CHAPTER-1

Synthesis of some novel pyrimidine analogues for its biological screening
1.1 INTRODUCTION

Nitrogen heterocyclic compounds are of special interest as they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities. Pyrimidines, being an integral part of DNA and RNA impart diverse pharmacological activities. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research.

1.1.1 Chemistry of pyrimidine

Pyrimidine is the most important member of all the diazine as this ring system occurs widely in living organisms. Pyrimidine can be thought as a cyclic amine and also be called as m-diazine or 1,3-diazine. Pyrimidine was first isolated by Gabriel and Colman in 1899. Pyrimidine (1) is a six member heterocyclic compound containing four carbon and two nitrogen atoms. In pyrimidine, each of the four \( sp^2 \) hybridized carbon atoms features a \( p \) orbital perpendicular to the plane of the ring and each \( p \) orbital contains one \( \pi \) electron. The nitrogen atoms are likewise \( sp^2 \) hybridized each containing one \( \pi \) electron in their \( p \) orbital for a total of six \( \pi \) electrons. The lone pair electrons do not contribute to the aromatic \( \pi \) electron sextet.

The pyrimidine ring is essentially flat. The depletion of electron density at the position ortho and para to the electronegative nitrogen atoms is more marked in the pyrimidine than the other two diazine i.e. pyrazine and pyridazine. This is because the two N atoms of this 1,3-diazines are so positioned that their individual effects reinforce each other and thus act in unison. That is why the resultant effect is greater in the pyrimidine than its isometric diazines, in which the electronic effects of the two N atoms instead of adding up, in fact partly antagonize each other. So the marked position (*) C-2, C-4 and C-6 as shown in structure (2) become strong electropositive centers, while the position C-5 retains some electronegativity, though only to a lesser extent. At the same time, the localization of \( \pi \)-electrons at the two N atoms results in decreased aromaticity of the ring. Thus pyrimidine is not truly aromatic; whatever
little appearance of aromaticity is left in the system is at C-5 carbon. This leads to a sharp fall in its stability reflected by low resonance energy i.e. 26 kcal/mol. The electron density diagram in which the distribution of electrical charges i.e. the gain or loss of the π-electrons at each atom of the ring has been calculated and shown in structure (3).

The unshared electrons at the two nitrogen atoms contribute to the low basicity of pyrimidine (pKa = 1.31) compared to pyridine (pKa = 5.2), bearing a single imine nitrogen atom. The basicity of pyrimidine is intermediate to that of the other two isometric diazine i.e. pyridazine (pKa = 2.33) and pyrazine (pKa = 0.6).

1.1.2 Therapeutic uses of pyrimidine

During the last three decades, several pyrimidine derivatives have been developed as chemotherapeutic agents and have found wide clinical applications. Some of them are listed below according to their clinical use.

1. Antineoplastic and anticancer agents\(^4\): 5-Fluorouracil (4), 5-Thiouracil (5).

2. Antifungal\(^5\): 5-Fluorocytosine (6)

3. Antimalarial\(^6\): Pyrimethamine (7)

4. Antibacterial\(^6\): Trimethoprim (8)

5. Antifolate\(^6\): Methotrexate (9)

6. Sulfa drug\(^7\): Sulfamerazine (10), Sulfadimidine (11)
7. Anthelmentic\textsuperscript{8}: Pyrantel pamoate (12)

8. Antiviral and anti-AIDS\textsuperscript{9, 10}: 5-Iododeoxyuridine (13), Stavudine (14), Zidovudine (15), Zalcitabine (16), Lamivudine (17)

9. Antibiotics\textsuperscript{11, 12}: Bacimethrin (18), Puromycin (19)

10. Antitubercular drug\textsuperscript{13, 14}: Capreomycin (20)

1.1.3 General methods for the synthesis of pyrimidine

Synthesis of pyrimidine has been of great interest to chemists because of its varied biological and pharmacological activities. The first example of pyrimidine synthesis was the synthesis of barbituric acid, in 1878, from malonic acid and urea.
methods of synthesis of pyrimidine are classified on the basis of components employed in the cyclization of pyrimidine.

1.1.3.1 One component synthesis

This involves the intramolecular cyclization of certain open chain intermediates to yield the pyrimidine nucleus. This method can be further classified according to the position of the bond formed during the cyclization.

A. 1,2(2,3)-bond formation

One of the examples of this type of synthesis is the base catalyzed cyclization of the N-cyanoamine derivative (21) to the aminopyrimidine derivatives (22).\(^{15}\)

\[
\begin{align*}
\text{ArHNC} & \quad \xrightarrow{\text{R}_1=\text{-H, -X}} \\
\text{NH}_2 & \quad \quad \text{NH}_2
\end{align*}
\]

B. 3,4(1,6)-bond formation

The pyrimidine synthesis occurs by condensation of 1,3-dicarbonyl compounds with amidines and proceeds via vinylamidine intermediate, which undergo intramolecular cyclization to form 3,4(1,6)-bond. Vinylamidine intermediate (23) has been cyclized under acidic and basic condition to give different pyrimidine derivatives (24) and (25) respectively.\(^{16}\)

\[
\begin{align*}
\text{EtOOC} & \quad \xrightarrow{\text{Dry } \text{HCl} \text{ Dioxane}} \\
\text{Cl} & \quad \quad \text{EtOOC}
\end{align*}
\]

C. 4,5 (6,5) bond formation

N-vinyl-N-acylbenzamidine (26) on heating in pyridine in the presence of \(p\)-toluene sulfonylchloride cyclizes to give 4-aminopyrimidines (27).\(^{17}\)
1.1.3.2 Two component synthesis

This is the most versatile and widely used method of pyrimidine synthesis. It involves the condensation of two reactants. One of the components used may contribute three, four or five atoms of the pyrimidine ring system, while the other contributes three, two or one atom respectively. For example, the first component (29) may be amidines derivative in which the choice of R governs the nature of the 2-substituent in the pyrimidine. The R may be H (formamidine), amino (guanidine), hydroxyl (urea), thiol (thiourea), alkyl, aryl, alkoxy etc. The choice of R’ in second component (28) governs the nature of the 5-substituent (usually hydrogen) of pyrimidine ring (30). X and Y are the carbonyl or nitrile groups.18

1.1.3.3 Three component synthesis

Very few reports are available for three component synthesis. Generally, each of the three components, contributes two atoms each, for cyclization to produce pyrimidine ring. The reaction of alkyne derivatives (31) with two molecules of nitrile (32) in the presence of boron trifluoride gives substituted pyrimidine (33).19

Another example is cyclization of α-haloketones (34) with two molecules of nitriles (35) in the presence of trifluoromethanesulfonic acid (Tf₂O) under mild condition to give alkyl and aryl-5-halopyrimidines (36). Moreover, it has the advantage that the starting ketones are more easily available than the alkynes.20
CHAPTER 1

INTRODUCTION

\[ \text{R}_1 \text{C}=\text{O} \quad \text{X} + \text{R}_2 \equiv \text{N} \xrightarrow{\text{TF-O, CH}_2\text{Cl}_2, \text{RT}} \]

(34) \quad (35) \quad (36)

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1.2 AIM AND RATIONALE OF WORK

Pyrimidine and their derivatives are endlessly pulling attention of the medicinal chemists in view of long and notable history extending from the day of their discovery as, important constituents of nucleic acid. The important pyrimidine compounds have diverse applications as bactericidal, fungicidal, anti-inflammatory, antihypertensive, analgesic, antiviral, antimalarial, antifilarial, antioxidant, anthelmintic, anti-HIV and anti tumor agents.

They are also used as calcium channel blockers, α-antagonist and neuropeptide antagonist. Alkaloids containing the dihydropyrimidine structure have been isolated from various marine sources which have displayed interesting biological activities. In particular, the batzelladine alkaloids have been found to be potent HIV gp-120-CD4 inhibitors.

The development of simple synthetic routes for widely used organic compounds from readily available reagents is one of the major tasks in organic synthesis. Nowadays, the one step methods involving three component condensation using different reagents and catalysts are popular in synthetic organic chemistry for the synthesis of heterocyclic compounds. These single step methods are more convenient as compared with two step strategies as they require shorter reaction times, easy product isolation and give higher yields and recoveries of the product. The various synthetic routes for pyrimidine derivatives have been reviewed. Recently, various methods are reported concerning the synthesis of pyrimidine derivatives, few one pot synthesis have also been published by three component condensation reaction using aromatic aldehydes, ethyl cyanoacetate or malononitrile and urea or thiourea or guanidine.

To the best of our knowledge, there are very rare reports on one step synthesis of pyrimidine derivatives using aromatic aldehydes, malononitrile and thiourea or urea. Therefore, we tried to synthesize 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidines by three-component condensation of aromatic aldehydes, malononitrile and thiourea or urea in order to investigate new biological active compounds.
1.3 REVIEW OF LITERATURE

1.3.1 SYNTHETIC REVIEW

Pyrimidine does not normally serve as a starting point for preparation of substituted pyrimidines. These compounds are generally prepared by five types of ring synthesis I, II, III, IV and V according to the nature of the fragments which combines together to form the pyrimidine nucleus.\textsuperscript{50}

1.3.1.1 Synthesis from N-C-C-C-N and C- fragment

1. Sasse\textsuperscript{51} has reported synthesis of 6-thioxopyrimidin-4(3H)-one (2) by treating N-methyl-2-thiocarbamoylacetamide (1) with ethyl formate. He also reported condensation reaction of malondiamide derivative (3) with ethyl chloroformate to produce methylthiopyrimidine-2,4-(1H,3H)-dione (4).

\[
\text{Sasse}^{51} \text{ has reported synthesis of 6-thioxopyrimidin-4(3H)-one (2) by treating N-methyl-2-thiocarbamoylacetamide (1) with ethyl formate. He also reported condensation reaction of malondiamide derivative (3) with ethyl chloroformate to produce methylthiopyrimidine-2,4-(1H,3H)-dione (4).}
\]

2. Weis and Rosenbach\textsuperscript{52} reported synthesis of pyrimidine derivatives (7) by heating acetyl acetone (5) and benzaldehyde in presence of two equivalent of ammonium acetate via the intermediate (6).
3. Sato et al.\textsuperscript{53} reported the reaction of β-aminocrotonamide (8) with succinic anhydride (9) to yield β-succinamido crotonamide (10), which in turn undergoes cyclization in basic medium to give 3,4-dihydro-6-methyl-4-oxo-2-pyrimidinyl propanoic acid (11).

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\(\text{\small \includegraphics[scale=0.5]{reaction1.png}}\)};
  \node (b) at (2,0) {\(\text{\small \includegraphics[scale=0.5]{reaction2.png}}\)};
  \node (c) at (4,0) {\(\text{\small \includegraphics[scale=0.5]{reaction3.png}}\)};
\end{tikzpicture}
\end{center}

1.3.1.2 Synthesis from N-C-N-C and C-C fragment

1. Krechl et al.\textsuperscript{54} reported synthesis of substituted pyrimidine-5-carbonitriles (13a,b) and ethyl pyrimidine-5-carboxylate (14) by the reaction of methyl-N-aminocarbonyl imidates (12a) or N-aminothiocarbonyl imidates (12b) with malononitriles, methyl cyanoacetate or diethyl malonate by refluxing in presence of alkoxide.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\(\text{\small \includegraphics[scale=0.5]{reaction4.png}}\)};
  \node (b) at (2,0) {\(\text{\small \includegraphics[scale=0.5]{reaction5.png}}\)};
  \node (c) at (4,0) {\(\text{\small \includegraphics[scale=0.5]{reaction6.png}}\)};
\end{tikzpicture}
\end{center}

2. Mazumdar and Mahajan\textsuperscript{55} reported the reaction of 1,3-diaza derivative (15) with ketene derivative (16) to give pyrimidine derivatives (17).

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\(\text{\small \includegraphics[scale=0.5]{reaction7.png}}\)};
  \node (b) at (2,0) {\(\text{\small \includegraphics[scale=0.5]{reaction8.png}}\)};
  \node (c) at (4,0) {\(\text{\small \includegraphics[scale=0.5]{reaction9.png}}\)};
\end{tikzpicture}
\end{center}
3. Guzman et al.\textsuperscript{56} reported cycloaddition reaction between diazadiene (18) and alkynes derivatives (19) to yield pyrimidine derivative (20).

\[
\begin{align*}
\text{NMe}_2\text{N} & \quad \text{Cl}_2\text{C} \equiv \text{NH} \\
\text{R} & \quad \text{R'} \quad \text{C} \equiv \text{C} \quad \text{COR} \quad \text{38-98\%} \\
\text{(18)} & \quad \text{(19)} & \quad \text{(20)}
\end{align*}
\]

1.3.1.3 Synthesis from C-C-C and N-C-N fragment

1. Grimoux\textsuperscript{57} has reported the first synthesis of pyrimidine nucleus from the condensation of urea (21) with malonic acid (22) in the presence of phosphorus oxychloride, it was named barbituric acid (23).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{H}_2\text{N} & \quad \text{O} \\
\text{(21)} & \quad \text{(22)} & \quad \text{(23)}
\end{align*}
\]

2. Pinner\textsuperscript{58} has reported the condensation reaction of benzamidine (24) with ethylacetoacetate in alkaline solution (25) to yield 4-hydroxy-6-methyl-2-phenyl-pyrimidine (26).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{C}_6\text{H}_5 \\
\text{C}_6\text{H}_5 & \quad \text{NaO} \\
\text{(24)} & \quad \text{(25)} & \quad \text{(26)}
\end{align*}
\]

3. Breaux and Zwikelmaier\textsuperscript{59} reported, enamino ester (28) when condensed with amidine derivatives (27) yield ethyl pyrimidine-5-carboxylate derivative (29).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{R} \\
\text{R} & \quad \text{COOEt} \\
\text{(27)} & \quad \text{(28)} & \quad \text{(29)}
\end{align*}
\]

4. Lemieux and Puskas\textsuperscript{60} reported that 4-carbethoxy-2,6-dihydroxypyrImidine (32) can be obtained by the reaction of urea (21) with diethyloxaloacetate (30).
presumably via intermediacy of 5-carbethoxymethylenehydention (31)\textsuperscript{61} that rearrange to give (32).

![Chemical structures](image)

5. Rupe \textit{et al.}\textsuperscript{62} reported, ethyl cyanoacetate (33) and urea or thiourea undergoes cyclization in alkaline medium via the intermediacy of the open form (34) to yield the corresponding 4-aminopyrimidine derivatives (35).

![Chemical structures](image)

6. Hussain \textit{et al.}\textsuperscript{63} reported that heating a mixture of ethyl cyanoacetate (33) with aldehydes and S-methylisothiourea (36) give corresponding 4-aryl-5-cyano-2-methylthio-6-oxopyrimidine derivative (37).

![Chemical structures](image)

7. Lorente \textit{et al.}\textsuperscript{64} reported reaction of cyanoolefine (38) with guanidine, urea, thiourea or S-methyl thiourea which gives 4-aminopyrimidine derivatives (39).

![Chemical structures](image)

8. Ertan \textit{et al.}\textsuperscript{65} reported cyclocondensation reaction of benzaldehyde derivatives (40) with urea or thiourea and acetoacetate derivative (41) in the presence of HCl according to Biginelli reaction to give pyrimidine derivatives (42).
9. Brown et al.\textsuperscript{66} reported that thiourea react readily with \( \beta \)-diketones to give pyrimidine of higher yields than urea, as a typical example, reaction of thiourea (43) with benzoylacetone (44) in acidic ethanol gives 6-methyl-4-phenyl-2(1\( H \))-pyrimidinethione (45).

![Chemical structure](image)

1.3.1.4 Synthesis from C-C-C-N and C-N fragment

1. Baddiley et al.\textsuperscript{67} reported synthesis of 4-amino-5-cyanopyrimidine (48) by condensation of malononitrile and formamidine (46) via elimination of ammonia and formation of aminomethylene compound (47) as an intermediate.

![Chemical structure](image)

2. Elnagdi et al.\textsuperscript{68} reported reaction of benzoylacetonitrile (49) with two moles of trichloroacetonitrile to give 2,4-bis-trichloromethyl-5-cyano-6-phenylpyrimidine (51) via the intermediate (50).

![Chemical structure](image)
3. Kashima et al.\textsuperscript{69} reported reaction of enaminoester (52) and phenylisocyanate in refluxing DMF to yield corresponding pyrimidine derivatives (53).

![Image]

4. Elnagdi et al.\textsuperscript{70} reported cyclization reaction of enaminonitrile ester (54) and trichloroacetonitrile to give corresponding pyrimidine derivatives (55).

![Image]

5. Ried and Beller\textsuperscript{71} reported reaction of ethyl 2-chloro-3,3-dicyanoacrylate (56) with N,N-dialkylcyanamide (57) to give chloropyrimidine (59) via the formation of intermediate (58).

![Image]

6. Assy and Moustafa\textsuperscript{72} reported reaction of aryl isothiocyanate with cyanothioacetamide (60) to yield pyrimidinethione derivatives (61).

![Image]

7. Harris et al.\textsuperscript{73} reported the synthesis of 6-chloro-2,4-diphenylpyrimidine (63) by reaction of cinnamoinitrile (62) with benzamide in the presence of phosphorous oxychloride.
8. Alberola et al.\textsuperscript{74} reported reaction of cyanamide with 4-aminopent-3-en-2-one (64) and their substituted derivatives in aqueous solution to form the 2-aminopyrimidine (65) in high yield.

9. Elnagdi et al.\textsuperscript{75} reported reaction of ethyl 3-amino-2,4-dicyanocrotonate sodium (66) with 2,2,2-trichloroacetonitrile, while the amino nitrogen initiates the reaction by addition to the cyano group of the acetonitrile, eventually forms substituted pyrimidine derivative (67).

1.3.1.5 Synthesis from N-C-C and C-N-C fragment

1. Goerdeler and Wieland\textsuperscript{76} reported addition of nucleophilic carbon of enaminoketone or enaminoester (68) to the electrophilic carbon of aryl isothiocyanate or alkoxy carbonyl isothiocyanate (69) to yield intermediate (70), which in turn cyclize in basic medium to give the corresponding pyrimidine thione derivative (71).
2. O’Callaghan and McMurry\textsuperscript{77} reported, aminocrotonates (73) reacts with two moles of aromatic aldehyde derivatives (72) in the presence of ammonium acetate to give benzoate salts of pyrimidine derivative (74).

\[
\text{CHO} \quad \frac{2}{\text{R}} \quad \text{COOR} \quad \xrightarrow{\text{AcONH}_4, \text{EtOH}} \quad \text{HN} \quad \text{COOR} \quad \text{R}_1
\]

\( R = -\text{CH}_3, -\text{C}_2\text{H}_5; R_1 = -4\text{H}, 3\text{-Cl}, 4\text{-Cl}, 3\text{-CH}_3, 3\text{-OCH}_3 \)

3. Cocco \textit{et al.}\textsuperscript{78} reported the synthesis of 2-amino-4-dialkylamino- or -4-ethoxy-6-methylthio-5-pyrimidincarbonitriles (77) by base-induced reaction between 3-amino-3-(dialkylamino)propenenitriles (75) and \( N\)-[bis (methylthio)methylene] cyanamide (76).

\[
\text{X} \quad \text{CN} \quad + \quad \text{H}_3\text{CS} \quad \text{=N-CN} \quad \xrightarrow{\text{Base}} \quad \text{X} \quad \text{CN} \quad \text{N} \quad \text{CN} \quad \text{SCH}_3 \quad \text{H}_2\text{N}
\]

\( R = -\text{OEt}, \text{pyrrolidino, piperidino, morpholino, 4-methylpiperazino} \)

### 1.3.1.6 Synthesis from N-C-C-C-N-C fragment:

1. Barluenga \textit{et al.}\textsuperscript{79} reported Hoffman type degradation of appropriate diamides (78) to afford pyrimidine (80) presumably via the initial formation of an isocyanate intermediate (79).

\[
\text{NaOCl} \quad \xrightarrow{\text{H}_2\text{NOCN}} \quad \text{H}^+ \quad \xrightarrow{} \quad \text{HN} \quad \text{H}_2\text{N}
\]

2. Barluenga \textit{et al.}\textsuperscript{79} reported addition of HCl to N-cyano group of (E)-N',2-dicyanobutanimidamide (81) whereby the nitrogen becomes nucleophilic and add to the appropriately positioned C-cyano group with formation of the 2,6-diamino-2-chloro-pyrimidine (82).
3. Nishio and Omote\textsuperscript{80} reported formation of 4,6-diphenyl-1-propylpyrimidine-2(1\textit{H})-one (84), where uriedo nitrogen in the propylurido substrate (83) is made nucleophilic by running the cyclization reaction under alkaline conditions.

4. Elghandour \textit{et al.}\textsuperscript{81} reported intramolecular cycloaddition reaction of amino group to the activated double bond in the thiourea derivative (85) to yield 6-substituted-1-phenyl-2-thioxo-2,3-dihydropyrimidin-4(1\textit{H})-one (86).

1.3.2 BIOLOGICAL REVIEW

1. Keche \textit{et al.}\textsuperscript{82} evaluated a series of novel 4-(3-(trifluoromethyl)phenylamino-6-(4-(3-arylureido/arylthioureido/arylsulfonamido)-pyrimidine derivatives as anti-inflammatory and antimicrobial agents.
From antimicrobial results it can be concluded that compounds (1) and (2) exhibited excellent potent antibacterial activity than ciprofloxacin against *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Escherichia coli* (EC) and *Salmonella typhimurium* (ST). The compounds (3) and (4) exhibited comparable to or even higher antifungal activity than miconazole against *Candida albicans* (CA), *Aspergillus niger* (AN), *Fusarium solani* (FS) and *Aspergillus flavus* (AF).

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/mL)</th>
<th>SA</th>
<th>BS</th>
<th>EC</th>
<th>ST</th>
<th>CA</th>
<th>AN</th>
<th>FS</th>
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<td>Ciprofloxacin</td>
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<tr>
<td>Miconazole</td>
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<td>20</td>
<td>15</td>
<td>15</td>
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</tr>
</tbody>
</table>

The synthesized compounds were also screened for its anti-inflammatory activity and compound (5) was more active while (6) exhibited similar activity against proactive kinase TNF-α and interleukin-6 with respect to the standard dexamethasone at 10 µM concentration.

2. Ramesh and Bhalgat\textsuperscript{83} reported biological screening of some novel dihydropyrimidines and their dimethylated adducts (7). Some of these novel derivatives showed moderate to potent *in-vitro* antioxidant, anti-inflammatory, antimicrobial and anthelmintic activity.
3. Tale et al.\textsuperscript{84} screened a series of novel 3,4-dihydropyrimidin-2(1H)-one urea derivatives (8) for its biological activity. Biological activity evaluation study revealed that among all the compounds screened, compounds having chloro and flouro group found to have promising anti-inflammatory activity (68-62\% TNF-\(\alpha\) and 92-86\% IL-6 inhibitory activity at 10 \(\mu\)M). Interestingly some compounds revealed promising antimicrobial activity at MIC of 10-30 \(\mu\)g/mL against selected pathogenic bacteria and fungi.

4. Mishra et al.\textsuperscript{85} evaluated some 3,4-dihydropyrimidine-2-one (9) as antimicrobial agent and their antibacterial activity was screened against \textit{Proteus mirabilis} and \textit{Pseudomonas aeruginosa}.

5. Pore et al.\textsuperscript{86} reported antibacterial and antifungal activities of N1-substituted 5-cyanopyrimidine derivatives (10) by cup plate method in the concentration of 25 \(\mu\)g against \textit{S. aureus} and \textit{E. coli}.

6. Agarwal et al.\textsuperscript{87} screened novel amino(substituted)pyrimidones for treating inflammation and immunological disorders. Compound (11) showed 75.76\% COX-2 inhibition in \textit{in-vitro} evaluation of COX-2 inhibition activity.

7. Deshmukh et al.\textsuperscript{88} evaluated 2-amino-5-cyano-6-hydroxy-4-arylpyrimidines (12) for its anti-bacterial activity against \textit{Escherichia coli} and \textit{Staphylococcus aureus}.
8. Singh et al.\textsuperscript{89} studied a series of pyrimidine derivatives (13) bearing amine substituents at C-2 position obtained from Biginelli 3,4-dihydropyrimidin-2(1H)-ones and the effect of structural variation on anti-TB activity against \textit{Mycobacterium tuberculosis} H\textsubscript{37}Rv strain and antiviral activity in a series of cell cultures was also evaluated.

9. Sondhi et al.\textsuperscript{90} synthesized various pyrimidine derivatives and screened for anti-inflammatory and analgesic activity. In this series two compounds have exhibited 39% and 40% anti-inflammatory activity and one compound (14) has shown 75% analgesic activity at 100 mg/kg dose.

10. Clive et al.\textsuperscript{91} reported substituted pyrimidine (15) as selective COX-2 inhibitors which showed IC\textsubscript{50}=18 nM against COX 2 and IC\textsubscript{50}>>91000 nM against COX 1.

\begin{center}
\includegraphics[width=\textwidth]{images}
\end{center}

\(R_1 = \text{alkyl}; R_3 = \text{NHH}_2, \text{alkyl}; R_2 = \text{tetrahydropyranyl}, \text{tetrahydrofuran-3-yl}\)

11. Carlos et al.\textsuperscript{92} reported the synthesis of various pyrimidine derivatives (16) for the treatment of inflammatory, immune system disorders. These compounds were found to have IC\textsubscript{50} in the range of 10 nM to 20 nM.

12. Sondhi et al.\textsuperscript{93} evaluated a number of pyrimidine derivatives (17) for its pharmacological activity. Anti-inflammatory activity of synthesized compounds was comparable while analgesic activity was found to be better than that of standard drug.
13. Venu et al.\textsuperscript{94} reported anti-inflammatory activity and its ulcerogenic effect of various 2-(2-aroylaroxy)-4,6-dimethoxypyrimidine derivatives (18). All the compounds have shown superior anti-inflammatory activity in the range 21.5-48.6\% at a dose of 40 mg/kg.

14. Chimenti et al.\textsuperscript{95} prepared various 3-cyano-4,6-diaryl-pyridin-2(1H)-one derivatives (19) and screened for its calcium channel blocking activity. On preliminary pharmacological tests all compounds were found to be active and some of them show calcium antagonistic activity superior or comparable to diltiazem.

15. Bhatt and Kulkarni\textsuperscript{96} reported the synthesis of some new pyrimidine derivatives (20) as anticancer agents. Some of these compounds showed moderate to considerable anticancer activity.

16. Guillemont et al.\textsuperscript{97} synthesized some novel diarylpyrimidine analogues (21) and evaluated for their antiviral activity against human immunodeficiency virus type-1.

17. Hopkins et al.\textsuperscript{98} reported synthesis of pyrimidinone derivatives. In this series compound TNK-6123 (22) was found as active against drug resistant HIV mutant.

18. Rovnyak et al.\textsuperscript{99} examined a series of novel dihydropyrimidine as calcium channel blockers that contain a basic group attached to either C-5 or N-3 of the heterocyclic ring. Dihydropyrimidine (23) was equipotent to nifedipine and amlodipine \textit{in-vitro}.

19. Yarim et al.\textsuperscript{100} 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, 2-nitrophenyl derivative (24) and 2-bromophenyl
derivative (25) have potent antispasmodic activity on BaCl$_2$ stimulated rat ileum.

![Chemical structures]

20. Bryzgalov et al.$^{101}$ studied antiarrhythmic activity of 4,6-di(het)aryl-5-nitro-3,4-dihydropyrimidin-(1H)-2-ones (26) towards 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.

21. Sujatha et al.$^{102}$ studied cardiovascular effects of a series of 4-(substituted)-3,4-dihydropyrimidinone derivatives (27) on isolated perfused frog heart at different dose levels and compared with the activity of digoxin. The interaction of 3,4-dihydropyrimidinones with β-blocker and calcium channel blocker was also investigated. Some of the compounds emerged as the most interesting compound in this series with potential cardiotonic activity.

![Chemical structures]

22. Sato et al.$^{103}$ reported 2-aminopyrimidine as histamine H$_4$ receptor antagonist. Compound (28) in the form of hydrochloride salt showed IC$_{50}$$< 20$ nM.
1.4 OBJECTIVES

✓ To search the novelty of proposed molecules and reaction conditions from various chemical databases like scifinder, Sciverse scopus, chemsynthesis, chemspider, etc.

✓ To synthesize the target compounds, 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives as per scheme by conventional, microwave assisted and green chemistry method.

✓ To monitor the purity and progress of the reaction using TLC technique.

✓ To purify the compounds by recrystallization using suitable solvents.

✓ To characterize the newly synthesized compounds by physical methods like melting point, R$_f$ value and spectral analysis like IR, NMR and Mass spectra.

✓ To evaluate the antimicrobial activity of newly synthesized compounds against various gram (+)ve, gram (-)ve bacteria, fungi and acid fast bacteria.

✓ To perform *in-vitro* anti-inflammatory activity and antioxidant activity of newly synthesized compounds
1.5 REACTION SCHEME

1.5.1 CONVENTIONAL METHOD

$$\text{ArCHO} + \text{H}_2\text{N-C-NH}_2 + \text{CNCH}_2\text{CN} \xrightarrow{\text{Con. HCl, EtOH, Reflux}} \begin{array}{c} \text{Ar} \\ \text{X} \end{array} \begin{array}{c} \text{CN} \\ \text{N} \\ \text{NH}_2 \\ \text{X} = \text{O, 1a-1l} \\ \text{X} = \text{Y, 2a-2l} \end{array}$$

1.5.2 MICROWAVE ASSISTED METHOD

$$\text{ArCHO} + \text{H}_2\text{N-C-NH}_2 + \text{CNCH}_2\text{CN} \xrightarrow{\text{Con. H}_2\text{SO}_4, \text{EtOH, MWT}} \begin{array}{c} \text{Ar} \\ \text{X} \end{array} \begin{array}{c} \text{CN} \\ \text{N} \\ \text{NH}_2 \\ \text{X} = \text{O, 1a-1l} \\ \text{X} = \text{Y, 2a-2l} \end{array}$$

1.5.3 GREEN CHEMISTRY METHOD

$$\text{ArCHO} + \text{H}_2\text{N-C-NH}_2 + \text{CNCH}_2\text{CN} \xrightarrow{\text{CH}_3\text{COONa, Water, Reflux}} \begin{array}{c} \text{Ar} \\ \text{X} \end{array} \begin{array}{c} \text{CN} \\ \text{N} \\ \text{NH}_2 \\ \text{X} = \text{O, 1a-1l} \\ \text{X} = \text{Y, 2a-2l} \end{array}$$
1.6 MATERIALS AND METHODS

1.6.1 GENERAL

- All reagents and solvents used were of laboratory (LR) grade, obtained from SD fine chemicals (Mumbai, India) and Merck (Mumbai, India) and were used without further purification.

1.6.2 MEASUREMENTS

- The progress of the reaction and purity of the synthesized compounds were checked on the precoated silica gel F254 plates obtained from Merck (Mumbai, India) using chloroform and ethyl acetate (7:3) as mobile phase. Iodine chamber and UV lamp (λ = 254 nm) were used for visualization of the spots.

- Melting points were determined in an open capillary tube on Chemline CL726 melting point apparatus and were uncorrected.

- Double beam Shimadzu 1800 UV spectrophotometer was used for the determination of absorbance.

- The IR spectra (νmax, cm⁻¹) were recorded on Shimadzu FT-IR 157 spectrophotometer as KBr discs.

- ¹H NMR (δ, ppm) spectra were recorded in CDCl₃ or DMSO-d₆ with Tetramethysilane (TMS) as internal standard on Bruker advance III NMR spectrophotometer at 500 MHz.

- Mass spectra were determined using direct inlet probe on a Shimadzu GC-MS QP 2010 mass spectrometer.

1.6.3 EXPERIMENTAL PROCEDURES

1.6.3.1 Synthetic procedure for preparation of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives (1a-1l and 2a-2l):

Conventional method:

Aromatic aldehyde (0.02 mol), malononitrile (0.02 mol) and urea or thiourea (0.022 mol) were dissolved in 25 mL absolute ethanol in a 100 mL round bottom flask. The
resulting reaction mixture was heated at reflux using a water bath. The reaction mixture was poured on the crushed ice (about 200 g) after the completion of the reaction monitored by TLC. On stirring separation of desired product takes place. The solid was filtered off, dried and recrystallized from suitable solvent.

**Microwave assisted method:**

A mixture of substituted aldehyde (0.005 mol), malononitrile (0.005 mol), urea or thiourea (0.005 mol) and conc. \( \text{H}_2\text{SO}_4 \) (1-2 drops) in absolute ethanol (5 mL) were taken in 10 mL beaker and irradiated in the domestic type microwave oven 900 W with a frequency 2450 MHz (Kenstar OM-25 DCE, India) for 2-4 min (one pulse each of 30 sec). After the completion of reaction (reaction monitoring by TLC), the reaction-mixture was then allowed to stand at room temperature and the product formed was filtered off, washed with ethanol, dried and recrystallized from suitable solvent.

**Green chemistry method:**

A mixture of substituted aldehyde (0.02 mol), malononitrile (0.02 mol), urea or thiourea (0.02 mol) and sodium acetate (0.02 mol) in water (50 mL) and ethanol (5 mL) was refluxed with stirring for 3.5-6 h (the progress of the reaction being monitored by TLC). The product precipitated from the reaction mixture after cooling was filtered and recrystallized from suitable solvent.

**Table 1.1 IUPAC name of pyrimidine derivatives**

<table>
<thead>
<tr>
<th>Compds.</th>
<th>IUPAC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>4-amino-2-hydroxy-6-phenylpyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1b</td>
<td>4-amino-2-hydroxy-6-styrylpyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1c</td>
<td>4-amino-2-hydroxy-6-(4-hydroxyphenyl)pyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1d</td>
<td>4-amino-6-(2-chlorophenyl)-2-hydroxypyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1e</td>
<td>4-amino-6-(4-chlorophenyl)-2-hydroxypyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1f</td>
<td>4-amino-6-(4-(dimethylamino)phenyl)-2-hydroxypyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1g</td>
<td>4-amino-2-hydroxy-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile</td>
</tr>
<tr>
<td>1h</td>
<td>4-amino-2-hydroxy-6-(4-hydroxy-3-methoxyphenyl)pyrimidine-5-carbonitrile</td>
</tr>
</tbody>
</table>
1i 4-amino-2-hydroxy-6-(2-nitrophenyl)pyrimidine-5-carbonitrile
1j 4-amino-2-hydroxy-6-(4-nitrophenyl)pyrimidine-5-carbonitrile
1k 4-amino-2-hydroxy-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile
1l 4-amino-2-hydroxy-6-(3-nitrophenyl)pyrimidine-5-carbonitrile
2a 4-amino-2-mercapto-6-phenylpyrimidine-5-carbonitrile
2b 4-amino-2-mercapto-6-styrylpyrimidine-5-carbonitrile
2c 4-amino-6-(4-hydroxyphenyl)-2-mercaptopyrimidine-5-carbonitrile
2d 4-amino-6-(2-chlorophenyl)-2-mercaptopyrimidine-5-carbonitrile
2e 4-amino-6-(4-chlorophenyl)-2-mercaptopyrimidine-5-carbonitrile
2f 4-amino-6-(4-(dimethylamino)phenyl)-2-mercaptopyrimidine-5-carbonitrile
2g 4-amino-2-mercapto-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile
2h 4-amino-6-(4-hydroxy-3-methoxyphenyl)-2-mercaptopyrimidine-5-carbonitrile
2i 4-amino-2-mercapto-6-(2-nitrophenyl)pyrimidine-5-carbonitrile
2j 4-amino-2-mercapto-6-(4-nitrophenyl)pyrimidine-5-carbonitrile
2k 4-amino-2-mercapto-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile
2l 4-amino-2-mercapto-6-(3-nitrophenyl)pyrimidine-5-carbonitrile
1.7 BIOLOGICAL SCREENING

1.7.1 ANTIMICROBIAL ACTIVITY

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. The Micro dilution broth susceptibility test is among the first to be developed and still serves as a reference method.

The MICs of synthesized compounds were carried out by broth micro dilution method according to reported procedure.\textsuperscript{104} Antibacterial activity was screened against two gram (+)ve bacteria (\textit{Staphylococcus aureus}, \textit{Bacillus subtilis}) and two gram (-)ve bacteria (\textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}). Antifungal activity was screened against one fungal species (\textit{Candida albicans}).

Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Serial dilutions of antimicrobial agents were made in Muller Hinton broth, after which a standardized bacterial/fungal suspension was added. Quantities of compound serially diluted in test tubes. One control test tube was prepared without compound which serves as growth control. All test tubes were inoculated with a calibrated suspension of the microorganism to be tested and incubated at 37°C for 24 h.

In primary screening 1000, 500, 200 and 100 µg/mL concentrations of the synthesized drugs were taken. The active drugs found in this primary screening were further diluted to obtain 75, 50 and 25 µg/mL concentrations and tested for antimicrobial activity. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. The highest dilution showing at least 99% inhibition was taken as MIC. The result of this is much affected by the size of the inoculums. All the test tubes contained $10^8$ colony forming unit (CFU) microorganism and 50 µL sample.

1.7.2 ANTITUBERCULAR ACTIVITY

There are three components which are important for screening of antitubercular activity of the synthesized compounds.

A. \textbf{Media:} These are the mixture of growth nutrient which are required for the smooth growth of the mycobacteria.
Ideal characteristics of media

1. Support rapid and luxuriant growth of small numbers of mycobacteria.
2. Inhibit the growth of contaminants.
3. Economical and simple to prepare.
4. Enable the performance of drug susceptibility tests.

Classification of media

1. Egg base media eg. Lowestein Jensen egg media
   
   **Advantages:**
   
   - It may be stored in the refrigerator for several months provided it was made from fresh eggs and if the caps are tightly closed to minimize drying by evaporation.
   - Less likely to contaminate and support good growth.
   
   **Disadvantage:**
   
   - Drug susceptibility tests are difficult to perform.

2. Agar base media eg. Middle brook 7H10 and 7H11 media
   
   **Advantages:**
   
   - It is a clear medium that examined with the aid of dissecting microscope.
   - Drug susceptibility tests performed easily.
   
   **Disadvantages:**
   
   - Exposure of media to daylight causes the release of formaldehyde gas in concentration sufficient to inhibit growth of mycobacteria.
   - Preparation and handling of media is difficult.

B. Types of mycobacterial culture:

1. *Mycobacterium tuberculosis* (ATCC 25177 / H$_{37}$Ra)
2. *Mycobacterium tuberculosis* (ATCC 27294 / H₃₇Rv)

C. **Drug:** For the screening of antitubercular compounds the drug has to be dissolved in the medium prior to incubation with the mycobacterium. To facilitate the dissolution generally DMSO or DMF is used as solvent in minimum quantity. In the total protocol for the antitubercular screening DMSO/DMF is also used as control. The quantity of DMSO used to dissolve compound should not change the composition and solidification pattern of media used.

**Methods used for susceptibility testing of antitubercular drugs:**

A. **BACTEC assay**\(^{105, 106}\)

1. Radiometric assay method

2. Non radiometric assay method

Based on the radiometric studies of metabolism of *M. tuberculosis* in the 1970s, the Bactec 460 instrument was developed. The method was developed based on the observation that mycobacteria catabolize fatty acid substrate to carbon dioxide. This system permits the automated detection of \(^{14}\text{CO}_2\).

In the Bactec system, the growth medium (Middlebrook 7H11) is supplement with \(^{14}\text{C}-\text{palmitic acid at a high radiospecific activity. If mycobacteria are present in the specimen inoculated in to the Bactec vial, the radioactively labeled palmitic acid is converted in to }^{14}\text{CO}_2\text{, which is detected by the instrument. In recent years, the Bactec 460 instrument is replaced by the non radiometric Bactec 460 instrument for routine blood cultures; however, the Bactec 460 version is retained because the sensitivity needed to detect mycobacteria species can be achieved only with the radiometric system.**

B. **Microplate alamar blue assay (MABA)**\(^{107, 108}\)

The alamar blue oxidation-reduction dye is a general indicator of cellular growth and viability; the blue, nonfluorescent, oxidized form becomes pink and fluorescent upon reduction. Growth can therefore be measured with a fluorimeter or spectrophotometer. It is nonradioactive method used for susceptibility testing of antitubercular drug.
C. Oxygen quenched fluorescent method\textsuperscript{109}

A fluorescent compound is embedded in silicone on the bottom of the MGIT tubes. Fluorescent indicator generally used is tri-4,7-diphenyl-1,10-phenanthroline ruthenium chloride pentahydrate. The tubes are fused with 10% CO\textsubscript{2} and capped with polypropylene caps. The fluorescent compound is sensitive to the presence of O\textsubscript{2} dissolved in broth. Large amount of dissolved O\textsubscript{2} quenches emissions from the compound and very less or no fluorescence is observed but in the presence of actively respiring microbes which consume oxygen, fluorescence can be observed using 365 nm UV transilluminator or long UV light (Wood’s lamp). Precaution should be taken when observing fluorescence. Use of long wave illuminator is mandatory.

Growth can also be detected by presence of non homogenous turbidity or small grains or flakes in the culture medium. The medium components are substances essential for rapid growth of Mycobacteria. Oleic acid is utilized by tubercle bacilli in its metabolic process. Albumin acts as a protective agent by binding free fatty acid which must be present in the medium.

Test compounds were evaluated for \textit{in-vitro} antitubercular activity. MICs were determined and interpreted for \textit{Mycobacterium tuberculosis} H\textsubscript{37}Rv according to the reported procedure by macro dilution reference method\textsuperscript{110} using L. J. media. Lowenstein Jensen media\textsuperscript{111} was prepared by dissolving potassium dihydrogen phosphate (1.2 g), magnesium sulphate (0.12 g), magnesium citrate (0.3 g), L-asparagine (1.8 g), glycerol/sodium pyruvate (6.0 mL/3.6 g) and malachite green 2\% (16 mL) in a beaker in 300 mL distilled water by heating the mixture. To this mixture known quantities of egg homogenate (500 mL) and benzyl penicillin (1,000,000 IU/mL) (1 mL) were added aseptically after cooling to about 40°C. The media was then coagulated at 90°C for 60 minutes. Finally the media was sterilized by autoclaving at 121°C for 15 minutes at 15 PSI pressure, afterwards mixture was cooled to 45°C and transferred to sterile petri plates to form uniform layer and allow it to solidify.

A culture of \textit{M. tuberculosis} H\textsubscript{37}Rv growing on L. J. medium was harvested in 0.85% saline in bijou bottles. Compounds were taken at concentrations of 100, 50 and 25 μg/mL in DMSO. The bottles then inoculated with test compounds and incubated at 37°C for 24 h followed by streaking of \textit{M. tuberculosis} H\textsubscript{37}Rv (5*10\textsuperscript{4} bacilli per tube).
These bottles were then incubated at 37°C and inspected for growth twice a week for a period of three weeks. Readings were taken at the end of 3 weeks. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The appearance of turbidity was considered as growth and indicates resistance to the compound.

1.7.3 ANTI-INFLAMMATORY ACTIVITY

The synthesized compounds were screened for *in-vitro* anti-inflammatory activity using inhibition of albumin denaturation technique according to reported method with slight modification. The standard drug and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5%. Test solution (1 mL, 100 mg/mL) was mixed with 1 mL of 1% albumin solution in phosphate saline buffer and incubated at 27°C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with UV-Vis spectrophotometer. The percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The diclofenac was used as standard drug. The percentage inhibition was calculated using the formula.

\[
\% \text{ Inhibition of denaturation} = \frac{\text{At} - \text{Ac}}{\text{Ac}} \times 100
\]

Where, At = Mean absorbance of test compound

Ac = Mean absorbance of control

1.7.4 ANTIOXIDANT ACTIVITY

1.7.4.1 Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate saline buffer (pH 7.4). 1 mL of various dilutions (12.5, 25, 50, 100 mg/mL) of the test samples or standard, ascorbic acid in methanol were added to 2 mL of hydrogen peroxide solution in the phosphate saline buffer. The absorbance was measured at 230 nm after 10 min.
1.7.4.2 Nitric oxide scavenging activity

The reaction mixture containing sodium nitroprusside (10 mM, 4 mL), phosphate saline buffer (pH 7.4, 1 mL) and test samples or ascorbic acid solution in dimethyl sulphoxide (1 mL) at various concentrations (12.5, 25, 50, 10 mg/mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of reaction mixture withdrawn, 1 mL sulphanillic acid reagent added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 mL of naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 640 nm.\textsuperscript{116}

In above antioxidant activity determination methods the experiments were performed in triplicate and average mean was taken. The percentage inhibition was calculated by the following formula. The IC\textsubscript{50} value was derived from the percentage inhibition at different concentration.

\[
\% \text{ Inhibition} = [1 - (At/Ac)] \times 100
\]

Where, \(At\) = Mean absorbance of test compound
\(Ac\) = Mean absorbance of control

1.7.4.3 Reducing power determination

The reducing power of test samples was determined according to reported method.\textsuperscript{117} The test compounds and standard drug, ascorbic acid were dissolved in N,N-dimethyl formamide (DMF) to get different dilutions (12.5, 25, 50, 100 mg/mL). 1 mL of these dilutions then mixed with 2.5 mL of (pH 6.6) 0.2 M phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The upper layer of solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm, against reagent blank solution. The IC\textsubscript{50} value was derived from the percentage inhibition at different concentration.

\[
\% \text{ Inhibition} = [(At/Ac) - 1] \times 100
\]

Where, \(At\) = Mean absorbance of test compound
\(Ac\) = Mean absorbance of control.
1.8 RESULTS

1.8.1 PHYSICAL DATA

Table 1.2 Physical data of pyrimidine derivatives

<table>
<thead>
<tr>
<th>Compds.</th>
<th>X</th>
<th>Ar</th>
<th>Molecular Formula</th>
<th>Molecular Weight (g)</th>
<th>Recrystallization Solvent</th>
<th>Rf*</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>O</td>
<td>C_{6}H_{5}</td>
<td>C_{11}H_{8}N_{4}O</td>
<td>212.21</td>
<td>Ethanol</td>
<td>0.72</td>
<td>179-180</td>
</tr>
<tr>
<td>1b</td>
<td>O</td>
<td>C_{6}H_{5}CH=CH-</td>
<td>C_{13}H_{10}N_{4}O</td>
<td>238.24</td>
<td>Ethanol</td>
<td>0.67</td>
<td>151-153</td>
</tr>
<tr>
<td>1c</td>
<td>O</td>
<td>4-OHC_{6}H_{4}-</td>
<td>C_{11}H_{4}N_{4}O_{2}</td>
<td>228.21</td>
<td>EtOH:H_{2}O (1:1)</td>
<td>0.51</td>
<td>173-175</td>
</tr>
<tr>
<td>1d</td>
<td>O</td>
<td>2-ClC_{6}H_{4}-</td>
<td>C_{11}H_{7}ClN_{4}O</td>
<td>246.65</td>
<td>Ethanol</td>
<td>0.65</td>
<td>180-182</td>
</tr>
<tr>
<td>1e</td>
<td>O</td>
<td>4-ClC_{6}H_{4}-</td>
<td>C_{11}H_{7}ClN_{4}O</td>
<td>246.65</td>
<td>Ethanol</td>
<td>0.68</td>
<td>162-164</td>
</tr>
<tr>
<td>1f</td>
<td>O</td>
<td>4-N(CH_{3})<em>{2}C</em>{6}H_{5}-</td>
<td>C_{13}H_{13}N_{5}O</td>
<td>255.28</td>
<td>Ethanol</td>
<td>0.58</td>
<td>191-192</td>
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<tr>
<td>1g</td>
<td>O</td>
<td>4-OMeC_{6}H_{4}-</td>
<td>C_{12}H_{10}N_{4}O_{2}</td>
<td>242.23</td>
<td>Ethanol</td>
<td>0.60</td>
<td>156-157</td>
</tr>
<tr>
<td>1h</td>
<td>O</td>
<td>4-OH-3-OMe-C_{6}H_{4}-</td>
<td>C_{12}H_{10}N_{4}O_{3}</td>
<td>258.23</td>
<td>Ethanol</td>
<td>0.52</td>
<td>194-196</td>
</tr>
<tr>
<td>1i</td>
<td>O</td>
<td>2-NO_{2}C_{6}H_{4}-</td>
<td>C_{11}H_{3}N_{2}O_{3}</td>
<td>257.20</td>
<td>Ethanol</td>
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<td>199-200</td>
</tr>
<tr>
<td>1j</td>
<td>O</td>
<td>4-NO_{2}C_{6}H_{4}-</td>
<td>C_{11}H_{3}N_{2}O_{3}</td>
<td>257.20</td>
<td>Ethanol</td>
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<td>1k</td>
<td>O</td>
<td>3-OMeC_{6}H_{4}-</td>
<td>C_{12}H_{10}N_{4}O_{2}</td>
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<td>Ethanol</td>
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<td>167-169</td>
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<td>1l</td>
<td>O</td>
<td>3-NO_{2}C_{6}H_{4}-</td>
<td>C_{11}H_{3}N_{3}O_{3}</td>
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<td>Ethanol</td>
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<td>160-162</td>
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<tr>
<td>2a</td>
<td>S</td>
<td>C_{6}H_{5}-</td>
<td>C_{11}H_{8}N_{4}S</td>
<td>228.27</td>
<td>Ethanol</td>
<td>0.76</td>
<td>152-153</td>
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<tr>
<td>2b</td>
<td>S</td>
<td>C_{6}H_{5}CH=CH-</td>
<td>C_{13}H_{10}N_{4}S</td>
<td>254.31</td>
<td>Ethanol</td>
<td>0.73</td>
<td>118-120</td>
</tr>
</tbody>
</table>
1.8.2 SPECTRAL DATA

Table 1.3 Spectral data of pyrimidine derivatives

<table>
<thead>
<tr>
<th>Compds.</th>
<th>IR (KBr, ν, cm⁻¹)</th>
<th>¹H NMR (CDCl₃, δ, ppm)</th>
<th>Mass (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3473, 3233, 2221, 1635, 1286, 1170</td>
<td>5.44 (s, 2H, NH₂), 7.55-7.59 (t, 2H, aro. CH), 7.65-7.68 (t, 1H, aro. CH), 7.81 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH)</td>
<td>212 (M⁺)</td>
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* Mobile Phase = Toluene : Ethyl acetate (7:3)
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<th>3410, 3238, 3086, 2225, 1687, 1307</th>
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<td>5.34 (s, 2H, NH₂), 7.26-7.27 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.42-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH)</td>
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<tr>
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<td>7.82 (s, 1H, OH)</td>
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<td>DMSO-\textit{d}₆: 5.40 (s, 2H, NH₂), 6.86-6.87 (d, 2H, aro. CH), 7.05 (s, 1H, OH), 7.48-7.50 (d, 2H, aro. CH), 7.65 (s, 1H, OH)</td>
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<td>3463, 3225, 2226, 1578, 1290, 749</td>
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<td>5.18 (s, 2H, NH₂), 7.46-7.49 (m, 1H, aro. CH), 7.57-7.58 (d, 2H, aro. CH), 8.19-8.21 (d, 1H, aro. CH), 7.82 (s, 1H, OH)</td>
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<td>5.30 (s, 2H, NH₂), 7.53-7.56 (d, 2H, aro. CH), 7.76 (s, 1H, OH), 7.87-7.89 (d, 2H, aro. CH)</td>
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<td>3.17 (s, 6H, (CH₃)₂), 5.33 (s, 2H, NH₂), 6.70-6.72 (d, 2H, aro. CH), 7.48 (s, 1H, OH), 7.83-7.84 (d, 2H, aro. CH)</td>
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<td>3450, 3237, 2224, 1570, 1263, 1177</td>
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<td>3.94 (s, 3H, CH₃), 5.50 (s, 2H, NH₂), 7.03-7.05 (d, 2H, aro. CH), 7.68 (s, 1H, OH), 7.93-7.95 (d, 2H, aro. CH)</td>
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<td>3.98 (s, 3H, CH₃), 5.14 (s, 2H, NH₂), 7.01-7.03 (d, 1H, aro. CH), 7.26 (s, 1H, OH), 7.30-7.32 (dd, 1H, aro. CH), 7.63 (s, 1H, OH), 7.73 (d, 1H, aro. CH)</td>
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<td>5.18 (s, 2H, NH₂), 7.82-7.85 (m, 2H, aro. CH), 7.89-7.92 (m, 1H, aro. CH), 8.37-8.39 (dd, 1H, aro. CH), 7.87 (s, 1H, OH)</td>
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<td>5.13 (s, 2H, NH₂), 7.91 (s, 1H, OH), 8.09-8.11 (d, 2H, aro. CH), 8.40-8.43 (d, 2H, aro. CH)</td>
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Saurashtra University

Dipen K. Sureja
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<th>1l</th>
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<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
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<td>3450, 3239, 3079, 2228, 1681, 1315</td>
<td>3499, 3323, 3219, 2220, 1634, 1294</td>
<td>3421, 3234, 2221, 1596, 1260, 760</td>
<td>3421, 3228, 2232, 1585, 1215, 617</td>
<td>3419, 3242, 2223, 1570, 1299, 1173</td>
<td>3446, 3249, 2229, 1563, 1278, 1183</td>
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<td>k</td>
<td>3.93 (s, 3H, CH₃), 5.33 (s, 2H, NH₂), 7.00-7.02 (d, 1H, aro. CH), 7.45-7.50 (m, 3H, aro. CH)</td>
<td>5.24 (s, 2H, NH₂), 7.77-7.79 (dd, 1H, aro. CH), 7.84 (s, 1H, OH)</td>
<td>4.81 (s, 1H, SH), 5.48 (s, 2H, NH₂), 7.56-7.59 (t, 2H, aro. CH), 7.64-7.68 (t, 1H, aro. CH)</td>
<td>4.82 (s, 1H, SH), 5.23 (s, 2H, NH₂), 7.25-7.26 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.43-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH)</td>
<td>DMSO-d₆: 4.65 (s, 1H, SH), 5.42 (s, 2H, NH₂), 6.85-6.86 (d, 2H, aro. CH), 7.12 (s, 1H, OH), 7.49-7.50 (d, 2H, aro. CH)</td>
<td>4.62 (s, 1H, SH), 5.26 (s, 2H, NH₂), 7.46-7.48 (m, 1H, aro. CH), 7.57-7.60 (d, 2H, aro. CH)</td>
<td>4.74 (s, 1H, SH), 5.46 (s, 2H, NH₂), 7.52-7.54 (d, 2H, aro. CH), 7.85-7.87 (d, 2H, aro. CH)</td>
<td>3.16 (s, 6H, (CH₃)₂), 4.67 (s, 1H, SH), 5.26 (s, 2H, NH₂), 6.71-6.72 (d, 2H, aro. CH), 7.82-7.84 (d, 2H, aro. CH)</td>
<td>3.92 (s, 3H, CH₃), 4.68 (s, 1H, SH), 5.09 (s, 2H, NH₂), 7.03-7.05 (d, 2H, aro. CH), 7.93-7.94 (d, 2H, aro. CH)</td>
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<td>m</td>
<td>7.50 (m, 3H, aro. CH), 7.65 (s, 1H, OH)</td>
<td>8.13 (dd, 1H, aro. CH), 8.52 (m, 1H, aro. CH), 8.62 (s, 1H, aro. CH)</td>
<td>7.68 (t, 1H, aro. CH), 7.93-7.95 (d, 2H, aro. CH),</td>
<td>4.82 (s, 1H, SH), 5.23 (s, 2H, NH₂), 7.25-7.26 (d, 1H, Ar-CH=CH-), 7.28-7.29 (d, 1H, Ar-CH=CH-), 7.43-7.48 (m, 3H, aro. CH), 7.61-7.62 (d, 2H, aro. CH)</td>
<td>7.12 (s, 1H, OH), 7.49-7.50 (d, 2H, aro. CH)</td>
<td>7.58 (d, 2H, aro. CH), 8.19-8.20 (d, 1H, aro. CH)</td>
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<tr>
<td>1l</td>
<td>242 (M⁺)</td>
<td>257 (M⁺)</td>
<td>228 (M⁺)</td>
<td>254 (M⁺)</td>
<td>244 (M⁺)</td>
<td>262 (M⁺), 264 (M+2)</td>
<td>262 (M⁺), 264 (M+2)</td>
<td>271 (M⁺)</td>
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<td></td>
</tr>
</tbody>
</table>
CHAPTER 1

RESULTS

Saurashtra University

Dipen K. Sureja

Table 1.4 Comparison between various synthetic methods for preparation of pyrimidine derivatives

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Conventional Method</th>
<th>Microwave Assisted Method</th>
<th>Green Chemistry Method</th>
</tr>
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<tbody>
<tr>
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<td>Reaction time (h)</td>
<td>% Yield*</td>
<td>Reaction time (min)</td>
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<tr>
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<td>3.0</td>
<td>78</td>
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<td>2.5</td>
<td>60</td>
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<td>1c</td>
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<td>74</td>
<td>3.0</td>
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<tr>
<td>1d</td>
<td>2.0</td>
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<td>1e</td>
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<td>79</td>
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1.8.3 COMPARISON BETWEEN VARIOUS SYNTHETIC METHODS
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<th>Yield refers to pure isolated product</th>
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<td>83</td>
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### 1.8.4 ANTIMICROBIAL ACTIVITY

Table 1.5 Antimicrobial activity of pyrimidine derivatives

<table>
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<th>Compds.</th>
<th>MIC (µg/mL)</th>
<th>Gram (+)ve bacteria</th>
<th>Gram (-)ve bacteria</th>
<th>Fungi</th>
<th>Acid fast</th>
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<td>P. aeruginosa</td>
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<td>100</td>
</tr>
<tr>
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</tr>
<tr>
<td>1c</td>
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<td>200</td>
<td>200</td>
<td>100</td>
</tr>
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### 1.8.5 ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

Table 1.6 Anti-inflammatory and antioxidant activity of pyrimidine derivatives

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<th>Compds.</th>
<th>Anti-inflammatory activity</th>
<th>Antioxidant activity</th>
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<td>*Absorbance Mean ± S.D.</td>
<td>% Inhibition of denaturation</td>
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<td>Control</td>
<td>0.1980 ± 0.024</td>
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<td>1a</td>
<td>0.3038 ± 0.010</td>
<td>53.43</td>
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<tr>
<td>1b</td>
<td>0.2885 ± 0.024</td>
<td>45.71</td>
</tr>
<tr>
<td>1c</td>
<td>0.3021 ± 0.046</td>
<td>52.58</td>
</tr>
<tr>
<td>1d</td>
<td>0.3429 ± 0.016</td>
<td>73.18</td>
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<tr>
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</tr>
<tr>
<td>1e</td>
<td>0.3584 ± 0.040</td>
<td>81.01</td>
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<td>1f</td>
<td>0.2399 ± 0.021</td>
<td>21.16</td>
</tr>
<tr>
<td>1g</td>
<td>0.2464 ± 0.001</td>
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<td>1h</td>
<td>0.2825 ± 0.020</td>
<td>42.68</td>
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<td>1i</td>
<td>0.3311 ± 0.004</td>
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<td>1j</td>
<td>0.3405 ± 0.003</td>
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<td>1k</td>
<td>0.2680 ± 0.010</td>
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<td>1l</td>
<td>0.3239 ± 0.024</td>
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<tr>
<td>2a</td>
<td>0.3012 ± 0.046</td>
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</tr>
<tr>
<td>2b</td>
<td>0.2853 ± 0.016</td>
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<td>2c</td>
<td>0.2961 ± 0.025</td>
<td>49.55</td>
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<tr>
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<td>2e</td>
<td>0.3547 ± 0.004</td>
<td>79.14</td>
</tr>
<tr>
<td>2f</td>
<td>0.2363 ± 0.025</td>
<td>19.34</td>
</tr>
<tr>
<td>2g</td>
<td>0.2401 ± 0.001</td>
<td>21.26</td>
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<tr>
<td>2h</td>
<td>0.2785 ± 0.010</td>
<td>40.66</td>
</tr>
<tr>
<td>2i</td>
<td>0.3256 ± 0.020</td>
<td>64.44</td>
</tr>
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<td>2j</td>
<td>0.3374 ± 0.024</td>
<td>70.40</td>
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<td>2k</td>
<td>0.2602 ± 0.036</td>
<td>31.41</td>
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<td>2l</td>
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<td>60.96</td>
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<tr>
<td>Diclofenac Na</td>
<td>0.3630 ± 0.003</td>
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<tr>
<td>Ascorbic Acid</td>
<td>---</td>
<td>---</td>
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</table>

*Average of triplicate reading; S.D. = Standard Deviation
1.9 DISCUSSION

Biginelli in 1893 reported one-step synthesis of 3,4-dihydropyrimidin-2(1H)-one by three-component condensation of aldehydes, ethyl acetoacetate and urea in alcohol using strong mineral acid\(^{118}\). However, this method suffered from drawbacks that the lower yields and longer reaction time especially with aliphatic as well as substituted aromatic aldehydes was observed. The reaction remained ignored almost for a century but with the confirmation that dihydropyrimidinones possess diverse and important biological properties, the interest in their synthesis has been greatly increased from last decades. The scope of the original Biginelli reaction was gradually extended by variation of all three building blocks, allowing access to a large number of multi functionalized dihydropyrimidinones. The several protocols and different reaction conditions like PPA, AlCl\(_3\), H\(_3\)BO\(_3\), conc. HCl, BF\(_3\).OEt\(_2\), NH\(_4\)Cl, CAN, NBS, triflates of lanthanide compounds and Bi, In, Cu along with microwave irradiation etc. have been tried\(^{119-130}\). A few methods also involve the use of ionic liquids\(^{131}\).

However, the multi-step methods lack the simplicity of one-pot methods and are no longer used but for the improvements as one-pot procedures continue with use of different protocols. However, out of several methods and those involving different catalyst suffer from drawbacks that the use of expensive reagents like triflates of Bi, Cu, lanthanides etc., prolonged reaction time and strongly acidic conditions, unsatisfactory yields and tedious workup procedures (e.g. acidic alumina) for the isolation of pure product in good yields. Many catalysts though are effective; their preparation procedures are difficult (e.g. ferric oxide nanocomposites). This requires the development of a new protocol for high yield and the use of inexpensive reagent, which requires shorter reaction time and with easier workup procedure. The facts and usefulness of Biginelli reaction inspired us to synthesize such type of new compounds to investigate promising biological activities.

✓ Preparation of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto) pyrimidines:

Compounds 6-amino-5-cyano-4-substituted-2-hydroxypyrimidines (1a-1l) and 6-amino-5-cyano-4-substituted-2-mercaptopyrimidines (2a-2l) were prepared using
three different reaction conditions like conventional heating, microwave assisted synthesis and green chemistry method.

**Conventional method:**

Aromatic aldehyde, malononitrile, urea or thiourea and conc. hydrochloric acid were mixed thoroughly in absolute ethanol and reaction mixture was refluxed on water bath. The reaction progress was monitored using TLC on precoated silica gel F$_{254}$ plates. After the completion of reaction, the mixture was poured on crushed ice. The separated solid was filtered, dried and recrystalized from suitable solvents. The IUPAC names and physical data of all the newly synthesized compounds are summarized in Table 1.1 and Table 1.2 respectively.

During the progress of reaction, the activated arylmethylene malononitrile is likely to be formed via a Knoevenagel condensation reaction of aromatic aldehydes and malononitrile, which subsequently reacted with urea or thiourea to form desired product. In the presence of conc. HCl, reaction proceeds smoothly giving desired products in short time and in a quantitative yield. It was observed that the electron donating groups as well as electron withdrawing groups present in aryl aldehydes does not affect the yield of the reaction.

Structures of all the synthesized compounds 1a-1l and 2a-2l were established on the basis of spectral data. The IR, $^1$H NMR and mass spectra supported the structure of various synthesized pyrimidines are recorded in Table 1.3. All the spectral data showed that the synthesized compounds were in full agreement with the proposed structures. For example, IR measurements which showed the presence of CN at region 2232-2211 cm$^{-1}$ and two sharp bands at 3492-3420 and 3310-3220 cm$^{-1}$ due to asymmetric and symmetric vibrations of the NH$_2$ group. In the $^1$H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts and multiplicities. $^1$H NMR spectrum of 1g showed a singlet at $\delta$ 3.94 ppm corresponding to -OCH$_3$ group; a broad singlet at 5.50 ppm due to 2H of primary NH$_2$ group. The doublet at 7.03-7.05 ppm and the doublet at 7.93-7.95 ppm are due four aromatic protons. The phenolic proton appears at 7.68 ppm. The mass spectrum of compound 1g showed molecular ion peak at at m/z 242 [M$^+$] corresponding to its molecular formula. The possible mass fragmentation pattern for compound 1g is given below, which confirmed its chemical structure.
Microwave assisted method:

In modern laboratories organic transformations should be rapidly executed and products readily purified. Clearly there will be a continuing need for the definition of novel reaction routes to both multifunctional scaffolds for lead generation and drug like heterocyclic structures. In this field controlled microwave irradiation has proved to be a powerful tool for both speeding up chemical optimizations and for efficient preparation of new target compounds. Thus microwave-assisted heating under controlled conditions has been shown to be an invaluable technology for medicinal chemistry and drug discovery applications since it often dramatically reduces reaction times, typically from days or hours to minutes or even seconds. The smaller volume of solvent required, contributed to a savings in cost and diminished the waste disposal problem.

A mixture of aromatic aldehyde, malononitrile and urea or thiourea in ethanol in presence of an acid catalyst i.e. con. sulfuric acid under microwave irradiation
resulted in the formation of final product. This present route, besides being advantageous in simple reaction conditions and easy work-up procedures, less time consuming and eco-friendly, has resulted in better yields over the conventional methods. All the three synthetic methods are compared in terms of yield and reaction time. The results are presented in Table 1.4.

**Green chemistry method:**

Introduced in the early 1990’s, green chemistry is defined as the utilization of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products. The concept of green chemistry is enshrined in a set of 12 principles, such as the prevention of waste, the design of energy efficient processes, the use of safe, environmentally benign solvents where possible, the use of renewable feed stocks etc.

Nowadays, organic reactions in water as solvent have attracted much attention, because of its usefulness as a cheap, safe and environment friendly solvent. The enhanced reactivity and selectivity observed in some reactions have been rationalized by various authors as being a consequence of hydrophobic effects and enforced hydrophobic interactions. Recently, Knoevenagel condensation has been reported in water using aromatic aldehydes and malononitrile.

A novel three-component reaction of aromatic aldehydes, malononitrile and urea or thiourea in water at reflux and in the presence of an equivalent amount of sodium acetate for 3-6 h, allowing the one-pot formation of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidines in good yields has been developed. In order to optimize the reaction conditions for preparing desire compounds, the synthesis of 6-amino-5-cyano-4-phenyl-2-hydroxypyrimidine was carried out under different reaction conditions. Reaction of benzaldehyde, malononitrile and urea in DMSO at reflux in the presence of a catalytic amount of triethylamine was too slow and the yield was low, for example, even after 16 h final product was obtained in only 40% yield, as compared to yield of 76% in 6 h under thermal aqueous conditions.

The complete process represents an example of a one-pot process with sequential steps where reagents and catalysts were mixed together and experimental conditions were adjusted in such a way as to promote the reaction cascade. Thus the benzylidenemalononitrile containing an electron-poor C=C double bond was
produced by rapid Knoevenagel condensation of malononitrile with the aromatic aldehyde, the formation of the benzylidenemalononitrile being monitored by TLC.

\[
\begin{align*}
\text{Ar} & \quad + \quad \text{H} - \text{H} - \text{HCN} \\
\text{Ar} & \quad \text{CN} \\
\text{CN} & \quad \text{HN} \\
\text{HN} & \quad \text{NH}_2 \\
\text{X} & \quad \text{H}_2\text{O} \\
\text{X} & \quad \text{H}_2\text{O} \\
\text{Ar} & \quad \text{H} - \text{CN} \\
\text{Ar} & \quad \text{H} - \text{CN} \\
\text{CN} & \quad \text{HN} \\
\text{HN} & \quad \text{NH}_2 \\
\text{Ar} & \quad \text{H} - \text{CN} \\
\text{Ar} & \quad \text{H} - \text{CN} \\
\text{CN} & \quad \text{HN} \\
\text{HN} & \quad \text{NH}_2
\end{align*}
\]

The second step was followed by Michael addition, cycloaddition, isomerization, aromatization to afford the 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto) pyrimidines. Intermediate was not stable and was not isolated from the reaction mixture. It must be easily oxidized by air to produce final compound. We believed that the driving force for such a transformation is the aromaticity of these final products.

✓ **Antimicrobial screening:**

The antimicrobial activities of the compounds 1a-1l and 2a-2l were tested against gram (-)ve bacteria (Escherichia coli, Pseudomonas aeruginosa), gram (+)ve bacteria (Bacillus subtilis, Staphylococcus aureus) and one fungi (Candida albicans). The results were recorded as MIC value. The results of preliminary antimicrobial testing of compounds 1a-1l and 2a-2l are shown in Table 1.5. From the data, it is clear that all the compounds possess weak to good activity against all the tested strains of microorganisms. Amongst all the synthesized derivatives in series, compound 1d, 1i, 1j, 2d, 2i and 2j exhibited good antibacterial activity. Compound 1j was found to be the most potent against all the tested strains of microorganism. However, it shows lower MIC values against gram (-)ve bacteria compared to gram (+)ve bacteria. Moreover, compounds 1e, 1j and 2i exhibited potent activity against C. albicans. All the results also revealed that the substitution on benzene ring plays an important role on the potency of synthesized compounds. From data, it can be concluded that 2-chloro and 2-nitro substituent on benzene ring increases potency of the synthesized compounds, while 4-nitro substitution shows best potency as antimicrobial agent.
Antitubercular screening:

All the synthesized compounds were screened for their antitubercular activity against *Mycobacterium tuberculosis* H₃⁷Rv by the broth dilution method according to recommended procedure by the National Committee for Clinical Laboratory Standards for the determination of minimum inhibitory concentration (MIC). The results of antitubercular activity are shown in Table 1.5. Compounds 1c and 1j were most active (MIC < 25 μg/mL) while compounds 1d and 2j also show moderate activity (MIC = 50 μg/mL). The other compounds were less active. The antitubercular activity results correlated well with those of antimicrobial activity. The results of antitubercular activity revealed that compounds 1d, 1j and 2j which have 2-chloro and 4-nitro group as substitution (electron withdrawing group) and 1c which has 4-hydroxyl group (electron releasing group) on phenyl ring enhanced the activity of pyrimidine derivatives while the other groups, such as 4-chloro, 4-dimethylamino, 4-methoxy, 3-methoxy, 3-nitro and 2-nitro groups substituted on phenyl ring did not influence the activity. Among the 4-nitro substituted compounds (1j and 2j) compound 1j, which has a hydroxyl group at 2nd position of pyrimidine ring system, exhibited the highest activity. This suggests that electron withdrawing groups and hydroxyl groups substituted at 4th position on phenyl ring are responsible for the good antitubercular activity. It is interesting to note that some of the currently used antitubercular drugs such as pyrazinamide, isoniazid and ethionamide, also possess electron withdrawing groups, while *p*-amino salicylic acid bears a free hydroxyl group in its structure.

Anti-inflammatory activity:

All of the newly obtained compounds 1a-1l and 2a-2l were tested for *in-vitro* anti-inflammatory activity. Compared to the standard, diclofenac sodium, they have shown acceptable anti-inflammatory activity. *In-vitro* anti-inflammatory activity of synthesized compounds is given in Table 1.6. The results revealed that the compounds, 1d, 2d, 1e, 2e, 1j and 2j exhibited very good anti-inflammatory activities. Amongst all the tested compounds 1e found to be more potent. The compounds 1i, 2i, 1j and 2j have showed good activity, while other compounds showed weak to moderate activities. The results also showed that different substitution on aromatic ring system affect the anti-inflammatory activity. Electron withdrawing group like chlorine and nitro at any position shows good anti-inflammatory activity while
electron releasing group shows weak to moderate activity. Furthermore, it can also be concluded that hydroxyl group is slightly more active compared to mercapto group at 2\textsuperscript{nd} position of pyrimidine ring system.

✓ **Antioxidant activity:**

All the synthesized compounds 1a-1l and 1a-2l were screened for their \textit{in-vitro} antioxidant activity by various methods such as scavenging of hydrogen peroxide, scavenging of nitric oxide radical and reducing power determination. \textit{In-vitro} antioxidant activity of synthesized compounds is given in Table 1.6. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. Particularly, hydroxyl derivatives showed good promising antioxidant activity as compared to that of standard, ascorbic acid due the availability of free hydroxyl group. The compound 1c and 1h are more potent among the tested series. This may be due to additional hydroxyl group present on phenyl ring in the structure. Compounds 1k, 1g, 2c and 2h also showed moderate to good antioxidant activity.
A simple, quick and efficient method for the synthesis of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives by three-component condensation of aromatic aldehydes, malononitrile and thiourea or urea in presence of con. HCl has been developed. Ease of separation of pure product, selectively and in high yields in comparison to the two-step strategies, are few of the unique features of this method.

A novel, microwave assisted eco-friendly convenient route, for the synthesis of 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives has been developed which gave excellent yields in short reaction times.

A new, rapid and simple multicomponent cyclocondensation protocol for the synthesis of biologically active 6-amino-5-cyano-4-substituted-2-(hydroxy/mercapto)pyrimidine derivatives has also been developed using water as a solvent at reflux temperature in good quantitative yields.

All the compounds synthesized were characterized by physical methods like melting point, thin layer chromatography and spectral analysis like IR, NMR and Mass spectra.

All the synthesized compounds have been investigated for their in-vitro antioxidant, anti-inflammatory, antibacterial, antifungal and antitubercular activity.

Among the newly synthesized compounds, highest antioxidant activity for compounds 1c, anti-inflammatory activity for compound 1e, antibacterial activity for compound 1j, antifungal activity for compounds 1e, 1j and 2i and antitubercular activity for compounds 1c and 1j were observed.

Accordingly, these novel classes of pyrimidine derivatives emerged as a valuable lead series that might be useful as antioxidant, anti-inflammatory, antibacterial, antifungal and antitubercular agents and hence promising candidates for further efficacy evaluation.
1.11 REFERENCES


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