CHAPTER-2

Synthesis of isoxazole and dihydroidoxazoles
2.1. Theoretical

Among the family of heterocyclic compounds the five membered isoxazoles and isoxazolines/dihydroisoxazoles provide a valuable scaffold in medicinal chemistry as well as a useful synthon in organic synthesis. In addition, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis. Isoxazole (35) is five membered heterocyclic ring containing nitrogen and oxygen atoms in the 1,2 positions. Isoxazolines/dihydroisoxazoles (36) are partially saturated analogs of isoxazole.

Isoxazole and isoxazolines have diverse useful bioactivities and are widely used as key intermediate in the construction of some natural products and related structures. So many methodologies exist towards the synthesis of isoxazoles and dihydroisoxazoles and most of them endeavor nitrile oxide cycloaddition as a key step. The addition of a 1,3-dipole to an alkene/alkyne for the synthesis of 5-membered ring is a classic reaction in organic chemistry. The general application of 1,3-dipoles in organic chemistry was first established by Huisgen. Isoxazole and isoxazoline heterocycles are derived from the [3+2] cycloaddition of a nitrile oxide to an alkene and alkyne, respectively. The nitrile oxide 1,3-dipole is generated in situ in Mukaiyama reaction from the corresponding primary nitroalkane and phenyl isocyanate. Here, nitrile oxide is generated by dehydration of primary nitroalkanes. This method is however the most useful due to its easy set up as described by Mukaiyama and coworker in 1960, 1,4-phenylene diisocyanate was used as dehydrating agent. By using 1,4-phenylene diisocyanate, polyurea byproduct was obtained which may be removed from product by simple filtration because this polyurea byproduct was insoluble in the reaction solvent (benzene and tetrahydrofuran).
Bhosale et al.\textsuperscript{10} reported the synthesis of derivatives of isoxazole (40) and isoxazolines (41) via 1,3-dipolar cycloaddition by reaction of aldoximes (37) with Magtrieve\textsuperscript{TM} (CrO\textsubscript{2}) in presence of dipolarophile (38) or (39).

\[
\text{N}=\begin{array}{c}
\text{O} \\
\text{X} \\
\text{X'}
\end{array}
\text{O} \\

\begin{array}{c}
\text{R}_1 \\
\text{X} \\
\text{X'}
\end{array}
\]

R\textsubscript{1}: 4-Cl-Ph; X: -Ph, -CH\textsubscript{2}-OTBS, -CH\textsubscript{2}-OTHP, CH\textsubscript{2}N(H)Boc, TMS-;
X': -Ph, -CO\textsubscript{2}Et, -CN, -Bn, -OAc

Kadnor et al.\textsuperscript{11} showed the synthesis of fluorinated isoxazolines (45) by the reaction of fluorinated chalcones (44) and hydroxyl amine hydrochloride in presence of acetic acid. These fluorinated chalcones were prepared by the Claisen-Schmidt reaction of fluorinated ketone (42) and substituted aromatic aldehyde (43).
Govindappa et al.\textsuperscript{12} outlined the synthesis of new cycloadducts, 3-aryl-5-(4-methoxyphenyl)-isoxazole-4-carbonitriles (48) by the 1,3-dipolar cycloaddition reaction of nitrile oxides with 3-(4-methoxyphenyl)propionitrile (46). The nitrile oxides were generated \textit{in situ} by the oxidative dehydrogenation of aromatic aldoximes (47).

\[ \text{CN} \quad \text{OH} \quad \text{CH}^{+} \]
\[
\begin{array}{c}
\text{CAT, EtOH} \\
100^\circ \text{C, 3h}
\end{array}
\]

\begin{align*}
R_1, R_2: & \quad H, H; H, CH_3; H, OCH_3; \\
& \quad OCH_3, OCH_3; H, F; H, Cl \\
\text{CAT:} & \quad \text{Chloramine-T}
\end{align*}

Denmark and Kallemeyn\textsuperscript{13} reported sequential [3+2] cycloaddition and cross-coupling reactions for the synthesis of 3,4,5-trisubstituted isoxazoles. Initially, isoxazolylsilanols (51) were prepared by the reaction of alkynyl dimethyl silyl ethers (50) and alkyl/aryl nitrile oxides (49). In the final step, 3,4,5-trisubstituted isoxazoles (53) were synthesized by the cross-coupling reaction of heterocyclic silanols (51) with number of aryl iodides (52).
Mayo et al.\textsuperscript{14} carried out 1,3-dipolar cycloaddition of nitrile oxide (55) with unsymmetrically substituted norbornenes (56) to give isoxazoline derivatives (57). The nitrile oxide generated by nitromethylbenzene (54) using Hassner’s method [(BOC)\textsubscript{2}O and DMAP].

Nitrogen and oxygen containing heterocyclic compounds have received considerable attention due to their wide range of biological and pharmacological activities. Isoxazoles and isoxazolines have been reported\textsuperscript{15,16} to possess antitubulin as well as antiinflammatory activity. Dihydroisoxazole derivatives are reported\textsuperscript{17} as antinociceptive compounds. Also, medicinal activity like anxiolytic activity has been reported\textsuperscript{18} for isoxazole derivatives.
Isoxazole and dihydroisoxazoles

The isoxazole and isoxazoline derivatives are also largely employed in the therapeutics and pharmaceutical areas such as antimicrobial, antibiotic, ulcerogenic, insecticidal, antituberculous, and anticancer. For many years, drugs based on isoxazole derivatives such as sulfamethoxazole (58), sulfafurazole (59), acivicin (60), oxacillin (61), leflunomide (62), and micaflungin have been in commercial use. Acivicin possesses antitumor and antileishmanial activity while isoxaflutole (63) is used as herbicidal drug.

\[
\begin{align*}
(58) & \quad \begin{array}{c}
\text{SO} \\
\text{NH}_2 \\
\end{array} \\
(59) & \quad \begin{array}{c}
\text{SO} \\
\text{NH}_2 \\
\end{array} \\
(60) & \quad \begin{array}{c}
\text{Cl} \\
\text{NH}_2 \\
\end{array} \\
(61) & \quad \begin{array}{c}
\text{NH} \\
\text{CO} \\
\text{COOH} \\
\end{array} \\
(62) & \quad \begin{array}{c}
\text{CF}_3 \\
\text{O} \\
\end{array} \\
(63) & \quad \begin{array}{c}
\text{O=S=S} \\
\end{array}
\end{align*}
\]
Isoxazole moiety is found in the COX II inhibitors \textsuperscript{20} bextra (valdecoxib) (64) and parecoxib (65). In several natural products such as aerothionon, homoaerothionin, aerophobin 1, epi-fistularin-3 and calafianin, isoxazoline ring is a common feature.

\[\text{ISOXAZOLE AND DIHYDROISOXAZOLES}\]

[34]
2.2. Synthesis and Characterization of Novel Long Chain Isoxazole and Dihydroisoxazole Derivatives: In Vitro Antimicrobial Activity*  

Various biological applications such as antimicrobial, pesticidal, anticancer and antifungal activities have been reported for seed oils, long chain alkenoic acids and their derivatives. These observations and our interest in the chemistry of hetero-fatty acids prompted us to synthesize isoxazole derivatives of fatty acids with different substituent at 4- and 5- positions. For this reason, the present strategy for the synthesis of new compounds is aimed in the direction of developing new isoxazole derivatives to inhibit the growth of gram-positive, gram-negative bacteria and most pathogenic fungi. After synthesis the compounds were tested for their antimicrobial activity by disk diffusion assay and MIC by broth micro dilution method against bacterial and fungal strains.

2.3. Results and Discussion

2.3.1. Chemistry

The long chain alkynoic acid and alkenoates used as the starting materials and were prepared from the corresponding long chain alkynoic acids. The long chain alkynoic acid (66) was synthesized using the procedure of Kannan et al. The esters of fatty acids (70-73) were prepared by refluxing the fatty acid in methanol in the presence of catalytic amount of concentrated sulfuric acid. Nitrile oxide was generated in situ from 1-nitrobutane employing 1,4-phenylene diisocyanate.

The 1,3-dipolar cycloaddition of nitrile oxide to terminal alkyne/alkene give isoxazole/dihydroisoxazole, respectively. On the other hand, the 1,3-dipolar cycloaddition of nitrile oxide to internal alkenes gives an inseparable isomeric mixture of dihydroisoxazole derivatives. This transformation was most effective when excess base (3 equivalents of triethylamine) was employed and 1-nitrobutane added dropwise over 6-8 hours while heating. After refluxing, the reaction was quenched with water. The polyurea (polymer) was removed by filtration. Products were purified by column chromatography and identified by using different spectral techniques. The signals of products in the $^1$H and $^{13}$C NMR spectra were successfully assigned. The high resolution mass (electron ionization) spectral studies have further confirmed their structures. The reaction sequences are outlined in Schemes 2 and 3.

\[
\begin{align*}
\text{[36]} &
\end{align*}
\]
Scheme 3: Synthesis of 3,5-disubstituted and 3,4,5-trisubstituted-4,5-dihydroisoxazoles
The structure of (74-77) is evident from their spectral data. The structure of compound (74) is given below:

![Structure of 5-(carbomethoxyoctyl)-3-propyl-4,5-dihydroisoxazole, (74)](image)

Compound (74), 5-(carbomethoxyoctyl)-3-propyl-4,5-dihydroisoxazole, showed IR absorption bands at 2931 cm\(^{-1}\) (C-H stretching), 1740 cm\(^{-1}\) (ester C=O stretching), 1457 (C=N stretching). The \(^1\)H NMR was more informative in assigning the structure. Diagnostic peaks for cyclic proton were appeared at 4.46 (1H, m, CH\(_2\)CH\(_2\) ring), 2.91 (1H, dd, \(J_{\text{H-H}} = 16.70\) Hz, \(J_{\text{H-H}} = 10.00\) Hz, CH-CH\(_2\) ring), 2.49 (1H, dd, \(J_{\text{H-H}} = 16.80\) Hz, \(J_{\text{H-H}} = 8.10\) Hz, CH-CH\(_2\) ring), 2.25 (4H, two triplets partially merged, -CH\(_2\)COOCH\(_3\), -CH\(_2\)CH\(_2\)CH\(_3\)), 1.60 (6H, m, CH\(_2\)CH\(_2\)CH\(_3\), (CH\(_2\))\(_3\)-CH\(_2\), CH\(_2\)CH\(_2\)COOCH\(_3\)), 1.25 (10H, br.s, CH\(_2\) Chain), 0.92 (3H, t, \(J = 7.30\) Hz, CH\(_3\)). In \(^13\)C NMR peaks at 174.2, 156.7, 79.9, 51.3, 42.0, 35.2, 34.0, 29.7, 29.3, 29.2, 29.1, 29.0, 25.5, 24.8, 19.7, 13.7 were observed. The mass spectra showed characteristic molecular ion peak in accord with the molecular formula. Similarly other compounds were characterized from their spectral data.

### 2.3.2. Biology

#### 2.3.2.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus*, *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (clinical isolates) bacterial strains. The susceptibility was assessed on the basis of the diameter of zone of inhibition against gram-positive and gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls (Table 3).
The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good antibacterial activity. Among all the synthesized compounds, compound (76) showed highest antibacterial activity (25 µg/mL) nearly equivalent to standard drug ciprofloxacin. Compounds (76 and 77) showed good inhibition against *S. pyogenes*, *S. aureus* and *E. coli* species. In general, all the compounds were more effective against gram-positive bacteria as compared to gram-negative bacteria.

### Table 3: Antibacterial activity of all the newly synthesized compounds

<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&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>69</td>
<td>21.6±0.4</td>
</tr>
<tr>
<td>74</td>
<td>20.2±0.3</td>
</tr>
<tr>
<td>75</td>
<td>22.3±0.4</td>
</tr>
<tr>
<td>76</td>
<td>23.2±0.2</td>
</tr>
<tr>
<td>77</td>
<td>22.4±0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>SP: *Streptococcus pyogenes*, <sup>b</sup>SA: *Staphylococcus aureus*, <sup>c</sup>PA: *Pseudomonas aeruginosa*, <sup>d</sup>KP: *Klebsiella pneumonia*, <sup>e</sup>EC: *Escherichia coli*. Positive control (standard): ciprofloxacin; negative control: DMSO; antibacterial activity measured by the halo zone test (unit, mm)

Minimum inhibitory concentrations (MICs) were determined by broth micro dilution method. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 4.
Table 4: MIC and MBC results of all the newly synthesized compounds

<table>
<thead>
<tr>
<th>Compound 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>SA&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MIC, MBC</td>
<td>MIC, MBC</td>
</tr>
<tr>
<td>69</td>
<td>50, 100</td>
<td>50, 100</td>
</tr>
<tr>
<td>74</td>
<td>50, 100</td>
<td>50, &gt;100</td>
</tr>
<tr>
<td>75</td>
<td>25, 50</td>
<td>25, 100</td>
</tr>
<tr>
<td>76</td>
<td>25, 50</td>
<td>25, 50</td>
</tr>
<tr>
<td>77</td>
<td>25, 50</td>
<td>25, 100</td>
</tr>
<tr>
<td>Standard</td>
<td>12.5, 12.5</td>
<td>6.25, 12.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>SP: Streptococcus pyogenes  
<sup>b</sup>SA: 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: ciprofloxacin

2.3.2.2. Antifungal studies

All the newly synthesized compounds were tested for in vitro antifungal activity against standard species of *Candida* and clinical isolates of *Candida* which includes *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis*. The results are presented in Tables 5 and 6. All the synthesized compounds had good antifungal effects against tested clinical species of *Candida*.

Among all the synthesized compounds, compounds (76 and 77) showed excellent antifungal activity (32 µg/mL) against *Candida tropicalis* and *Candida albicans*, which were resistant to fluconazole and itraconazole as well as against standard species of *Candida*.
### Table 5: *In vitro* antifungal activity of all the synthesized compounds against standard species of *Candida*

<table>
<thead>
<tr>
<th>Comp. Codes</th>
<th>Tested fungi (MIC 90% and MFC µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
</tr>
<tr>
<td>69</td>
<td>&gt;256</td>
</tr>
<tr>
<td>74</td>
<td>&gt;256</td>
</tr>
<tr>
<td>75</td>
<td>128</td>
</tr>
<tr>
<td>76</td>
<td>128</td>
</tr>
<tr>
<td>Flu.</td>
<td>&gt;256</td>
</tr>
<tr>
<td>lt.</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

<sup>a</sup>CA: *Candida albicans*, <sup>b</sup>CT: *Candida tropicalis*, <sup>c</sup>CG: *Candida glabrata*, <sup>d</sup>CP: *Candida parapsilosis*, <sup>e</sup>CK: *Candida krusei*, <sup>f</sup>CD: *Candida dubliniensis*. Flu. = Fluconazole, lt. = Itraconazole

### Table 6: *In vitro* antifungal activity of all the synthesized compounds against clinical species of *Candida*

<table>
<thead>
<tr>
<th>Compound Codes</th>
<th>Tested fungi (MIC 90% and MFC µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
</tr>
<tr>
<td>69</td>
<td>&gt;256</td>
</tr>
<tr>
<td>74</td>
<td>&gt;256</td>
</tr>
<tr>
<td>75</td>
<td>128</td>
</tr>
<tr>
<td>76</td>
<td>32</td>
</tr>
<tr>
<td>77</td>
<td>64</td>
</tr>
<tr>
<td>Flu.</td>
<td>8</td>
</tr>
<tr>
<td>lt.</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup>CA: *Candida albicans*, <sup>b</sup>CT: *Candida tropicalis*, <sup>o</sup>CP: *Candida parapsilosis*, Flu. = Fluconazole, lt. = Itraconazole, * = resistant to fluconazole and itraconazole, R = resistant
Also, molecular docking studies have been performed on peptide deformylase (PDF) of *Escherichia coli* and CYP 450-14DM of *Candida albicans* to evaluate the possible mode of action of the molecules in the active site of the receptor. The results of the docking experiments for the newly synthesized compounds (69, 74-77), with the 30s ribosomal subunit were found to be in accordance with the in vitro antimicrobial activity data obtained. The binding sites of the compounds were found to be in close proximity to the binding site of the standard drug ciprofloxacin. It was interesting to observe that even though the core structure of all the compounds was the same, the degree of interaction and binding patterns were found to be different. We anticipate that this kind of docking and antipathogenic screening studies would help in designing of novel drugs that targets microbial protein synthesis. Moreover, the mode of action of the long chain isoxazole and dihydroisoxazole derivatives showed that the affinity of the lead molecules for Cytochrome P450-14DM was mainly attributed to hydrogen and their non-bonding interaction with the active site residues of Cytochrome P450-14DM protein and binding with the heme, which was similar to that of azoles antifungal agents. All the results and detailed studies of the molecular docking of these newly synthesized compounds are given in the published paper.

### 2.3.3. Conclusion

The proposed method for the synthesis of novel long chain isoxazole derivatives was simple and all the synthesized compounds obtained in good yield. The report of the study confirms traditional usage of these compounds as antimicrobial agents. However, large data must be generated through the pharmacognostic studies before going for commercialization. This present study showed that all the title compounds were exhibiting antibacterial activities. These compounds were evaluated against yeast. Among the synthesized compounds, compounds (76 and 77) showed antimicrobial activity against all tested microorganisms which is almost equivalent to the standard drugs ciprofloxacin and fluconazole. Also, compounds (76 and 77) had antifungal activity against tested clinical species of *Candida* which were resistant to fluconazole as well as itraconazole. It was interesting to observe that even though the core structure of all the compounds was the same, the biological activities were found
Isoxazole and dihydroisoxazoles

to be different against different microbial strains. The studies presented here provide a new structural type for the development of novel antimicrobial agents.
2.4. Experimental

2.4.1. Chemistry

Physical and Spectroscopic Measurements

Anhydrous conditions were achieved by drying flasks and other equipments in the oven. Reagents were of commercial grade and used without further purification. When needed, solvents were dried and distilled before use. The source of olefinic and hydroxy-olefinic fatty acids, instrumental details of IR, NMR and mass spectrometry are already given in experimental section of chapter 1 (page number 19). The eluent was a mixture of petroleum ether and diethyl ether in different proportions for different compounds. 1-Nitrobutane and 1,4-phenylene diisocyanate were purchased from Sigma Aldrich. Triethylamine, tetrahydrofuran (THF) and benzene were purchased from Merck, Mumbai.

General Procedure for the Synthesis of Long Chain Alkynoic Acid, (66)

The long chain alkynoic acid (66) used as the starting material was prepared by the previously reported method.

Synthesis of 3,5-disubstituted isoazole derivative (69) of long chain alkynoic acid:

1,4-Phenylene diisocyanate (68) (0.003 mole) was added to long chain alkynoic acid (66) (0.001 mole) in dry tetrahydrofuran (THF) (20 mL). Triethylamine (0.003 mole) was added to the reaction mixture and this was heated to 80°C. 1-Nitrobutane (67) (0.003 mole) was added in portions over a period of 6-8 hours, and then the reaction was heated for additional 2 hours. The precipitate was observed. After heating, the reaction mixture was cooled. The reaction was quenched with water (≈ 6 mL) and then allowed to stir at room temperature for 1 hour. The polyurea (polymer) was removed by filtration and washed with THF. The solvent was evaporated on a water bath and then worked up with diethyl ether and water. The ether layer was dried over anhydrous sodium sulfate. The excess of solvent was removed by heating on water
bath. Further, product (69) was purified by silica gel column with petroleum ether and increasing concentration of diethyl ether as eluent.

The spectroscopic and analytical data for the synthesized compound (69) is presented below:

5-(Carboxyoctyl)-3-propylisoxazole, (69)

White solid; M.P. = 79-80°C; yield: 80%

IR (KBr, cm⁻¹): 3285 (O-H stretching), 2918 (C-H stretching), 1702 (C=O stretching), 1465 (C=N stretching).

¹H NMR (CDCl₃, δppm): 12.61 (1H, s, COO⁻), 6.98 (1H, s, CH ring), 2.35 (2H, t, J = 7.52 Hz, CH₂COOH), 2.18 (4H, two triplets merged together, (CH₂)₃-CH₂, CH₂CH₂CH₂), 1.63 (2H, m, CH₂CH₂COO⁻), 1.54 (2H, m, CH₂CH₂CH₂CH₃), 1.28 (10H, br.s, CH₂ chain), 0.90 (3H, t, J = 7.21 Hz, CH₃).

¹³C NMR (CDCl₃, δppm): 176.1, 167.8, 82.1, 80.8, 44.2, 35.8, 32.6, 29.8, 29.6, 29.3, 29.0, 27.4, 25.6, 22.9, 14.1.


Synthesis of 3,5-disubstituted and 3,4,5-trisubstituted-4,5-dihydroisoxazole derivatives (74-77) of long chain alkenoates:

1,4-Phenylene diisocyanate (68) (0.03 mole), 1-nitrobutane (67) (0.01 mole) and long chain alkenoates (70-73) (0.01 mole) were dissolved in dry benzene (30 mL). Triethylamine (25-50 drops) was added. The reaction mixture became turbid and a precipitate was observed. After 8 hours at reflux the reaction mixture was cooled. The reaction was quenched with water (≥ 5 mL) and allowed to stir at room temperature for 1 hour. The polyurea (polymer) was removed by filtration and washed with benzene. The solvent was evaporated on a water bath and then worked up with diethyl ether and water. The ether layer was dried over anhydrous sodium sulfate and excess
solvent was evaporated on water bath. Further, the products (74-77) were purified by silica gel column with petroleum ether and increasing concentration of diethyl ether as eluent. All these novel compounds were characterized from their spectral data.

The spectroscopic and analytical data for the synthesized compounds (74-77) are presented below:

5-(Carbomethoxyoctyl)-3-propyl-4,5-dihydroisoxazole, (74)

Colourless oily liquid; yield: 85%

IR (KBr, cm⁻¹): 2931 (C-H stretching), 1740 (C=O stretching), 1457 (C=N stretching).

¹H NMR (CDCl₃, δH): 4.46 (1H, m, CH₂-CH ring), 3.60 (3H, s, OCH₃), 2.91 (1H, dd, J₁₂,₋₁₉ = 16.70 Hz, J₁₉₋₁₂ = 10.00 Hz, CH-CH₂ ring), 2.49 (1H, dd, J₉₋₁₂ = 16.80 Hz, J₁₂₋₉ = 8.10 Hz, CH-CH₃ ring), 2.25 (4H, two triplets partially merging, CH₂COOCH₃, CH₂CH₂CH₃), 1.60 (6H, m, CH₂CH₂CH₃, (CH₂)₅-CH₂, CH₂CH₂COOCH₃), 1.25 (10H, br.s, CH₂ Chain), 0.92 (3H, t, J = 7.30 Hz, CH₃).


5-(Carbomethoxyheptyl)-4-octyl-3-propyl-4,5-dihydroisoxazole, (75)

Colourless oily liquid; yield: 85%

IR (KBr, cm⁻¹): 2928 (C-H stretching), 1743 (C=O stretching), 1462 (C=N stretching).

¹H NMR (CDCl₃, δH): 5.26 (2H, m, H/C-CH ring), 3.59 (3H, s, O-CH₃), 2.54 (2H, t, J = 7.60 Hz, CH₂COOCH₃), 2.42 (2H, t, J = 7.60 Hz, CH₂CH₂CH₃), 1.95 (4H, m, H₂C-
Isoxazole and dihydroisoxazoles

CH-CH-CH₂, 1.70 (2H, m, CH₂CH₂COOCH₃), 1.56 (2H, m, CH₂CH₂CH₃), 1.20 (20H, br.s, CH₂ Chain), 0.91 (3H, t, J = 7.20 Hz, CH₂CH₂CH₃), 0.81 (3H, dist.t, CH₃).

¹³C NMR (CDCl₃, δ₀): 173.8, 159.2, 80.1, 52.8, 43.0, 35.5, 34.8, 33.4, 32.0, 31.2, 30.9, 29.9, 29.8, 29.6, 29.4, 29.2, 29.1, 26.6, 25.3, 24.4, 22.7, 18.8, 13.9.


5-(Carboxymethoxyheptyl)-4-[(2'R)-2'-hydroxoctyl]-3-propyl-4,5-dihydroisoxazole, (76)

Yellow oily liquid; yield: 80%

IR (KBr, cm⁻¹): 3328 (O-H stretching), 2924 (C-H stretching), 1738 (C=O stretching), 1447 (C=N stretching).

¹H NMR (CDCl₃, δ₁H): 5.32 (2H, m, H₳-CH ring), 4.20 (1H, m, CH-OH), 3.60 (3H, s, O-Cl₃), 2.65 (2H, t, J=7.54 Hz, CH₂COOCH₃), 2.35 (2H, t, J = 7.45 Hz, CH₂CH₂CH₃), 2.10 (4H, m, H₂CHC-CH₂CH₃), 1.80 (1H, m, CH-OH), 1.68 (2H, m, CH₂CH₂COOCH₃), 1.42 (2H, m, CH₂CH₂CH₃), 1.30 (18H, br.s, CH₂ chain), 0.95 (3H, t, J = 7.25 Hz, CH₂CH₂CH₃), 0.87 (3H, dist.t, CH₃).

¹³C NMR (CDCl₃, δ₁C): 176.4, 158.7, 80.0, 72.1, 52.8, 43.4, 39.5, 35.6, 34.8, 33.5, 32.3, 29.9, 29.7, 29.5, 29.3, 29.2, 29.0, 27.4, 25.4, 24.9, 21.9, 19.5, 14.0.


5-[(8'R)-8'-Hydroxy(carboxymethoxydecyl)-4-pentyl-3-propyl-4,5-dihydroisoxazole, (77)

Yellow oily liquid; yield: 75%

IR (KBr, cm⁻¹): 3294 (O-H stretching), 2918 (C-H stretching), 1734 (C=O stretching), 1468 (C=N stretching).
**Isoxazole and dihydroisoxazoles**

$^1$H NMR (CDCl$_3$, $\delta$): 5.34 (1H, m, HC-CH ring), 4.41 (1H, m, CH-OH), 3.69 (3H, s, O-CH$_3$), 2.55 (2H, t, $J = 7.45$ Hz, CH$_2$COOCH$_3$), 2.29 (2H, t, $J = 7.50$ Hz, CH$_2$CH$_2$CH$_3$), 1.98 (4H, m, H$_2$CHC-CHCH$_2$), 1.78 (1H, m, CH-OFI), 1.67 (2H, m, CH$_2$CH$_2$COOCH$_3$), 1.40 (2H, m, CH$_2$CH$_2$CH$_3$), 1.30 (18H, br.s, CH$_2$ chain), 0.96 (3H, t, $J = 7.25$ Hz, CH$_2$CH$_2$CH$_3$), 0.87 (3H, dist.t, CH$_3$).

$^{13}$C NMR (CDCl$_3$, $\delta$): 175.4, 156.9, 81.0, 73.1, 51.8, 42.0, 39.0, 34.9, 34.2, 33.4, 31.9, 29.8, 29.6, 29.4, 29.3, 29.2, 29.0, 26.8, 24.9, 23.9, 21.9, 19.8, 13.9.


2.4.2. Biology

2.4.2.1. Antibacterial Assay

The antibacterial activity of the synthesized compounds was completed by the disk diffusion method and measured by halo zone test$^{26,27}$. The susceptibility was assessed on the basis of the diameter of zone of inhibition against gram-positive and gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls.

A standard inoculum (1-2 X $10^7$ c.f.u./mL 0.5 McFarland standards) was spread onto the surface of sterile agar plates. The discs measuring 6 mm in diameter were prepared using Whatman no. 1 filter paper and were 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 the nutrient agar medium. Ciprofloxacin (30 $\mu$g) 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 the diameter of the zone of inhibition against gram-positive and gram-negative strains of bacteria.

The minimum inhibitory concentration (MIC) of synthesized compounds against bacterial strains was performed by the micro dilution test and the results were observed visually and spectrophotometrically. The nutrient broth, which contained
logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5 \times 10^5$ c.f.u./mL of actively dividing bacteria cells. The cultures of the bacterial strains 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 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.

2.4.2.2. Antifungal Assay

Bacterial and fungal strains were obtained from microbiology laboratory, Aligarh Muslim University, as gift, where these strains were already characterized. Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), oatmeal, and RPMI 1640 were used for agar dilution and macrodilution methods. The clinical isolates of fungi including *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* were purified and subcultures on SC, SCC, and PDA media before testing. To obtain the stock solutions of the compounds, 200 mg/mL of the compound was dissolved in DMSO.

Agar dilution assay and micro dilution method were used to establish the MIC as well as minimum fungicidal concentration (MFC) of synthetic derivatives. The compounds were diluted in solid and broth media to obtain a final concentration from 0.0312 to 256 mg/mL, using PDA and RPMI 1640 media. The inocula of the yeasts were prepared from 1-10 days mature colonies grown. Fluconazole and itraconazole or griseofulvin were used as positive and the solvents of the compounds as negative blanks.
2.5. References


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