CHAPTER-5

Synthesis of oxazolines and thiazolines
5.1. Theoretical

Oxazolines are five membered cyclic compounds having imino-ether linkage (\(-\text{N} = \text{C} - \text{O}\)-) group. In terms of saturation, oxazoline (155) exists between oxazole (154) and oxazolidine (156). The first successful synthesis of oxazoline was reported in 1889. Oxazolines (also called oxazolyls) have been found to possess wide range of chemical applications, specially for carboxylic acids as protecting group and in asymmetric catalysis as ligands. Depending on the position of double bond, three structural isomers of oxazoline are possible, despite that only 2-oxazolines (155) are common. 4-Oxazolines (158) are rare and 3-oxazolines (157) are even less common.

Similarly, thiazoline is the structural analogue of oxazoline where the oxygen is replaced by sulfur. Thiazoline (160) exists between thiazole (159) and thiazolidine (161) with reference to saturation. Like oxazolines, depending on the location of the double bond existence of three different thiazoline rings are possible i.e. 2-thiazoline (160), 3-thiazoline (162) and 4-thiazoline (163). Thiazoline was first prepared by dialkylation of thioamides.

\[ R: \text{Organyl group} \]
Numerous methods have been discovered for the synthesis of oxazoline and thiazoline derivatives\(^3\)-\(^8\). Padmavathi et al.\(^9\) reported the synthesis of oxazoline and thiazoline derivatives (165, 166) by treatment of arylethenesulfonyleacetic acid methyl ester (164) with 2-amino ethanol or 2-amino-ethanthiol in the presence of n-butyllithium and anhydrous samarium (III) chloride in toluene, respectively.

Katritzky et al.\(^10\) outlined the microwave reactions for the synthesis of 2-substituted-2-oxazolines (169) and 2-substituted-2-thiazolines (171) by the treatment of 2-amino-2-methyl-1-propanol (168) or 2-amino-ethanthiol hydrochloride (170) with N-acylbenzotriazoles (167) in the presence of thionylchloride.
Oxazolines and thiazolines

\[
\begin{align*}
&\text{RCOBt} + \text{(168)} \rightarrow \text{microwaves} \rightarrow \text{O} \\
&\text{(167)} &\text{(169)} \\
&\text{R: } 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{CH}_3\text{OC}_6\text{H}_4, 4-\text{NO}_2\text{C}_6\text{H}_4, 4-\text{ClC}_6\text{H}_4, 2-\text{ClC}_6\text{H}_4, \text{phenyl,} \\
&1-\text{naphthyl, } 2-\text{furyl, } 2-\text{phenylethenyl, } 1-(6-\text{methoxy-2-naphthyl})\text{ethyl} \\
&\text{Bt: } \text{[Diagram of structure]} \\
\end{align*}
\]

\[
\begin{align*}
&\text{RCOBt} + \text{(170)} \rightarrow \text{microwaves} \rightarrow \text{S} \\
&\text{(167)} &\text{(171)} \\
&\text{R: } 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{CH}_3\text{OC}_6\text{H}_4, 4-\text{NO}_2\text{C}_6\text{H}_4, 4-\text{ClC}_6\text{H}_4, 2-\text{ClC}_6\text{H}_4, \\
&\text{phenyl, } 2-\text{furyl, } 2-\text{phenylethenyl} \\
\end{align*}
\]

Dowex-50W-hydrogen ion exchange resin was used by Bazgir et al.\textsuperscript{11} as reusable catalyst for the synthesis of oxazoline and thiazoline derivatives (174) through aryl nitriles (172) and 2-amino-alcohol or 2-amino-ethanthiol (173) condensation reaction.

\[
\begin{align*}
&\text{ArCN} + \text{(172)} \rightarrow \text{dowex-50W} \rightarrow \text{solvent-free/80°C} \rightarrow \text{Ar} \\
&\text{(173)} &\text{(174)} \\
&\text{Ar, X: } \text{C}_6\text{H}_5, \text{O}; 4-\text{Cl-C}_6\text{H}_4, \text{O}; 3-\text{Cl-C}_6\text{H}_4, \text{O}; 4-\text{Me-C}_6\text{H}_4, \text{O}; 4-\text{CN-C}_6\text{H}_4, \text{O}; \\
&\text{C}_6\text{H}_5, \text{S}; 4-\text{Cl-C}_6\text{H}_4, \text{S}; 4-\text{Me-C}_6\text{H}_4, \text{S}; 4-\text{CN-C}_6\text{H}_4, \text{S}; 4-\text{MeO-C}_6\text{H}_4, \text{S}; \\
&4-\text{NO}_2\text{C}_6\text{H}_4, \text{S} \\
\end{align*}
\]

Nitrogen containing heterocycles have gained importance because they possess many biological activities and pharmaceutical properties. The use of fatty acid substrates as starting material has become significant because of their own biological activities such as pesticidal, antimicrobial, antidepressant, antitumor activities\textsuperscript{12-15}. Significant biological activities exhibited by oxazolines, thiazolines and their corresponding
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oxidized derivatives i.e. oxazoles and thiazoles which are commonly found in marine sources. The summary of four years literature survey in the review\textsuperscript{16} showed the isolation, synthetic and biological studies of these natural products. Among them, the derivatives of oxazoline and thiazoline have been used as therapeutic agents and have biological properties such as antihypertensive\textsuperscript{17}, antidiabetic\textsuperscript{18}, antitumor\textsuperscript{19, 20}, antialzheimer\textsuperscript{21}, antihypercholesterolemic\textsuperscript{22} and antiinflammatory\textsuperscript{23} activities. 2-Oxazolines and thiazolines are important substructures in a large number of biologically active natural products\textsuperscript{24-27}. Micacocidin (175) is an antibiotic having potent antimycoplasma activity containing two thiazoline and one thiazolidine rings. Micacocidin was isolated from the culture broth of \textit{Pseudomonas} sp. no. 57-250\textsuperscript{28}.

\begin{center}
\begin{tikzpicture}
  \node (thiazoline) at (0,0) {\includegraphics[width=1.0\textwidth]{thiazoline.png}};
  \node at (-2,1.5) {Micacocidin (175)};
\end{tikzpicture}
\end{center}

Agyemang \textit{et al.}\textsuperscript{29} for the first time reported the presence of 2-ethyl-4-methyl-3-thiazoline (176) and 2-isopropyl-4-methyl-3-thiazoline (177) with other components in nature through an analysis of sesame seed oil. The thiazoline derivatives also play important role in pharmaceutical drug discovery\textsuperscript{29}.

\begin{center}
\begin{tikzpicture}
  \node (thiazoline) at (0,0) {\includegraphics[width=0.5\textwidth]{thiazoline.png}};
  \node at (-2,1.5) {Thiazoline derivatives (176) and (177)};
\end{tikzpicture}
\end{center}

Drug aminorex/aminoxafen (2-amiino-5-aryl oxazoline) (178) having oxazoline moiety. Aminorex is a weight loss (anorectic) stimulant drug. Frump\textsuperscript{31} outlined the
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applications of oxazolines in the field of protective coatings, surface active agents, gasoline and lube oil additives, corrosion inhibitors, antifoam agents, textile chemicals, adhesives and binders, stabilizers for chlorinated hydrocarbons, stabilizers for aqueous formaldehyde solutions, protective films in polish formulations, foam stabilizers, photography, plasticizers and agriculture.

![Chemical Structure](image)

Enteric bacterial infections are responsible for morbidity and mortality globally in developing countries and areas such as the Indian sub-continent, part of South America and tropical part of Africa\textsuperscript{32, 33}. In case of \textit{E. coli} infection amoxicillin, norfloxacin and ciprofloxacin are generally used but have harsh side effects\textsuperscript{34}. Toxicity and resistance to the drugs also play important role in the treatment failure\textsuperscript{35}. For this reason the present stratagem for the synthesis of new compounds was aimed in the direction of developing new oxazoline and thiazoline derivatives that inhibits the growth of gram-positive, gram-negative bacteria and fungi.
5.2. Synthesis, Characterization of Long Chain Oxazolines and Thiazolines: In Vitro Antimicrobial Activity

Many of the previously known methods involve expensive reagents, long reaction time, low yield, strong acidic conditions and use of toxic organic solvents. Therefore to avoid these limitations and due to biological importance of oxazoline and thiazoline derivatives as mentioned earlier, the synthesis of new compounds was aimed in the direction of developing new oxazoline and thiazoline fatty acid ester derivatives that inhibits the growth of gram-positive, gram-negative bacteria and fungi. The method described here have advantages like short reaction time, high yields of products, usage of cheap starting material for the synthesis of these novel hetero-fatty acid ester analogs.

5.3. Results and discussion

5.3.1. Chemistry

Previously in our laboratory, fatty acids and their derivatives have been reported as antimicrobial agents. It has been suggested that the incorporation of the fatty acid chain may increase antimicrobial activity of certain organic moieties. The synthesis, characterization and antimicrobial activities of oxazoline and thiazoline derivatives of fatty acid esters are described in this chapter. The synthesis of 2-amino-5-substituted and 2-amino-4,5-disubstituted oxazolines (183-186) and thiazolines (187-190) were carried out by refluxing urea and thiourea, respectively, with dibromo derivatives (179-182) of olefinic and hydroxy-olefinic fatty acid esters in methanol (Scheme 7).

All reactions were monitored by thin layer chromatography. The nucleophilic substitution of urea and thiourea to dibromo derivatives of olefinic and hydroxy-olefinic fatty acid esters gives inseparable isomeric mixture of oxazolines and thiazolines, respectively. The products (183-190) were purified on silica gel column with petroleum ether and diethyl ether as eluent. The synthesized compounds were identified on the basis of IR, $^1$H NMR, $^{13}$C NMR and mass spectra.
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\[
R_1 - \text{CH} - \text{CH} - R_2
\]

\[
\text{Br} \quad \text{Br}
\]

(179-182)

\[
\text{NH}_2\text{CONH}_2 \xrightarrow{\text{MeOH, reflux}} \text{NH}_2\text{CSNH}_2
\]

\[\text{R}^+ \quad \text{N} \quad \text{H} \quad \text{Z} \quad \text{N} \quad \text{O}
\]

(183-186)

(187-190)

Scheme 7: Synthesis of 2-amino-5-substituted and 2-amino-4,5-disubstituted oxazolines and thiazolines

<table>
<thead>
<tr>
<th>Compound Codes</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>179, 183, 187</td>
<td>H</td>
<td>( \text{H}_2\text{C} \quad \text{CH}_2 \quad \text{COOCH}_3 )</td>
</tr>
<tr>
<td>180, 184, 188</td>
<td>( \text{CH}_2 )</td>
<td>( \text{H}_2\text{C} \quad \text{CH}_2 \quad \text{COOCH}_2 )</td>
</tr>
<tr>
<td>181, 185, 189</td>
<td>( \text{CH}_2 \quad \text{OH} )</td>
<td>( \text{H}_2\text{C} \quad \text{COOCH}_3 )</td>
</tr>
<tr>
<td>182, 186, 190</td>
<td>( \text{CH}_2 )</td>
<td>( \text{H}_2\text{C} \quad \text{CH}_2 \text{OH} \quad \text{COOCH}_3 )</td>
</tr>
</tbody>
</table>
The structure of compound (183) is given below:

![Structure of 2-amino-5-(carbomethoxyoctyl)-1,3-oxazoline (183)](image)

A detailed spectral description for compound (183) is discussed below:

IR spectrum of compound (183) revealed 3320 cm\(^{-1}\) (N-H stretching), 1739 cm\(^{-1}\) (C=O in ester), 1437 (C=N ring stretching), 1362 (C-O-C). In the \(^1\)H NMR, the cyclic protons CH\(_2\)-CH were observed at \(\delta_H 4.16\) (tdd, 1H, \(J_{\text{H-H}} = 6.6\) Hz, \(J_{\text{H-H}} = 10.2\) Hz, CH\(_2\)-CF), 3.85 (dd, 1H, \(J_{\text{H-H}} = 10.2\) Hz, \(J_{\text{H-H}} = 1.2\) Hz, \(H\text{-C-CH}\)), 3.64 (dd, 1H, \(J_{\text{H-H}} = 17.1\) Hz, \(J_{\text{H-H}} = 3.6\) Hz, \(H\text{-C-CH}\)), a sharp singlet at 3.66 was observed for three methyl ester protons and the two amine protons were observed at \(\delta_H 2.32\) as a singlet. The structure of compound (183) was further confirmed by \(^13\)C NMR spectral data which showed peaks at \(\delta_C\) 179.8, 163.4, 79.2, 64.7, 51.4, 36.3, 35.9, 34.0, 29.8, 29.1, 28.7, 26.7, 24.9. The mass spectra showed characteristic molecular ion peak in accord with the molecular formula.

Similarly, other compounds were characterized from their spectral data.

### 5.3.2. Biology

#### 5.3.2.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), Methicillin resistant *Staphylococcus aureus* (MRSA +Ve), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method and measured by halo zone test\(^{41,42}\). The MIC of synthesized compounds against bacterial
strains was performed by micro dilution test and results were observed visually and spectrophotometrically. The susceptibility was assessed on the basis of diameter of zone of inhibition against gram-positive and gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 10. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 11. All the compounds showed moderate to good inhibitory action against both types of bacteria. Compounds (185, 186, 189 and 190) showed good inhibition against \textit{S. pyogenes}, \textit{S. aureus} and \textit{E. coli} species. Compound (186) showed stronger antibacterial activity nearly equivalent to that of ciprofloxacin against the methicillin-resistant \textit{Staphylococcus aureus} (MRSA). In general, all the compounds were more effective against gram-positive bacteria as compared to gram-negative bacteria.

Table 10: Antibacterial activity of oxazoline and thiazoline derivatives of fatty acid esters

<table>
<thead>
<tr>
<th>Compound Codes</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive bacteria</td>
</tr>
<tr>
<td></td>
<td>SP(^b)\</td>
</tr>
<tr>
<td></td>
<td>MRSA(^b)</td>
</tr>
<tr>
<td>183</td>
<td>14.1±0.3</td>
</tr>
<tr>
<td>184</td>
<td>14.1±0.2</td>
</tr>
<tr>
<td>185</td>
<td>17.1±0.3</td>
</tr>
<tr>
<td>186</td>
<td>20.3±0.2</td>
</tr>
<tr>
<td>187</td>
<td>12.9±0.4</td>
</tr>
<tr>
<td>188</td>
<td>14.1±0.3</td>
</tr>
<tr>
<td>189</td>
<td>15.1±0.3</td>
</tr>
<tr>
<td>190</td>
<td>16.1±0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a)\text{SP: Streptococcus pyogenes, } \text{MRSA: methicillin resistant Staphylococcus aureus, } \text{PA: Pseudomonas aeruginosa, } \text{KP: Klebsiella pneumonia, } \text{EC: Escherichia coli.} 
Positive control (standard): ciprofloxacin and negative control (DMSO) measured by the halo zone test (unit, mm).
Table 11: MIC and MBC results of oxazoline and thiazoline derivatives of fatty acid esters

<table>
<thead>
<tr>
<th>Comp. Codes</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MRSA&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>183</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>184</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>185</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>186</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>187</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>188</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>189</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>190</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>SP: Streptococcus pyogenes,  
<sup>b</sup>MRSA: methicillin resistant Staphylococcus aureus,  
<sup>c</sup>PA: Pseudomonas aeruginosa,  
<sup>d</sup>KP: Klebsiella pneumonia,  
<sup>e</sup>EC: Escherichia coli.  
MIC (µg/mL) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/mL) = minimum bacterial concentration, i.e. the lowest concentration of the compound for killing the bacteria completely. Positive control (standard): ciprofloxacin.

5.3.2.2. Antifungal studies

Antifungal activity was also done by disk diffusion method<sup>43</sup>, <sup>44</sup>. For assaying antifungal activity Candida albicans, Aspergillus fumigatus, Penicillium marneffei and Trichophyton mentagrophytes (recultured) in DMSO strains were used. The fungal activity of each compound was compared with griseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The MIC of synthesized compounds against fungal strains was performed by micro dilution test and results were observed visually and spectrophotometrically. The fungal zones of inhibition values are given in Table 12. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 13. All the compounds showed...
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moderate to good inhibitory activity against the four strains of fungus. Among all the screened compounds, compounds (185, 186, 189 and 190) showed excellent antifungal activity, nearly equivalent to the control compound against *C. albicans*, *P. marneffei* and *A. fumigatus* fungal strains. The MFC of few compounds was found to be the same as MIC but in most of the compounds it was two or three or four folds higher than the corresponding MIC results. Compound (186) exhibited stronger antifungal activity against *Candida albicans* strains.

Table 12: Antifungal activity of oxazoline and thiazoline derivatives of fatty acid esters

<table>
<thead>
<tr>
<th>Compound Codes</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>183</td>
<td>21.1±0.3</td>
</tr>
<tr>
<td>184</td>
<td>17.5±0.3</td>
</tr>
<tr>
<td>185</td>
<td>22.9±0.2</td>
</tr>
<tr>
<td>186</td>
<td>28.2±0.2</td>
</tr>
<tr>
<td>187</td>
<td>19.3±0.3</td>
</tr>
<tr>
<td>188</td>
<td>17.8±0.6</td>
</tr>
<tr>
<td>189</td>
<td>24.8±0.5</td>
</tr>
<tr>
<td>190</td>
<td>26.9±0.3</td>
</tr>
<tr>
<td><strong>Standard</strong></td>
<td>30.0±0.0</td>
</tr>
<tr>
<td><strong>DMSO</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>CA: *Candida albicans*, <sup>b</sup>AF: Aspergillus fumigatus, <sup>c</sup>TM: Trichophyton mentagrophytes, <sup>d</sup>PM: Penicillium marneffei. Positive control (griseofulvin) and negative control (DMSO) measured by the halo zone test (unit, mm).
## Table 13: MIC and MFC of oxazoline and thiazoline derivatives of fatty acid esters

<table>
<thead>
<tr>
<th>Compound</th>
<th>CA°1</th>
<th>AF°2</th>
<th>TM°3</th>
<th>PM°4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codes</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>183</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>184</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>185</td>
<td>12.5</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>186</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>187</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>188</td>
<td>25</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>189</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>20</td>
</tr>
<tr>
<td>190</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

°A: Candida albicans, °'AF: Aspergillus fumigatus, °TM: Trichophyton mentagrophytes, °PM: Penicillium marneffei. MIC (µg/mL) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/mL) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

### 5.3.3. Conclusion

For the first time, the synthesis of 2-amino-5-substituted and 2-amino-4,5-disubstituted oxazoline and thiazoline derivatives of olefinic and hydroxy-olefinic fatty acid esters by nucleophilic substitution of urea and thiourea to the dibromo derivatives of olefinic and hydroxy-olefinic fatty acid esters is reported in this chapter. *In vitro* antimicrobial activity of the tested compounds shows that all these novel compounds were found to be active against different bacterial and fungal strains. Compounds (185, 186, 189 and 190) showed good antimicrobial activity nearly equivalent to that of ciprofloxacin and griseofulvin. The present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent antimicrobial agents.
5.4. Experimental

5.4.1. Chemistry

Physical and Spectroscopic Measurements

The sources of all the fatty acids and instrumentation details are the same as chapter 1 (page number 19). All reagents were generally used as received from commercial suppliers. When needed, solvents were dried and distilled before used. The esters of fatty acids (long chain alkenoates) were prepared by refluxing the fatty acid in methanol in the presence of catalytic amount of concentrated sulfuric acid. The dibromo derivatives of fatty acid esters were prepared following the literature method\(^4^5\). Reactions were monitored by TLC. Mixture of petroleum ether and diethyl ether was used as developing solvents in different proportions for different compounds and was visualized in an iodine chamber. Products were purified by column chromatography and the synthesized compounds were identified on the basis of IR, \(^1\)H NMR, \(^13\)C NMR and mass spectra.

**General procedure for preparation of dibromo derivatives (179-182) of fatty acid esters:**

Fatty acid ester (0.1 mole) was dissolved in carbon tetrachloride and an equimolar amount of bromine (added drop wise to the reaction mixture) at 0°C. The reaction was stirred until all the fatty acid ester was used.

**Synthesis of oxazoline (183-186) and thiazoline (187-190) derivatives of fatty acid esters:**

0.1 Mole of dibromo derivative of fatty acid ester (179-182) dissolved in methanol and equimolar amount of urea/thiourea was added. The mixture was refluxed on a paraffin bath. Reaction was continued till all reactants were consumed. After completion, excess of solvent was evaporated on a water bath and then worked up with diethyl ether-water. The organic layer was dried over anhydrous sodium sulfate.
and excess of solvent was removed by heating on water bath. Product was purified by column chromatography. All these novel compounds were yellow oily liquid and were characterized from their spectral data.

The spectroscopic and analytical data for the synthesized compounds (183-190) are presented below:

2-Amino-5-(carbomethoxyoctyl)-1,3-oxazoline, (183)

Yield: 85%

IR (KBr, cm\(^{-1}\)): 3320 (N-H stretching), 2931 (C-H stretching), 1739 (C=O in ester), 1437 (C=N ring), 1362 (C-O-C).

\(^1\)H NMR (CDCl\(_3\), \(\delta_r\)): 4.16 (tdd, 1H, \(J_{\text{n-ch}} = 6.6\) Hz, \(J_{\text{n-lz}} = 10.2\) Hz, \(J_{\text{n-c}} = 17.1\) Hz, CH\(_2\)-CH\(_2\)), 3.85 (dd, 1H, \(J_{\text{n-c}} = 10.2\) Hz, \(J_{\text{n-\eta}} = 1.2\) Hz, H\(_2\)-C-CH\(_3\)), 3.66 (s, 3H, OCH\(_3\)), 3.64 (dd, 1H, \(J_{\text{n-c}} = 17.1\) Hz, \(J_{\text{n-lz}} = 3.6\) Hz, H\(_2\)-C-CH\(_3\)), 2.32 (s, 2H, NH\(_2\)), 2.30 (t, \(J = 7.52\) Hz, 2H, -CH\(_2\)COOCH\(_3\)), 2.12 (m, 2H, -CH\(_2\)CH\(_2\)COOCH\(_3\)), 1.76 (m, 2H, -CH\(_2\)CH\(_2\)COOCH\(_3\)), 1.31 (br.s, 10H, (CH\(_2\))\(_5\)).

\(^13\)C NMR (CDCl\(_3\), \(\delta_C\)): \(\delta\) 179.8, 163.4, 79.2, 64.7, 51.4, 36.3, 35.9, 34.0, 29.8, 29.1, 28.7, 26.7, 24.9.

MS (ESI): m/z = 279.151 found [M+Na]\(^+\), Calculated [M+Na]\(^+\) = 279.287.

2-Amino-4-octyl-5-(carbomethoxyheptyl)-1,3-oxazoline, (184)

Yield: 80%

IR (KBr, cm\(^{-1}\)): 3322 (N-H stretching), 2927 (C-H stretching), 1740 (C=O in ester), 1461 (C=N ring), 1366 (C-O-C).
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\[ ^1H \text{NMR (CDCl}_3, \delta_H) \]: 4.20 (m, 2H, CH-CH ring), 3.65 (s, 3H, -OCH3), 2.31 (s, 2H, NH2), 2.24 (t, 2H, J = 7.75 Hz, CH2COOCH3), 1.56 (m, 4H, CH2-CH-CH2), 1.44 (m, 4H, CH2CH2COOCH3), 1.30 (br.s, 18H, (CH2)9), 0.87 (dist.t, 3H, CH3).


\[ \text{MS (ESI)} \]: m/z = 377.301 found [M+Na]+, calculated [M+Na]+ = 377.448.

2-Amino-4-(2'R)(2'-hydroxyoctyl)-5-(carbomethoxymethyl)-1,3-oxazoline, (185)

Yield: 82%

\[ \text{IR (KBr, cm}^{-1}) \]: 3461-3315 (O-H, N-H stretching), 2928 (C-H stretching), 1740 (C=O in ester), 1459 (C=N ring), 1363 (C-O-C).

\[ ^1H \text{NMR (CDCl}_3, \delta_H) \]: 4.05 (m, 1H, CH-OH), 3.98 (m, 2H, CH-CH ring), 3.66 (s, 3H, -OCH3), 2.32 (s, 2H, NH2), 2.20 (t, 2H, J = 7.14 Hz, CH2COOCH3), 2.06 (m, 1H, CH-OH), 1.59 (m, 4H, CH2-CH-CH2), 1.49 (m, 2H, CH2CH2COOCH3), 1.43 (br.s, 18H, (CH2)9), 0.88 (dist.t, 3H, CH3).

\[ ^13C \text{NMR (CDCl}_3, \delta_C) \]: 179.2, 162.9, 79.1, 72.4, 65.1, 52.9, 42.3, 36.1, 35.2, 34.6, 33.9, 31.7, 29.9, 29.4, 29.0, 28.8, 28.6, 27.7, 25.8, 14.1.

\[ \text{MS (ESI)} \]: m/z = 393.352 found [M+Na]+, calculated [M+Na]+ = 393.447.

2-Amino-4-pentyl-5-[(8'R)-8'-hydroxy(carbomethoxydecyl)-1,3-oxazoline, (186)

Yield: 65%

\[ \text{IR (KBr, cm}^{-1}) \]: 3475-3314(O-H, N-H stretching), 2925 (C-H stretching), 1739 (C=O in ester), 1455 (C=N ring), 1362 (C-O-C).

\[ ^1H \text{NMR (CDCl}_3, \delta_H) \]: 4.12 (m, 1H, CH-OH), 3.85 (m, 2H, CH-CH ring), 3.65 (s, 3H, -OCH3), 2.33 (s, 2H, NH2), 2.18 (t, 2H, J = 7.32 Hz, CH2COOCH3), 2.02 (m, 1H,
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CH-OH), 1.60 (m, 4H, CH₂-CH₂CH₂-CH₂), 1.53 (m, 2H, CH₂CH₂COOCH₃), 1.38 (br.s, 18H, (CH₂)₉), 0.84 (dist.t, 3H, CH₃).

¹³C NMR (CDCl₃, δ_C): 178.8, 164.7, 78.4, 73.2, 63.6, 52.9, 41.8, 37.3, 34.7, 33.4, 31.3, 29.8, 29.0, 28.7, 28.5, 28.4, 28.3, 26.9, 25.8, 14.0.


2-Amino-5-(carbomethoxyoctyl)-1,3-thiazoline, (187)

Yield: 83%

IR (KBr, cm⁻¹): 3324 (N-H stretching), 2929 (C-H stretching), 1738 (C=O in ester), 1435 (C=N ring), 1359 (C-S-C).

¹H NMR (CDCl₃, δ_H): 4.16 (tdd, 1H, J_H-CH₂ = 6.6 Hz, J_H-CH₂ = 10.2 Hz, J_H-N = 17.1 Hz, CH₂-CH₂), 3.85 (dd, 1H, J_H-N = 10.2 Hz, J_H-C₂H₅ = 1.2 Hz, H₂C-CH₂), 3.66 (s, 3H, OCH₃), 3.64 (dd, 1H, J_H-N = 17.1 Hz, J_H-C₂H₅ = 3.6 Hz, H₂C-CH₂), 2.32 (s, 2H, NH₂), 2.30 (t, J = 7.52 Hz, 2H, CH₂COOCH₃), 2.12 (m, 2H, CH₂CH₂COOCH₃), 1.76 (m, 2H, CH₂(CH₂)₅), 1.31 (br.s, 10H, (CH₂)₉).

¹³C NMR (CDCl₃, δ_C): δ 177.2, 165.9, 79.5, 62.2, 50.8, 35.8, 34.3, 33.8, 28.8, 28.6, 28.3, 25.8, 24.2.


2-Amino-4-octyl-5-(carbomethoxyheptyl)-1,3-thiazoline, (188)

Yield: 80%

IR (KBr, cm⁻¹): 3323 (N-H stretching), 2926 (C-H stretching), 1740 (C=O stretching), 1461(C=N ring), 1355 (C-S-C).
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$^1$H NMR (CDCl$_3$, $\delta_{HH}$): 4.20 (m, 2H, CH-CH ring), 3.66 (s, 3H, -OCH$_3$), 2.32 (s, 2H, NH$_2$), 2.30 (t, 2H, $J = 7.45$ Hz, CH$_2$COOCH$_3$), 1.57 (m, 4H, CH$_2$-CHCH-CH$_2$), 1.43 (m, 2H, CH$_2$CH$_2$COOCH$_3$), 1.29 (br.s, 18H, (CH$_2$)$_9$), 0.89 (dist.t, 3H, CH$_3$).

$^13$C NMR (CDCl$_3$, $\delta_{CC}$): 177.5, 164.9, 79.1, 63.9, 52.0, 36.3, 33.8, 31.5, 30.2, 29.9, 29.7, 29.5, 28.8, 28.7, 28.1, 28.0, 25.1, 24.6, 22.6, 14.0.


2-Amino-4-(2'R)-(2'-hydroxyoctyl)-5-(carbomethoxyheptyl)-1,3-thiazoline, (189)

Yield: 83%

IR (KBr, cm$^{-1}$): 3493-3317 (O-H, N-H stretching), 2930 (C-H stretching), 1738 (CO in ester), 1461 (C=N ring), 1358 (C-S-C).

$^1$H NMR (CDCl$_3$, $\delta_{HH}$): 4.02 (m, 1H, CH-OH), 3.95 (m, 2H, CH-CH ring), 3.66 (s, 3H, -OCH$_3$), 2.32 (s, 2H, NH$_2$), 2.20 (t, 2H, $J = 7.46$ Hz, CH$_2$COOH), 2.00 (m, 1H, CH-OH), 1.62 (m, 4H, CH$_2$-CHCH-CH$_2$), 1.38 (m, 2H, CH$_2$CH$_2$COOCH$_3$), 1.36 (br.s, 18H, (CH$_2$)$_9$), 0.79 (dist.t, 3H, CH$_3$).

$^13$C NMR (CDCl$_3$, $\delta_{CC}$): 178.8, 164.2, 79.8, 71.8, 62.7, 51.8, 38.8, 35.0, 34.8, 33.5, 31.4, 29.9, 29.5, 28.8, 28.5, 28.0, 27.7, 25.8, 14.0.


2-Amino-4-pentyl-5-{(8'R)-8'-hydroxy(carbomethoxydeceyl)-1,3-thiazoline, (190)

Yield: 68%

IR (KBr, cm$^{-1}$): 3493-3317 (O-H, N-H stretching), 2927 (C-H stretching), 1712 (C=O in ester), 1452 (C=N ring), 1340 (C-S-C).

$^1$H NMR (CDCl$_3$, $\delta_{HH}$): 4.20 (m, 1H, CH-OH), 3.83 (in, 2H, CH-CH ring), 3.66 (s, 3H, -OCH$_3$), 2.33 (s, 2H, NH$_2$), 2.30 (t, 2H, $J = 7.32$ Hz, CH$_2$COOCH$_3$), 2.14 (m, 1H,
CH-OH), 1.58 (m, 4H, CH₂-CHCH₂), 1.49 (m, 2H, CH₂CH₂COOCH₃), 1.37 (br.s, 18H, (CH₂)₉), 0.89 (dist.t, 3H, CH₃).

¹³C NMR (CDCl₃, δ): 178.9, 163.4, 79.1, 70.9, 63.1, 51.4, 38.4, 35.1, 34.5, 34.2, 33.4, 31.3, 29.0, 28.9, 28.6, 28.5, 28.3, 26.9, 25.7, 14.0.


5.4.2. Biology

5.4.2.1. Antibacterial assay

A standard inoculum (1-2 X 10⁷ c.f.u./mL 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140°C for 1 hour. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Ciprofloxacin (30 µg) was used as positive control. While the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 hours at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against gram-positive and gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 X 10⁵ c.f.u./mL of actively dividing bacteria cells. The cultures were incubated for 24 hours at 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bacterial concentration (MBC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 hours of incubation at
35°C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed.

5.4.2.2. Antifungal assay

Antifungal activity was also done by disk diffusion method. Sabourand's agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petridish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 hour using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The fungal activity of each compound was compared with standard drug. Inhibition zones were measured and compared with the controls.

The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4 - 6 \times 10^4$ c.f.u./mL. The cultures were incubated for 48 hours at 35°C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain the minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 hours of incubation at 35°C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed.
5.5. References


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