MICROWAVE ASSISTED SYNTHESIS OF 4-SUBSTITUTED POLYHYDROXYLIDENE-2-PHENYL OXAZOLIDINE-5-ONES


Department Of Pharmaceutical Chemistry, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada-520010, India.

ABSTRACT

A series of new oxazolidines were prepared by Erlenmeyer condensation of hippuric acid and polyhydroxylated aldehydes or ketones. The synthesized oxazolidines derivatives were characterised by means of IR, 1H-NMR, 13C-NMR spectral data. All the compounds were checked for their bioactive score using online software that is molinspiration programme and also tested for Cytochrome P-450 inhibition using ochem series. These compounds are having better bioactive score against enzyme and protease inhibition. These compounds were evaluated for their biological activities like antibacterial and antifungal activity. The compounds are more sensitive towards gram positive than gram negative bacteria. They possess significant activities when compared with standard fluconazole.

KEYWORDS: oxazolidines, synthesis, antimicrobial, antifungal activity.

1. INTRODUCTION

The synthesis and biological activities of oxazolidines derivatives occupy an important position in heterocyclic chemistry as well as in medicinal chemistry. 4-substituted polyhydroxylidine -2-phenyl oxazolidines 5-ones and its derivatives possess a wide range of various biological and pharmaceutical activities. They have a wide range of applications as Antimicrobial,1-3 anti convulsant,4 anti tubercular,5 antihyperglycemic,6 Analgesic, Anti tumour,7 Anti viral, Anti inflammatory,8 cardiotonic, anti cancer,9 Anti convulsant,10 tyrosine inhibitory property.
2. MATERIALS AND METHODS

The chemicals used for the synthesis were supplied by LOBA chemicals. Purity of the compounds was checked on thin layer chromatography (TLC) plates (Silica Gel G) using the solvent systems ethyl acetate:hexane (7:3). The spots were located under UV light (254 and 365 nm). Melting points were determined on Gallenkamp (MFB-600) melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded on a shimadzu FTIR-8300 spectrometer as KBr disk. The 1H-NMR and 13C-NMR spectra (solvent CDCl$_3$) were recorded on Bruker 400 MHz spectrophotometer using TMS as internal standard.

General synthesis (1-5)

The synthesis was illustrated in Scheme 1 Hippuric acid (0.01mole), Polyhydroxylated aldehydes or ketones (0.01moles), Acetic anhydride (0.03 moles), freshly prepared 10% Sodium acetate solution were added in a 100ml beaker at a time and micro-wave assisted condensation was carried out at 70 MHz for 3 minutes. After cooling 10 ml of ethanol was added and kept overnight at 5°C. The solid separated was filtered, washed with alcohol, and recrystallised. The synthesised compounds were identified by TLC, Melting point and spectroscopic studies. The compound data were given in Table 1.

**Compound I:** (4Z)-4-(2, 3, 4, 5, 6-pentahydroxyhexylidene)-2-phenyl 1,3oxazolidine-5-one: Yield 88 %, mp. 140-180 °C; FTIR (γ max, cm$^{-1}$) 3600 (NH- bonding), 3335 (OH), 1742 (C=O bending), 1547 (-C=C); $^1$H NMR: (400MHZ,CDCl$_3$) δ 3.37-3.98 (-CH), δ6.38-7.19 (m,5H,J8.12Ar-H), δ2.0 (-OH) δ3.56,3.81 (-CH$_2$); $^{13}$CNMR: (400MHZ,CDCl$_3$) δ127.0,128.6,126.8 (Ar-C), δ127.5,135.1 (C=C), δ176.3 (O= C), δ67.1,73,71.3,72.9 (O-CH),δ64.5(O-CH$_2$),δ8.1(CH$_3$)

**Compound II:** (4Z)-2-phenyl-4-(2,3,4,5-tetrahydroxypentylidene)-1,3-oxazolidine-5-one: Yield 75%, mp. 140-180 °C; FTIR (γ max, cm$^{-1}$) 3600 (-NH-bonding), 3335 (OH-bonding), 1735 (-C=O bonding), 1533 (-C=C bonding), $^1$H NMR: (400MHZ,CDCl$_3$) δ 3.38-3.98(O-CH),δ6.38-7.19 (m,5H,J8.12Ar-H)δ2.0(-NH),δ2.0(-OH)δ3.56,3.81(CH$_2$); $^{13}$CNMR:(400MHZ,CDCl$_3$)δ127.0,128.6,126.8(Ar-C),δ127.5,135.1 (C=C), δ176.3 (O= C), δ67.1,73,71.3,72.9 (O-CH),δ66.8,75.2,72.9(O-CH$_2$),δ64.6(O-CH$_2$).

**Compound III:** (4Z)-2-phenyl-4-(3,4,5,6-tetrahydroxy-2-oxohexylidene)-1,3oxazolidine-5-one: Yield 75 %, mp. 160-200 °C; FTIR (γ max, cm$^{-1}$) 1543 (-C=C bonding), 3333 (O-H
bonding), 1739 (=C-O bonding), 3590 (-NH-bonding); 1H NMR: (400MHZ,CDCl3) δ 3.69-4.16(O-CH3), δ6.38-7.19(m,5H,J8.12Ar-H)δ2.0(-NH), δ2.0(-OH)δ1.93(CH3); 13CNMR: (400 MHZ, CDCl3) δ127.0,128.6,126.8 (Ar-C), δ148.7(C=C), δ176.3,197.0 (O=O), δ71.0, 79.3 (O-CH).

**Compound IV**:

(4z)-2phenyl-4-\{4,5,6trihydroxy2(hydroxymethyl)oxan3yl\}oxypentane1,2,3,4tetrol)-1,3 oxazolidine-5-one: Yield 78 %, mp. 150-185 °C; FTIR (γ max, cm$^{-1}$) 1555 (-C=C bonding ), 1614 (=C-O bonding), 3336 (=O-H bonding), 3690 (-NH bonding)

1H NMR: (400MHZ, CDCl3) δ 3.40-3.98(O-CH3), δ6.38-7.19(m,5H,J8.12Ar-H)δ2.0(-NH), δ2.0(-OH)δ3.56,3.81(O-CH2); 13CNMR: (400MHZ, CDCl3) δ127.0,128.6,126.8 (Ar-C), δ124.7,148.7(=C=C), δ176.3,197.0 (O=O), δ71.0, 79.3 (O-CH).

**Compound V**:

(4Z)-4-(4-{[3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]methyl}-2,3,5,6-tetrahydroxyhexyldene)-2-phenyl-1,3-oxazolidine-5-one

Yield 78 %, mp. 150-185 °C; FTIR (γ max, cm$^{-1}$) 1555 (-C=C bonding ), 1614 (=C-O bonding), 3336 (=O-H bonding), 3690 (-NH bonding) 1H NMR: (400MHZ, CDCl3) δ 3.38-3.98(O-CH2), δ6.38-7.19(m,5H,J8.12Ar-H)δ2.0(-NH), δ2.0(-OH); 13CNMR: (400MHZ, CDCl3) δ127.0,128.6,126.8 (Ar-C), δ127.5,135.1 (C=C), δ176.3 (O= C), δ73.6,71.0,74.1,73.4 (O-CH), δ62.3,64.8 (O-CH2).

3. **INSILICO EVALUATION**

**MOLINSPIRATION**: Insilco evaluation was carried out by Molinspiration Cheminformatics program. Molinspiration Cheminformatics virtual screening methodology can efficiently separate drug-likeness from inactive structures. Molinspiration, web based software was used to obtain parameter such as drug likeness and bioactive score. The results were shown in Table 2 and 3.

**Online Chemical Modelling**: The Online Chemical Modelling Environment is a web-based platform that aims to automate and simplify the typical steps required for QSAR modelling. The platform consists of two major subsystems: the database of experimental measurements and the modelling framework. A user-contributed database contains a set of tools for easy input, search and modification of thousands of records. The results were shown in Table 4.
4. Invitro Evaluation

4.1 Antibacterial activity\textsuperscript{[11]}: All the synthesized compounds I-V were examined for \textit{invitro} antibacterial activity against an assortment of two gram-positive bacteria \textit{Staphylococcus aureusNCIM2901, Bacillus subtilis MTCC 441} and two Gram-negative bacteria \textit{Pseudomonas aeruginosa} and \textit{Proteus vulgaris MTCC 1771} by diffusion method. Fluconazole were used as an internal standard.

Nutrient agar (High media) was dissolved and distributed in 25ml quantities in boiling tubes and were sterilized in an autoclave at 121°C (15 lbs / sq.in) for 20 minutes. The medium was inoculated at one percent level using 18 hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for above 30min. In a size of 4 inches petridishes, five cups of 8mm diameter at equal distance were made in each plate. In the cups the test solutions of different concentrations were added and in another plate cups were made for standard and control. The plates thus prepared were left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37°C the plates were examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition measured and recorded. The results were represented in \textbf{Table 5}.

4.2. Antifungal activity\textsuperscript{[12]}: The antifungal activity of compounds was assayed against three different fungal strains \textit{Aspergillus niger MTCC 282, Penicillium chrysogenum MTCC5108}.

Potato dextrose agar\textsuperscript{[13]} (Hi-media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and were sterilized in an autoclave at 121 °C (15lbs/sq.in) for 20 minutes. The medium was inoculated with 1% 18hr old cultures of organisms aseptically in to sterile petridish and allowed to set at room temperature for about 30 minutes. At a size of 4 inches petridish 5 cups of 8mm diameter at equal distance were made in a petriplate with a sterile borer. The solutions of test concentrations (250μg/ml, 200μg/ml, 150μg/ml and 100μg/ml) and standard were added to respective cups aseptically and labelled accordingly. DMF as control did not show any inhibition. The plates were left for 90 minutes in refrigerator for diffusion and incubated for 72 hrs at 37°C ± 1°C. The plates were examined for inhibition zones. Fluconazole was used as standard. The experiments were performed in duplicate and the average diameters of the zones of inhibitions were summarized in \textbf{Table 6}. 
5. RESULTS AND DISCUSSION

The compounds were checked for their bioactive scores using online software, we found that they are having better bioactive scores against enzyme and protease inhibition. Based on this we have further under gone for anti microbial activities. All the compounds showed significant antibacterial and antifungal activity but more active towards gram positive bacteria. Among these the compound IV exhibited good anti bacterial and anti fungal activity. The molecules were also tested for Cytochrome P-450 inhibition and were found as non inhibitors.

<p>| Table: 1 |</p>
<table>
<thead>
<tr>
<th>Code</th>
<th>Compound</th>
<th>M.F</th>
<th>M.W</th>
<th>MP(°c)</th>
<th>%Yield</th>
<th>C%</th>
<th>H%</th>
<th>O%</th>
<th>N%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(4Z)-4-(2,3,4,5,6-pentahydroxyhexylidene)-2-phenyl 1,3oxazolidine-5-one</td>
<td>C_{15}H_{19}NO_{7}</td>
<td>339.44</td>
<td>140-180</td>
<td>88</td>
<td>55.38</td>
<td>5.84</td>
<td>34.43</td>
<td>4.31</td>
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<tr>
<td>II</td>
<td>(4Z)-2-phenyl-4-(2,3,4,5-tetrahydroxypentylidene)-1,3-oxazolidine-5-one</td>
<td>C_{14}H_{17}NO_{6}</td>
<td>309.318</td>
<td>150-170</td>
<td>75</td>
<td>56.94</td>
<td>5.80</td>
<td>32.51</td>
<td>4.74</td>
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<tr>
<td>III</td>
<td>(4Z)-2-phenyl-4-(3,4,5,6-tetrahydroxy-2-oxohexylidene)-1,3 oxazolidine-5-one</td>
<td>C_{15}H_{17}NO_{7}</td>
<td>337.328</td>
<td>160-200</td>
<td>75</td>
<td>55.73</td>
<td>5.30</td>
<td>34.64</td>
<td>4.33</td>
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<tr>
<td>IV</td>
<td>(4Z)-2phenyl-4-[[4,5,6trihydroxy2(hydroxymethyl)oxan3yl oxyl]pentane1,2,3,4tetrol] 1,3 oxazolidine-5-one</td>
<td>C_{20}H_{27}NO_{12}</td>
<td>472.431</td>
<td>150-185</td>
<td>78</td>
<td>50.74</td>
<td>5.75</td>
<td>40.55</td>
<td>2.46</td>
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<tr>
<td>V</td>
<td>(4Z)-4-(4-{[3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]methyl}-2,3,5,6-tetrahydroxyhexylidene)-2-phenyl-1,3-oxazolidine-5-one</td>
<td>C_{22}H_{31}NO_{11}</td>
<td>485.48</td>
<td>170-180</td>
<td>80</td>
<td>54.43</td>
<td>6.414</td>
<td>36.25</td>
<td>2.89</td>
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### Table 2: GENERAL PROPERTIES

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<th>Compound</th>
<th>Milogp</th>
<th>TPSA</th>
<th>natoms</th>
<th>MW</th>
<th>n ON</th>
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<th>Nviolations</th>
<th>n rot b</th>
<th>Volume</th>
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<td>5</td>
<td>0</td>
<td>5</td>
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<td>10</td>
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### Table 3: BIOACTIVITY SCORE

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<th>Compound</th>
<th>GPCR ligand</th>
<th>Ion channel inhibitor</th>
<th>Kinase inhibitor</th>
<th>Nuclear receptor ligand</th>
<th>Protease inhibitor</th>
<th>Enzyme inhibition</th>
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<tbody>
<tr>
<td>I</td>
<td>-0.18</td>
<td>-0.08</td>
<td>-0.32</td>
<td>0.02</td>
<td>-0.04</td>
<td>0.07</td>
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### Table 4:

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<th>COMPOUND</th>
<th>AQ.SOL.UBILITY</th>
<th>LOG IGC50</th>
<th>AMES</th>
<th>CYP3 A4</th>
<th>CYP2 D6</th>
<th>CYP2 C19</th>
<th>CYP2 C9</th>
<th>CYP2 C19</th>
<th>CYP1 A2</th>
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<td>I.</td>
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<td>_</td>
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<td>_</td>
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<tr>
<td>II.</td>
<td>1.5</td>
<td>0.09</td>
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<td>_</td>
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<tr>
<td>III.</td>
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<td>_</td>
<td>_</td>
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<td>IV.</td>
<td>0.62</td>
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<td>_</td>
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<tr>
<td>V.</td>
<td>0.62</td>
<td>-0.1</td>
<td>Inactive</td>
<td>_</td>
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</table>

+ inhibitor, _ noninhibitor

Aq-aqueous, IGC 50-Environmental toxicity

### Table 5: Antibacterial Activity

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<tr>
<th>Compound</th>
<th>B.subtilis</th>
<th>E.coli</th>
<th>P.aeroginosa</th>
<th>S.aureus</th>
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<tbody>
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<td>I</td>
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<td>14</td>
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<tr>
<td>IV</td>
<td>20</td>
<td>20</td>
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<tr>
<td>V</td>
<td>15</td>
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</table>

Standard
Table 6: Antifungal Activity

<table>
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<th>Aspergillus niger</th>
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<tbody>
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<tr>
<td>II</td>
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<td>V</td>
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<tr>
<td>Standard</td>
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</tbody>
</table>

Hippuric acid

Polyhydroxylated aldehyde or ketone

4-substituted polyhydroxylidene 2-phenl-oxazolidine-5-one

Scheme 1:

General synthesis of 4-substituted polyhydroxylidene 2-phenl-oxazolidine-5-one
6. ACKNOWLEDGEMENTS
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7. REFERENCES


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