 mol) in ethanol (15 ml) in the presence of etidronic acid (100 mg/0.01 mol) was irradiated under microwave oven for 10 min at 300W. The progress of reaction was monitored by TLC, after completion of reaction, the reaction mixture was allowed to stand at room temperature for several hours so precipitation was obtained. The product was filtered, washed with chilled methanol and isolated product was recrystallized from ethanol. Yield 78 %.
Similarly, other 4-aryl-6-isopropyl-N-(5-methyl-1,3-thiazol-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (2a-j) were prepared. The physical constants are recorded in Table-2a, page no.49.

[C] BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4- TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE.

Antimicrobial testing was carried out as described in Chapter-1, Section-I, Experimental [C], page no. 37. The results obtained from antimicrobial testing are recorded in Table-2b, page no. 50.
TABLE-2a: PHYSICAL CONSTANTS OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE

<table>
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<tr>
<th>Sr. No.</th>
<th>R</th>
<th>M.F. / M.W.</th>
<th>MP °C</th>
<th>Classical method</th>
<th>Microwave Method</th>
<th>% Composition Calcd./Found</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Yield</td>
<td>Time (hr.)</td>
<td>Yield</td>
<td>Time (min.)</td>
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<tr>
<td>2a</td>
<td>4-OMe</td>
<td>C_{19}H_{22}N_{4}O_{5}S_{2} 402.53</td>
<td>239-242</td>
<td>60 13</td>
<td>81 10</td>
<td>56.59 56.33</td>
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<tr>
<td>2b</td>
<td>H</td>
<td>C_{18}H_{20}N_{5}O_{3}S_{2} 372.51</td>
<td>227-229</td>
<td>56 15</td>
<td>85 8</td>
<td>58.04 57.81</td>
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<tr>
<td>2c</td>
<td>4-F</td>
<td>C_{18}H_{10}FN_{4}O_{3}S_{2} 390.50</td>
<td>217-218</td>
<td>55 14</td>
<td>76 7</td>
<td>55.36 55.09</td>
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<tr>
<td>2d</td>
<td>3-Cl</td>
<td>C_{18}H_{15}ClN_{6}O_{3}S_{2} 406.95</td>
<td>198-201</td>
<td>41 17</td>
<td>77 9</td>
<td>53.12 53.08</td>
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<tr>
<td>2e</td>
<td>2,3-diCl</td>
<td>C_{18}H_{19}Cl_{2}N_{4}O_{3} 441.40</td>
<td>224-226</td>
<td>49 16</td>
<td>63 16</td>
<td>48.98 48.71</td>
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<tr>
<td>2f</td>
<td>4-N(Me)_{2}</td>
<td>C_{20}H_{25}N_{5}O_{3}S_{2} 415.58</td>
<td>208-210</td>
<td>55 12</td>
<td>68 8</td>
<td>57.80 57.55</td>
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<tr>
<td>2g</td>
<td>2,5-diOMe</td>
<td>C_{20}H_{23}N_{6}O_{3}S_{2} 432.56</td>
<td>229-231</td>
<td>43 20</td>
<td>64 18</td>
<td>55.53 55.26</td>
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<tr>
<td>2h</td>
<td>4-NO_{2}</td>
<td>C_{18}H_{19}N_{5}O_{3}S_{2} 417.51</td>
<td>222-225</td>
<td>54 13</td>
<td>82 14</td>
<td>51.78 51.42</td>
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<tr>
<td>2i</td>
<td>3-NO_{2}</td>
<td>C_{18}H_{19}N_{5}O_{3}S_{2} 417.51</td>
<td>231-234</td>
<td>48 15</td>
<td>79 17</td>
<td>51.78 51.58</td>
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<tr>
<td>2j</td>
<td>4-OH</td>
<td>C_{18}H_{18}N_{5}O_{3}S_{2} 388.51</td>
<td>252-253</td>
<td>51 19</td>
<td>74 13</td>
<td>55.65 55.28</td>
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### TABLE-2b: BIOLOGICAL SCREENING OF 4-ARYL-6-ISOPROPYL-N-(5-METHYL-1,3-THIAZOL-2-YL)-2-THIOXO-1,2,3,4-TETRAHYDRO-PYRIMIDINE-5-CARBOXAMIDE

<table>
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<th>Sr. No.</th>
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<tr>
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<td>Minimal bactericidal concentration µg/ml</td>
<td>Minimal fungicidal concentration µg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gram +ve Bacteria</td>
<td>Gram –ve Bacteria</td>
</tr>
<tr>
<td>1</td>
<td>2a</td>
<td>200</td>
<td>200</td>
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<tr>
<td>2</td>
<td>2b</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>2c</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
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<td>250</td>
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<td>5</td>
<td>2e</td>
<td>500</td>
<td>100</td>
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<td>2f</td>
<td>500</td>
<td>500</td>
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<tr>
<td>7</td>
<td>2g</td>
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<td>200</td>
</tr>
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<td>8</td>
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<td>9</td>
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<td>500</td>
<td>500</td>
</tr>
<tr>
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**MINIMAL INHIBITION CONCENTRATION**

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<th>S.pyogenes</th>
<th>E.coli</th>
<th>P.aeruginosa</th>
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<tr>
<td>Gentamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.05</td>
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<tr>
<td>Ampicillin</td>
<td>250</td>
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<td>100</td>
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<tr>
<td>Chloramphenicol</td>
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<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>50</td>
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<td>Norfloxacin</td>
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**MINIMAL FUNGICIDAL CONCENTRATION**

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<th>C.Albicans</th>
<th>A.Niger</th>
<th>A.Clavatus</th>
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<tr>
<td>Nystatin</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Greseofulvin</td>
<td>500</td>
<td>100</td>
<td>100</td>
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ANTIBACTERIAL ACTIVITY:

From screening results, substituted Pyrimidines $2a$ (R= 4-OMe) & $2g$ (R= 2,5-di OMe) against *S.aureus*, $2e$ (R= 2,3-di Cl) against *E.coli* and $2d$ (R= 3-Cl) against *P.aeruginosa* possess very good activity compare to ampicillin. While $2c$ (R= 4-F) & $2d$ (R= 3-Cl) against *S.aureus*, $2e$ (R= 2,3-di Cl) against *S.pyogenus* and $2c$ (R= 4-F) against *E.coli* & *P.aeruginosa*, possess moderate activity as compare with ampicillin. The remaining compounds possess moderate to poor activity against all four bacterial species.

ANTIFUNGAL ACTIVITY:

Antifungal screening data shows that substituted Pyrimidine $2c$ (R= 4-F) exhibite promissing activity against *C.albicans* while $2a$ (R= 4-OMe) exhibite moderate activity against *A.niger* & *A.clavatus* compare to Greseofulvin. The remaining compounds exhibite moderate to poor activity.
REFERENCES

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62. Ji-Tai Li, Jun-Fen Han, Jin-Hui Yang, Tong-Shuang Li; *Ultrasonics Sonochem.* **10**(3), 119-122 (2003).
64. H. Kumar, A. Parmar; *Ultrasonics Sonochem.*, **15**(2), 129-132 (2007).
Studies on Biologically Active...

Chapter 1

Studies on Tetrahydropyrimidines


