Chapter – 3

Studies on 2-Azitidinone Derivatives
INTRODUCTION

Azetidin-2-one, also called β-Lactam, is a four member cyclic amide which was first synthesized by Staudinger\(^1\) in 1907. Because of the discovery of penicillin in 1928 by Fleming and its structural confirmation by X-ray crystallography\(^2\), the bioactivity of β-lactam compounds has been investigated intensively\(^3\). Before 1970, penicillin and cephalosporin were the antibiotics that attracted most synthetic effort. The four membered β-lactam (2-azetidinone) ring systems (I) has for many years been of great practical significance as the center of reactivity of penicillins (II) and Cephalosporin (III).

\[
\text{(I)} \quad \text{(II)} \quad \text{(III)}
\]

The basic skeleton commonly encountered in β-lactam antibiotics are the penam (IV) and cepham (V) and high reactivity of β-lactam ring system is essential for the antibiotic activity of these compounds.

\[
\text{(IV)} \quad \text{(V)}
\]

Much effort has been put in for the synthesis of different β-lactams rings to combat resistant bacteria and for better pharmacological results\(^4\). There has been considerable interest in other fused β-lactams such as clavulanic acid, thienamycin and related olivanic acid derivatives\(^5\).

**REACTIVITY**

Nonfused β-lactam containing natural products include the nocardicins\(^6\) (VI) and the monobactams\(^7\) (VII) as well as the more complex pachystermines\(^8\), firetoxin\(^9\) and bleomycins\(^10\).
Incorporation of an amide linkage into a four membered ring results in angle strain and some degree of inhibition of amide resonance, rendering β-lactams more susceptible than normal amides to nucleophilic attack at the carbonyl group. Not surprisingly, β-lactams undergo N(1)-C(2) cleavage on treatment with a variety of nucleophiles and this ability of a β-lactam to act as an acylating agent is generally considered to be, at least in part, responsible for the antibacterial properties of penicillin and cephalosporin. These strained bicyclic β-lactams inhibit bacterial cell wall biosynthesis, apparently by acylating transpeptidases.

**Mechanism**

Formation of β-lactam by addition of two atom component to imine function is one of the most important and versatile route of synthesis. The reaction may initially involve generation of ketene from various precursors. For the reaction of ketene with imine, two extreme mechanisms can be envisaged. Concerted (2 + 2) cycloaddition to give or the more generally accepted formation of a dipolar intermediate (N-acetyl derivative) which cyclizes to an azetidin-2-one. (Scheme-I)

Other mechanisms of β-lactam consists of the addition of C-N and =O component to form a ring substituted acetyl chloride with electron withdrawing substituents and at least one hydrogen at α-carbon add to imines in the presence of
amine bases\textsuperscript{12}. The mechanism take place by nonconcerted cycloaddition reaction as depicted in following (Scheme-II).

![Scheme-II](image)

The reaction may be experimented in many cases by treating a mixture of the imine and dichloro acetic acid with POCl\textsubscript{3} or POBr\textsubscript{3}\textsuperscript{13,14}. It has been observed that the acid chloride is formed as an intermediate through the phosphorus halide participate as an electrophillic catalyst as shown in following scheme (Scheme-III).

![Scheme-III](image)
SYNTHETIC ASPECTS

Microwave assisted synthesis of 2-Azetidinone derivative

Kidwai M. et al\textsuperscript{15} synthesized 2-Azetidinone derivatives.

Desai K. G. et al\textsuperscript{16} synthesized 2-Azetidinones derivatives.

Upadhyay A. et al\textsuperscript{17} synthesized 4-Aryl-3-chloro-1-[[5-nitroindazol-1-yl]acetamido]-2-oxoazetidines.

Dubey A. et al\textsuperscript{18} synthesized 2-azetidinone derivatives.

Conventional Synthesis of 2-Azetidinone derivative

Diazomethane was found to give β-lactams when treated with phenyl- and p-bromophenylisocyanates\textsuperscript{19}. Indolyl-3-isocyanate reacted similarly\textsuperscript{20}.
Joshi N. et al\textsuperscript{21} synthesized Azetidinones from Hydrazino s-triazines

\begin{center}
\includegraphics[width=\textwidth]{joshi.png}
\end{center}

Mulwad V. et al\textsuperscript{22} synthesized Indoloquinolones, Triazoloindoloquinolines and Its derivatives.

\begin{center}
\includegraphics[width=\textwidth]{mulwad.png}
\end{center}

Amr A. E. et al\textsuperscript{23} synthesized 2-Chloro-6-hydrazino-isonicotinic acid hydrazide.

\begin{center}
\includegraphics[width=\textwidth]{amr.png}
\end{center}

Kumar S. et al\textsuperscript{24} synthesized Tetrazolo[1,5-a]quinoxaline Based Azetidiones by conventional method.

\begin{center}
\includegraphics[width=\textwidth]{kumar.png}
\end{center}
Kumar S. et al.\textsuperscript{25} also synthesized 2-azetidinone by using triethylamine as organic base.

Shingade S. et al.\textsuperscript{26} synthesized 5-chloroindoline-2,3-dione derivatives using triethyl amine.

Kumar A. et al.\textsuperscript{27} synthesized quinazolinones derivatives based on 2-azetidinone.

Deyrup J. A. et al.\textsuperscript{28} synthesized 2-azetidinone from aziridine in the presence of thionylchloride or oxalylchloride, which undergoes ring expansion. The conversion is stereospecific and yields are good.

Hlubucek J. R. et al.\textsuperscript{29} synthesized 2-azetidinone by \textbf{Photolytic Wolf rearrangement} of 3-diazopyrrolidine-2,4-diones, in the presence of tert-butylcarbazate\textsuperscript{30}. This method was extended to the synthesis of azetidin-2-one,
which was found to be biologically inactive\textsuperscript{31}. The fused system under similar conditions produced, which was found to be highly unstable\textsuperscript{32}.

\[
\begin{align*}
\text{hv} & \quad \text{t-BuOCONHCO} \\
t-\text{BuOCONHNH}_2 & \quad \text{t-BuOCONNH}_2
\end{align*}
\]

**BIOLOGICAL SIGNIFICANCE**

Although 2-azetidinone derivatives possess wide spectrum of pharmacodynamic activities starting from sedative to hypnotic and also anticonvulsant\textsuperscript{33}, they are the drugs still most widely prescribed as antibiotics. A series of 7-\(\beta\)-(2-(amino pyrimidinyl)-2-oxyiminoacetamido]-cephalosporins have been described by Goto J. et al\textsuperscript{34} as useful antibiotics.

2-Azetidinone derivatives are also powerful antibacterials\textsuperscript{35}. A large number of 3-chloro-monocyclic \(\beta\)-lactams have been prepared by cycloaddition of \(\beta,\beta\)-disubstituted enamines with aryl isocyanates which possess powerful antibacterial activity\textsuperscript{36}. A comparative study of the bactericidal activity exhibited by bisnor penicillins and that of the corresponding penicillins has been studied by Driver M. J. et al\textsuperscript{37}.

\[
\begin{align*}
\text{COONa} & \\
\end{align*}
\]

Patel N. B. et al\textsuperscript{38} have synthesized 6-Bromo-3-(3-chloro-2-(substituted phenyl)-4-oxazetidin-1-yl)-2-(2,6-dichloro phenyl amino)benzyl) quinazolin-4(3H)-one derived from quinazolin-4(3H)-one and evaluated for **antimicrobial activity**.
Chikhalia K. H. et al\textsuperscript{39} have obtained pyrimidine based thiazolidinones and azetidinones as \textit{antimicrobial} and \textit{antitubercular} agents. Several 2-aryl-3-[4-(4-chlorophenyl)-6-(3,4,5-trimethoxy-phenyl)pyrimidin-2-yl-ureido]-4 thiazolidinones and 1-[4-(4-chloro phenyl) -6-(3,4,5 trimethoxyphenyl)pyrimidin-2-yl-ureido]- 3-chloro-4-aryl-2-azetidinones have been synthesized and tested for their antibacterial, antifungal and anti-tubercular activities against different microorganisms.

Shah et al\textsuperscript{40} synthesized azetidinones from hydrazine thieno[3,2-d] pyrimidines as potential antimicrobial agents. All the products have been evaluated for their in vitro growth inhibitory activity against several microbes like B. megatilis, B. subtilis, E. coli, A. aerogens and A. awamori. Most of the compounds exhibited maximum activity in the range of 21-27 mm against A. aerogens.

Kumar A. et al\textsuperscript{41} have synthesized 3-[4’-(p-chloro phenyl)-thiazol-2’-yl]-2-[(substituted azetidinone/thiazolidinone)-amino methyl]-6-bromoquinazolin-4 ones as \textit{anti-inflammatory agent}. 
Parmar et al\textsuperscript{42} reported synthesis of azetidinones from hydrazinopyrimidine as potential antimicrobial agents. All the products were evaluated for their in vitro growth inhibitory activity against several microbes like B. megaterium, B. subtilis, E. coli, P. fluorescens and A. awamori. All the compounds exhibited mild to moderate antimicrobial activity against all microorganisms which exhibited promising activity with ampicillin and chloramphenicol against P. fluorescence.

The literature survey reveals that azetidinones have diverse biological potential and have been used as important antibiotics since years. The drug resistance by microbes has resulted into constant effort by the chemists to search for newer alternatives. The easy synthetic routes, unique nature and capability to associate with varied heterocyclic moieties have always caught attention of the chemists, pharmacologists and researchers to explore for better alternatives for the existing drug molecules. Further, pyrimidinone derivatives are reported to have shown varied biological activities. In an attempt to explore for newer moieties to cope with the drug resistance by different bacteria, it was planned to synthesize and study activities of newer azetidinone containing pyrimidine heterocycles as described under the following sections.

**Section – I:** PREPARATION OF 2-BENZYLIDENEHYDRAZINYL-1,6-DIHYDRO-4-ISOBUTYL-1-METHYL-6-OXOPYRIMIDINE-5-CARBONITRILE

**Section – II:** PREPARATION OF 2-(4-ARYL-3-CHLORO-2-OXO AZETIDIN-1-YLAMINO)-1,6-DIHYDRO-4-ISOBUTYL-1-METHYL-6-OXOPYRIMIDINE-5-CARBONITRILE


**SECTION – I**

**PREPARATION OF 2-BENZYLIDENEHYDRAZINYL-1,6-DIHYDRO-4-ISO BUTYL-1-METHYL-6-OXOPYRIMIDINE-5-CARBONITRILE**

A large number of compounds either linear or heterocyclic, derived from azomethine group have been reported as active biological entities. Thus, with a view to getting better therapeutic agents, the preparation of Schiff’s bases of type (1) bearing pyrimidine nucleus was undertaken by the condensation of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile with different aromatic aldehydes.

![Chemical Structure](image)

The constitution of the synthesized products has been elucidated using elemental analysis, Infra-red and $^1$H nuclear magnetic resonance spectroscopy and supported by Mass spectrometry.

The products have been screened for their in vitro biological assay like antibacterial activities towards Gram positive and Gram negative bacterial strains at a concentration 32 µg/ml. The Biological activities of the synthesized compounds have been compared with standard drugs. The details have been cited in Chapter-8.
REACTION SCHEME

\[ \text{Reaction Scheme Image} \]
CHAPTER – 3  2-Azetidinone Derivatives

EXPERIMENTAL

A) Preparation of 1,2,3,4-tetrahydro-6-isobutyl-4-oxo-2-thioxopyrimidine-5-carbonitrile
   As per chapter-1, section-I, page no. – 33

B) Preparation of 1,6-dihydro-4-isobutyl-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile
   As per chapter-2, section-II(B), page no. – 74

C) Preparation of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile
   A mixture of 1,6-dihydro-4-isobutyl-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile (2.21gm, 0.01mol) and Hydrazine hydrate (3.5ml) in absolute alcohol (30ml) was refluxed for 6 hours. The reaction mixture was poured into crushed ice and the solid product obtained after neutralization with acetic acid was kept in water overnight. The product was isolated and crystallized from absolute alcohol. Yield-52%, MP-170°C; Anal. Calcd. for C_{10}H_{15}N_{5}O: C, 54.28; H, 6.83; N, 31.65; Found: C, 54.25; H, 6.82; N, 31.62.

D) Preparation of 2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile
   Take 2-hydrazinyl -1,6- dihydro -4- isobutyl -1-methyl -6-oxo pyrimidine -5-carbonitrile (2.21gm, 0.01mol), benzaldehyde (1.01ml, 0.01mol) in absolute alcohol (20ml) and add 2-3 drop of gl.acetic acid. The reaction mixture was refluxed for 3 hour. After completion of reaction, the reaction mixture was cool at room temperature and poured into crushed ice. Obtained solid product was filtered, dried and crystallized from absolute alcohol. Yield-69%, MP-200°C; Anal. Calcd. for C_{17}H_{19}N_{5}O: C, 66.00; H, 6.19; N, 22.64; Found: C, 65.98; H, 6.19; N, 22.62
   Similarly other schiff’s bases were prepared. The physical constants are recorded in Table – 3.1.

E) BIOLOGICAL EVALUATION
   The compounds were tested for bacterial growth inhibition activity against a primary panel including Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus. (details in chapter–8) The primary inhibition screen was done as a single point concentration for test compounds. Colistin and Polymyxin B were used as positive inhibitor controls for Gram-negative bacteria. Vancomycin and Daptomycin were used as positive inhibitor controls for Gram-positive bacteria.
PHYSICAL DATA

Table 3.1: Physical constants of 2-(benzylidenehydrazinyl)-1,6-di hydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>Comp.</th>
<th>R</th>
<th>M.F.</th>
<th>M.W.</th>
<th>MP °C</th>
<th>Yield %</th>
<th>N %</th>
</tr>
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<tr>
<td>SB-1</td>
<td>-C6H5</td>
<td>C17H19N5O</td>
<td>309</td>
<td>200</td>
<td>69</td>
<td>22.64</td>
</tr>
<tr>
<td>SB-2</td>
<td>-4-Cl-C6H4</td>
<td>C17H18N5OCl</td>
<td>343</td>
<td>206</td>
<td>55</td>
<td>20.37</td>
</tr>
<tr>
<td>SB-3</td>
<td>-4-OCH3-C6H4</td>
<td>C18H21N5O2</td>
<td>339</td>
<td>190</td>
<td>48</td>
<td>20.64</td>
</tr>
<tr>
<td>SB-4</td>
<td>-4-N(CH3)2-C6H4</td>
<td>C19H24N6O</td>
<td>352</td>
<td>212</td>
<td>53</td>
<td>23.85</td>
</tr>
<tr>
<td>SB-5</td>
<td>-3,4(OCH3)2-C6H3</td>
<td>C19H23N5O3</td>
<td>369</td>
<td>186</td>
<td>56</td>
<td>18.96</td>
</tr>
<tr>
<td>SB-6</td>
<td>-3-OCH3-4-OH-C6H3</td>
<td>C18H21N5O3</td>
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<td>206</td>
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<td>SB-7</td>
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<td>C18H21N5O</td>
<td>323</td>
<td>190</td>
<td>52</td>
<td>21.66</td>
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<td>SB-8</td>
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<td>C17H18FNSO</td>
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<td>170</td>
<td>58</td>
<td>21.39</td>
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<tr>
<td>SB-9</td>
<td>-C4H3O</td>
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<tr>
<td>SB-11</td>
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<td>C17H18N5OCl</td>
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<td>184</td>
<td>54</td>
<td>20.37</td>
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<tr>
<td>SB-12</td>
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<td>C17H18N6O3</td>
<td>354</td>
<td>222</td>
<td>61</td>
<td>23.72</td>
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Spectral studies

\(^1\)H NMR spectrum of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile.

NIVPH-03
PMR Spectral study of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxo pyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Signal Position (δ ppm)</th>
<th>Relative No. of Proton</th>
<th>Multiplicity</th>
<th>Inference</th>
</tr>
</thead>
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<td>0.93</td>
<td>6H</td>
<td>doublet</td>
<td>-CH-(CH$_3$)$_2$</td>
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<tr>
<td>2</td>
<td>2.18</td>
<td>1H</td>
<td>multiplet</td>
<td>-CH$_2$-CH-(CH$_3$)$_2$</td>
</tr>
<tr>
<td>3</td>
<td>2.38</td>
<td>2H</td>
<td>doublet</td>
<td>-CH$_2$-CH-</td>
</tr>
<tr>
<td>4</td>
<td>3.15</td>
<td>3H</td>
<td>singlet</td>
<td>-N-CH$_3$</td>
</tr>
</tbody>
</table>
IR Spectral study of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxo pyrimidine-5-carbonitrile

<table>
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<tr>
<th>System</th>
<th>Vibration Mode</th>
<th>Band Position (cm(^{-1}))</th>
<th>Ref. (Page no.-46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary Amine</td>
<td>N – H str.</td>
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<td>3500-3300</td>
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<td>C – N vib.</td>
<td>1308</td>
<td>1350-1280</td>
</tr>
<tr>
<td></td>
<td>N – H Wag</td>
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<td>750-700</td>
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<td>Alkane</td>
<td>C – H str. (asy)</td>
<td>2954</td>
<td>2975-2950</td>
</tr>
<tr>
<td></td>
<td>C – H str. (sym)</td>
<td>2868</td>
<td>2880-2860</td>
</tr>
<tr>
<td></td>
<td>C – H def.</td>
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<tr>
<td>Nitrile</td>
<td>C = N str.</td>
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<td>2260-2200</td>
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<td>Carbonyl</td>
<td>C = O str.</td>
<td>1676</td>
<td>1760-1665</td>
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<tr>
<td>N - CH(_3)</td>
<td>N – C str.</td>
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<td>1090</td>
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Mass spectrum of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxo pyrimidine-5-carbonitrile

Mass fragmentation pattern
$^1$H NMR spectrum of 2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile.
Expanded $^1$H NMR spectrum

PMR Spectral study of 2-(benzyldenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Signal Position ($\delta$ ppm)</th>
<th>Relative No. of Proton</th>
<th>Multiplicity</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
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<td>doublet</td>
<td>-CH-(CH$_3$)$_2$</td>
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<td>2</td>
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<td>multiplet</td>
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<td>3</td>
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<td>2H</td>
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<td>-CH$_2$-CH-</td>
</tr>
<tr>
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<td>3.24</td>
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<td>singlet</td>
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</tr>
<tr>
<td>5</td>
<td>7.45 &amp; 7.94</td>
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<td>multiplet</td>
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<tr>
<td>6</td>
<td>8.45</td>
<td>1H</td>
<td>singlet</td>
<td>-NH</td>
</tr>
<tr>
<td>7</td>
<td>10.97</td>
<td>1H</td>
<td>singlet</td>
<td>=CH</td>
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IR Spectral study of 2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
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<th>System</th>
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<tr>
<td>Secondary Amine</td>
<td>N – H str.</td>
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<td>30</td>
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<td></td>
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<td>C – H str.(sym.)</td>
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<tr>
<td>Nitrile</td>
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<td>Carbonyl</td>
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<td>33</td>
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<td>N - CH(_3)</td>
<td>N – C str.</td>
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<tr>
<td>Sciff base</td>
<td>C = N – C str.</td>
<td>1622</td>
<td>30</td>
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</tbody>
</table>
Mass spectrum of 2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

![Mass Spectrum Image]
RESULT AND DISCUSSION

Synthesis of substituted Schiff’s base has been carried out by the reaction of 2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile and different aldehydes. Total 12 compounds were synthesized and characterized using spectroscopic technique. These compounds were used as intermediates for the synthesis of azetidinone and thiazolidinone derivatives. All synthesized compounds were further studied for their antibacterial activities.
**SECTION – II**

**PREPARATION OF 2-(4-ARYL-3-CHLORO-2-OXO AZETIDIN-1-YLAMINO)-1,6-DIHYDRO-4-ISOBUTYL-1-METHYL-6-OXOPYRIMIDINE-5-CARBONITRILE**

During the past years, considerable evidence has been accumulated to demonstrate the efficacy of azetidinone derivatives in inducing variety of therapeutic activities. Led by these observations, azetidinones of type (2) have been synthesized by the condensation of different schiff’s bases with chloroacetyl chloride in the presence of basic catalyst triethylamine.

![Chemical Structure](image)

The constitution of the synthesized products has been elucidated using elemental analysis, Infra-red and $^1$H nuclear magnetic resonance spectroscopy and supported by Mass spectrometry.

The products have been screened for their in vitro biological assay like antibacterial activities towards Gram positive and Gram negative bacterial strains at a concentration 32 $\mu$g/ml. The Biological activities of the synthesized compounds have been compared with standard drugs. The details have been cited in Chapter-8.
REACTION SCHEME

\[
\text{O} \quad \text{O} \quad \text{CN} \\
\text{H}_2\text{N} \quad \text{NH}_2
\]

\[
\text{S} \\
\text{CHO}
\]

\[
\text{K}_2\text{CO}_3 \\
2\text{CH}_3\text{I} \quad \text{K}_2\text{CO}_3
\]

\[
\text{NH}_2\text{NH}_2
\]

\[
\text{R-CHO}
\]

\[
\text{ClCH}_2\text{COCl}
\]
EXPERIMENTAL

A) Preparation of **1,2,3,4-tetrahydro-6-isobutyl-4-oxo-2-thioxopyrimidine-5-carbonitrile**

As per chapter-1, section-I, page no. – 33

B) Preparation of **1,6-dihydro-4-isobutyl-1-methyl-2-(methylthio)-6-oxopyrimidine-5-carbonitrile**

As per chapter-2, section-II(B), page no. – 74

C) Preparation of **2-hydrazinyl-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile**

As per chapter-3, section-I(C), page no. – 98

D) Preparation of **2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile**

As per chapter-3, section-I(D), page no. – 98

E) Preparation of **2-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile**

To a well stirred mixture of chloroacetyl chloride (0.95ml, 0.012mol) and triethylamine (1.65ml, 0.012mol) in dry dioxane (15ml), was added a solution of 2-(benzylidenehydrazinyl)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile (2.21gm, 0.01M) in dry dioxane at 0 °C. The reaction mixture was then stirred at room temperature for 20-22 hrs. and kept for 2 days. The product was isolated and crystallized from ethanol. Yield – 62%, MP-176°C, Anal. Calcd. for C_{19}H_{20}ClN_{5}O_{2}: C, 59.14; H, 5.22; N, 18.15; Found: C, 59.12; H, 5.19; N, 18.15.

Similarly other azetidinone derivatives were prepared. The physical constants are recorded in Table – 3.2.

F) BIOLOGICAL EVALUATION

The synthesized compounds were tested for bacterial growth inhibition activity against a primary panel including Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus. (details in chapter–8) The primary inhibition screen was done as a single point concentration for test compounds. Colistin and Polymyxin B were used as positive inhibitor controls for Gram-negative bacteria. Vancomycin and Daptomycin were used as positive inhibitor controls for Gram-positive bacteria.
**PHYSICAL DATA**

![Chemical Structure](image)

Table – 3.2 : Physical constants of 2-(4-aryl-3-chloro-2-oxo azetidin-1-yl amino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>Comp.</th>
<th>R</th>
<th>M.F.</th>
<th>M.W.</th>
<th>MP °C</th>
<th>Yield %</th>
<th>N %</th>
<th>Calc.</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ-1</td>
<td>-C₆H₅</td>
<td>C₁₉H₂₀ClN₃O₂</td>
<td>385</td>
<td>176</td>
<td>62</td>
<td>18.15</td>
<td>18.15</td>
<td></td>
</tr>
<tr>
<td>AZ-2</td>
<td>-4-Cl-C₆H₄</td>
<td>C₁₉H₁₉Cl₂N₅O₂</td>
<td>420</td>
<td>164</td>
<td>66</td>
<td>16.66</td>
<td>16.65</td>
<td></td>
</tr>
<tr>
<td>AZ-3</td>
<td>-4-OCH₃-C₆H₄</td>
<td>C₂₀H₂₂ClN₃O₃</td>
<td>415</td>
<td>142</td>
<td>61</td>
<td>16.84</td>
<td>16.85</td>
<td></td>
</tr>
<tr>
<td>AZ-4</td>
<td>-4-N(CH₃)₂-C₆H₄</td>
<td>C₂₁H₂₅ClN₆O₂</td>
<td>429</td>
<td>134</td>
<td>69</td>
<td>19.59</td>
<td>19.52</td>
<td></td>
</tr>
<tr>
<td>AZ-5</td>
<td>-3,4(OCH₃)₂-C₆H₃</td>
<td>C₂₁H₂₄ClN₃O₄</td>
<td>446</td>
<td>158</td>
<td>72</td>
<td>15.71</td>
<td>15.68</td>
<td></td>
</tr>
<tr>
<td>AZ-6</td>
<td>-3-OCH₃-4-OH-C₆H₃</td>
<td>C₂₀H₂₂ClN₃O₄</td>
<td>432</td>
<td>204</td>
<td>75</td>
<td>16.22</td>
<td>16.19</td>
<td></td>
</tr>
<tr>
<td>AZ-7</td>
<td>-4-CH₃-C₆H₄</td>
<td>C₂₀H₂₂ClN₃O₂</td>
<td>400</td>
<td>122</td>
<td>61</td>
<td>17.51</td>
<td>17.47</td>
<td></td>
</tr>
<tr>
<td>AZ-8</td>
<td>-4-F-C₆H₄</td>
<td>C₁₉H₁₉ClFN₅O₂</td>
<td>404</td>
<td>202</td>
<td>59</td>
<td>17.34</td>
<td>17.33</td>
<td></td>
</tr>
<tr>
<td>AZ-9</td>
<td>-C₄H₃O</td>
<td>C₁₉H₁₈ClN₅O₃</td>
<td>375</td>
<td>166</td>
<td>52</td>
<td>18.64</td>
<td>18.61</td>
<td></td>
</tr>
<tr>
<td>AZ-10</td>
<td>-4-OH-C₆H₄</td>
<td>C₁₉H₂₀ClN₃O₃</td>
<td>402</td>
<td>214</td>
<td>68</td>
<td>17.43</td>
<td>17.42</td>
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<tr>
<td>AZ-11</td>
<td>-2-Cl-C₆H₄</td>
<td>C₁₉H₁₉Cl₂N₃O₂</td>
<td>420</td>
<td>186</td>
<td>66</td>
<td>16.66</td>
<td>16.65</td>
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</tr>
<tr>
<td>AZ-12</td>
<td>-4-NO₂-C₆H₄</td>
<td>C₁₉H₁₉ClN₆O₆</td>
<td>430</td>
<td>118</td>
<td>71</td>
<td>19.51</td>
<td>19.48</td>
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</tr>
</tbody>
</table>
SPECTRAL STUDIES

$^1$H NMR spectrum of 2-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-ylamino)-1,6-dihydro-4-isobuty1-1-methyl-6-oxopyrimidine-5-carbonitrile
Expanded $^1$H NMR spectrum

PMR Spectral study of 2-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-ylamino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Signal Position ($\delta$ ppm)</th>
<th>Relative No. of Proton</th>
<th>Multiplicity</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76-0.91</td>
<td>6H</td>
<td>double doublet</td>
<td>-CH$_2$-(CH$_3$)$_2$</td>
</tr>
<tr>
<td>2</td>
<td>1.73</td>
<td>1H</td>
<td>multiplet</td>
<td>-CH$_2$-CH$_2$-(CH$_3$)$_2$</td>
</tr>
<tr>
<td>3</td>
<td>2.00-2.39</td>
<td>2H</td>
<td>multiplet</td>
<td>-CH$_2$-CH-</td>
</tr>
<tr>
<td>4</td>
<td>3.23</td>
<td>3H</td>
<td>singlet</td>
<td>-N-CH$_3$</td>
</tr>
<tr>
<td>5</td>
<td>6.09</td>
<td>1H</td>
<td>doublet</td>
<td>N-CH</td>
</tr>
<tr>
<td>6</td>
<td>7.01</td>
<td>1H</td>
<td>doublet</td>
<td>CH-Cl</td>
</tr>
<tr>
<td>7</td>
<td>7.25 &amp; 7.75</td>
<td>5H</td>
<td>multiplet</td>
<td>Ar-H</td>
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</tbody>
</table>
IR Spectral study of 2-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl amino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

<table>
<thead>
<tr>
<th>System</th>
<th>Vibration Mode</th>
<th>Band Position (cm(^{-1}))</th>
<th>Ref. (Page no.-46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary Amine</td>
<td>N – H str.</td>
<td>3269</td>
<td>3500-3300</td>
</tr>
<tr>
<td></td>
<td>N – H def.</td>
<td>1606</td>
<td>1650-1550</td>
</tr>
<tr>
<td></td>
<td>C – N vib.</td>
<td>1365</td>
<td>1350-1280</td>
</tr>
<tr>
<td></td>
<td>N – H wag.</td>
<td>761</td>
<td>750-700</td>
</tr>
<tr>
<td>Alkane</td>
<td>C – H str. (asy.)</td>
<td>2968</td>
<td>2975-2950</td>
</tr>
<tr>
<td></td>
<td>C – H str. (sym.)</td>
<td>2870</td>
<td>2880-2860</td>
</tr>
<tr>
<td>Nitrile</td>
<td>C = N str.</td>
<td>2223</td>
<td>2260-2200</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>C = O str.</td>
<td>1636</td>
<td>1760-1665</td>
</tr>
<tr>
<td>N - CH(_3)</td>
<td>N – C str.</td>
<td>1091</td>
<td>1090</td>
</tr>
<tr>
<td>Halide</td>
<td>C – F str.</td>
<td>1230</td>
<td>1300</td>
</tr>
<tr>
<td>β - lactam</td>
<td>C = O str.</td>
<td>1697</td>
<td>1760-1730</td>
</tr>
<tr>
<td></td>
<td>C – Cl str.</td>
<td>783</td>
<td>830-560</td>
</tr>
</tbody>
</table>
Mass spectrum of 2-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl amino)-1,6-dihydro-4-isobutyl-1-methyl-6-oxopyrimidine-5-carbonitrile

Mass fragmentation pattern
RESULT AND DISCUSSION

In this section, total 12 newer azetidinone derivatives containing substituted pyrimidine were synthesized and characterized using spectroscopic technique like IR, $^1$H NMR, Mass spectroscopy and Elemental analysis. All synthesized compounds were further studied for their antibacterial activities. Some derivatives showed good activity against different bacterial strains. (details in chapter-8)
REFERENCES


