Synthesis of 2-[4-(substituted benzylidene)-5-Oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione Derivatives as Novel Potential Antimicrobial Agents

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ABSTRACT
In the present study, a series of new substituted oxazoline derivatives (4a-4h) were synthesized by the Erlenmeyer condensation of phthaloylglycylglycine with different aldehydes in the presence of anhydrous sodium acetate and acetic anhydride. The structure of newly-synthesized compounds were evaluated by elemental analyses and spectral (UV-Visible, IR, NMR, Mass) studies. All the synthesized derivatives were evaluated for their antimicrobial activity. Preliminary pharmacological evaluation revealed that the compound 4b, 4c, 4d, 4g and 4h showed better performance against Staphylococcus aureus, Staphylococcus epidermidis, Proteus mirabilis, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. The minimum inhibitory concentration (MIC) was determined for the target compounds as well as for standard drugs. Physicochemical similarity of the new derivatives with standard drugs was assessed by calculating from a set of 10 physicochemical parameters using software programs. The result demonstrated the potential and usefulness of developing novel oxazoline derivatives which would be effective against resistant and pathogenic bacterial and fungal strain.

Keywords: Oxazolone; Substituted benzylidene; Antibacterial activity; Antifungal activity
synthesized by the Erlenmeyer condensation of phthaloylglycylglycine with different aldehydes in the presence of anhydrous sodium acetate and acetic anhydride. Then the effects of these new novel oxazolone derivatives against resistant and pathogenic bacterial and fungal strain are examined.

**MATERIALS AND METHODS**

The melting points were measured, using digital melting point apparatus (Flora; Perfiz India) and were found to be uncorrected. The purity of compounds was checked by TLC. The $\lambda_{\text{max}}$ was calculated using double beam UV-Visible Shimadzu spectrophotometer. IR spectra ($\nu$, cm$^{-1}$) were recorded on Shimadzu FTIR 1800S spectrometer following nujol method. $^1$H NMR (δ, ppm) spectra were recorded by DMSO-D$_6$ solutions and TMS as an internal standard on a BRUKER AVANCE-II 400 NMR spectrometer. For mass spectra, solutions were made in HPLC grade methanol. Elemental analysis was performed on an ECS 4010 Elemental Combustion System. Structural similarity studies between standard drugs (cefixime, tosufloxacin tosylate) and targeted compounds were performed by Chem3D Ultra, molecular modelling software.

**Chemistry**

A new series of synthetic oxazolone were prepared from commercially available glycine and phthalic anhydride reaction product phthaloylglycylglycine. The synthetic route is outlined in Scheme 1, the titled compound 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4a-4h) was synthesized by reacting phthaloylglycylglycine with suitable aldehydes in presence of anhydrous sodium acetate and acetic anhydride in high yields [5,15,17]. The purity of the compounds was checked by TLC, elemental analyses and characterized by spectral data. The physical data and elemental analysis of the synthesized compounds are summarized in Table 1.

**Synthesis of Phthaloylglycine [1]**

A mixture of phthalic anhydride (9 g, 0.06 mol) and glycine (4.5 g, 0.06 mol) was fused in a boiling tube at 160-190°C (oil bath) for 20-30 min. The product obtained was cooled at room temperature and crystallized from water to get phthaloylglycine [1].

**Synthesis of phthaloylglycine chloride [2]**

A mixture of phthaloylglycine [1] (8 g, 0.039 mol) and thionyl chloride (16 mL) was refluxed gently for 30 min in a round bottom flask fitted with reflux condenser having a drying tube on the top. Excess thionyl chloride was removed by distillation under reduced pressure. The residual phthaloylglycine chloride [2] was crystallized from petroleum ether.

**Synthesis of Phthaloylglycylglycine [3]**

A solution of phthaloylglycine chloride (2) (4.2 g, 0.09 mol) in dioxane (25 mL) was added to the stirred suspension of glycine (1.55 g) and magnesium oxide (1.1 g) in water (50 mL). The temperature was kept at 4-5°C during addition, stirring continued for 10-15 min at 25°C and then acidified with hydrochloric acid. The mixture was cooled, the separated product was filtered, washed with cold water and crystallised from hot water to get phthaloylglycylglycine [3].

### Table 1. Physical data of the compounds (4a-4h)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Molecular Formula</th>
<th>Molecular weight (g/mol)</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$R_f$ Value</th>
<th>Calculated (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>H</td>
<td>C$<em>{19}$H$</em>{12}$N$_2$O$_4$</td>
<td>332.31</td>
<td>89.92</td>
<td>197-200</td>
<td>298</td>
<td>0.52</td>
<td>68.67 (68.65)</td>
</tr>
<tr>
<td>4b</td>
<td>4-OH, 3-OC$<em>{2}$H$</em>{4}$</td>
<td>C$<em>{20}$H$</em>{14}$N$_2$O$_2$</td>
<td>378.34</td>
<td>88.75</td>
<td>135-138</td>
<td>328</td>
<td>0.63</td>
<td>63.49 (63.51)</td>
</tr>
<tr>
<td>4c</td>
<td>4-CH$_{3}$</td>
<td>C$<em>{20}$H$</em>{14}$N$_2$O$_2$</td>
<td>346.34</td>
<td>80.91</td>
<td>138-141</td>
<td>238</td>
<td>0.52</td>
<td>69.36 (70.23)</td>
</tr>
<tr>
<td>4d</td>
<td>4-NO$_2$</td>
<td>C$<em>{19}$H$</em>{11}$N$_2$O$_2$</td>
<td>377.31</td>
<td>79.84</td>
<td>85-89</td>
<td>265</td>
<td>0.50</td>
<td>60.48 (60.51)</td>
</tr>
<tr>
<td>4e</td>
<td>3-NO$_2$</td>
<td>C$<em>{19}$H$</em>{11}$N$_2$O$_2$</td>
<td>377.31</td>
<td>80.92</td>
<td>60-63</td>
<td>297</td>
<td>0.53</td>
<td>60.48 (60.51)</td>
</tr>
<tr>
<td>4f</td>
<td>4-OH</td>
<td>C$<em>{19}$H$</em>{11}$N$_2$O$_2$</td>
<td>348.31</td>
<td>81.87</td>
<td>146-149</td>
<td>320</td>
<td>0.51</td>
<td>65.52 (66.15)</td>
</tr>
<tr>
<td>4g</td>
<td>2-OH</td>
<td>C$<em>{19}$H$</em>{11}$N$_2$O$_2$</td>
<td>348.31</td>
<td>82.92</td>
<td>120-123</td>
<td>220</td>
<td>0.49</td>
<td>65.52 (65.22)</td>
</tr>
<tr>
<td>4h</td>
<td>4-Cl</td>
<td>C$<em>{19}$H$</em>{11}$ClNO$_2$</td>
<td>366.75</td>
<td>82.90</td>
<td>160-163</td>
<td>299</td>
<td>0.47</td>
<td>62.22 (61.95)</td>
</tr>
</tbody>
</table>

(Mobile phase: chloroform: methanol (9:1, v/v)
(Comd.: compound, Mol.: molecular, m.p.: melting point)
Synthesis of 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione [4]

An equimolar mixture of phthaloylglycglycine and suitable aldehyde (15 mmol) in freshly-distilled acetic anhydride (10 cm³) containing fused anhydrous sodium acetate (1.2 g) was heated on a steam bath for 4 hours then cooled, yield the formation of yellow solid mass, now filtered off and washed with light petroleum (40–60°C). It was well dried, triturated with cold saturated sodium carbonate solution and again filtered. Then after washing with water, dried and recrystallized from suitable solvent to yield the compounds (4a-4h). All new titled compounds (4a-4h) were synthesized following the same procedure (Scheme I).

Synthesis Protocol

Synthesis of derivatives (1-3) was carried out by following the reported literature procedure [15]

Phthaloylglycine [1]

Yield 98%; m.p. 188–190°C, λmax 220 nm; IR (υ, cm⁻¹): 3558 (O–H stretch), 3050, 2980 (C–H stretch), 1801,1716 (phthalyl C=O), 1700 (carbonyl stretch),1527(C=C stretch), 671; ¹H NMR (400 MHz, DMSO; δ ppm): 10.69 (s, 1H, OH), 7.87 (m, 2H, ArH), 7.80 (m, 2H, ArH), 4.34 (s, 2H, CH₂).

Phthaloylglycine chloride [2]

Yield 97.5%; m.p. 133–135°C, λmax 224 nm; IR (υ, cm⁻¹): 3045, 2980 (C–H stretch),1782, 1710 (phthalyl C=O), 1705 (carbonyl stretch),1610 (C=C stretch), 790; ¹H NMR (400 MHz, DMSO; δ ppm): 8.01 (m, 2H, ArH), 7.74 (m, 2H, ArH), 4.92 (s, 2H, CH₂).

Phthaloylglycine [3]

Yield 93.5%; m.p. 193–195°C, λmax 248 nm; IR (υ, cm⁻¹): 3464 (O–H stretch), 3280 (N–H stretch), 3103, 2900 (C–H stretch), 1801,1734 (phthalyl C=O), 1711 (carbonyl stretch), 1642 (peptide stretch), 1622 (N–H bend), 1510 (C=C stretch), 746; ¹H NMR (400 MHz, DMSO; δ ppm): 10.27 (s, 1H, OH), 8.13 (m, 2H, ArH), 7.94 (m, 2H, ArH), 4.83 (s, 2H, CH₂), 4.18 (s, 2H, CH₂).

Synthesis of 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione derivatives (4a-4h) were carried out using the literature procedure (Madkour, 2002) and their spectral data are as given below:

2-[4-Benzylidene-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4a)

IR (υ, cm⁻¹): 3021, 2920 (C–H stretch), 1772, 1703 (phthalyl C=O),1767 (lactone C=O stretch),1685 (C=N stretch), 1620 (C=C stretch), 1125 (C=C–O stretch), 719; ¹H NMR (400 MHz, DMSO; δ ppm): 7.92 (m, 2H, ArH), 7.87 (m, 2H, ArH), 7.74 (m, 2H, ArH), 7.62 (m, 2H, ArH), 7.51 (m, 1H, ArH), 7.42 (s, 1H, =CH), 4.83 (s,2H, CH₂); MS: m/z 332.08, 333.08 (M+1), 334.09 (M+2).

2-[4-(Hydroxy-3-methoxy-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4b)

IR (cm⁻¹): 3500 (O–H stretch), 3066, 2890 (C–H stretch), 1767, 1718 (phthalyl C=O),1761 (lactone C=O stretch),1589 (C=N), 1589, 1517 (C=C stretch),1282, 1120 (C=C–O stretch), 893; ¹H NMR (400 MHz, DMSO; δ ppm): 7.93 (m, 2H, ArH), 7.87 (m, 2H, ArH), 7.74 (m, 2H, ArH), 7.62 (m, 2H, ArH), 7.51(m, 1H, ArH) 7.42 (s, 1H, =CH), 5.50 (s, 1H, OH), 4.83 (s, 2H, CH₂), 3.68 (s, 3H, OCH₃); MS: m/z 378.09, 379.09 (M+1), 380.09 (M+2).

2-[4-(4-Methyl-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4c)

IR (cm⁻¹): 3062, 2962 (C–H stretch), 1834, 1701 (phthalyl C=O), 1785 (lactone C=O stretch), 1627 (C=N), 1543 (C=C–O stretch), 734; ¹H NMR (400 MHz, DMSO; δ ppm): 7.90 (m, 2H, ArH), 7.86 (m, 2H, ArH), 7.82 (d, 2H, ArH), 7.57 (d, 2H, ArH), 7.24 (s, 1H, =CH), 4.85 (s,2H, CH₂), 3.10 (s,3H, CH₃); MS: m/z 346.10, 347.10 (M+1), 348.10 (M+2).

2-[4-(4-Nitro-benzylidene)]-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4d)

IR (cm⁻¹): 3010, 2974 (C–H stretch), 1834, 1766 (phthalyl C=O), 1790 (lactone C=O stretch), 1627 (C=N stretch), 1548 (C=C–O stretch ), 1458 (asymmetric N=O stretch), 1375 (symmetric N=O stretch), 1107 (C–O–C stretch); ¹H NMR (400 MHz, DMSO; δ ppm): 8.58 (d, 2H, ArH), 8.32 (d, 2H, ArH), 7.91 (m,2H, ArH), 7.84 (s,1H, =CH), 7.85 (t, 2H, ArH), 4.27 (s,2H, CH₂); MS: m/z 377.06, 378.07 (M+1), 379.07 (M+2).

2-[4-(3-Nitro-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4e)

IR (cm⁻¹): 3057, 2900 (C–H stretch), 1785, 1697 (phthalyl C=O), 1790 (lactone C=O stretch), 1639 (C=N stretch), 1627 (C=C–O stretch), 1475 (asymmetric N=O stretch), 1300 (symmetric N=O stretch),1049 (C–O–C stretch); ¹H NMR (400 MHz, DMSO; δ ppm): 8.60 (m, 1H, ArH), 8.39 (d, 1H, ArH), 8.10 (d, 1H, ArH), 7.92 (m, 2H, ArH), 7.82 (m, 1H, ArH), 7.75 (m, 1H, ArH), 7.32 (s,1H, =CH), 4.77 (s, 2H, CH₂); MS: m/z 377.06, 378.07 (M+1), 379.07 (M+2).

2-[4-(4-Hydroxy-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4f)

IR (cm⁻¹): 3406 (O–H stretch), 3032 (C–H stretch), 1791, 1714 (phthalyl C=O), 1777 (lactone C=O stretch), 1566 (C=N stretch), 1519 (C=C–O stretch), 1199 (C=C–O stretch); ¹H NMR (400 MHz, DMSO; δ ppm): 7.94 (m, 2H, ArH), 7.81 (m, 2H, ArH), 7.78 (d, 2H, ArH), 7.18 (s,1H,=CH), 6.65 (d, 2H, ArH), 4.87 (s, 1H, OH), 3.72 (s,2H, CH₂); MS: m/z 348.07, 349.08 (M+1), 350.08 (M+2).
2-[4-(2-Hydroxy-benzylidene)-5-oxo-4,5-dihydrooxazol-2-ylmethyl]-isoindole-1,3-dione (4g)

IR (cm\(^{-1}\)): 3460 (O–H stretch), 3000 (C–H stretch), 1808, 1728 (phthalyl C=O), 1765 (lactone C=O stretch), 1643 (C=N stretch), 1625 (C=C stretch), 1056 (C–O–C stretch); \(^1\)H NMR (400 MHz, DMSO; \(6\) ppm): 7.87 (m, 2H, ArH), 7.80 (m, 2H, ArH), 7.75 (d, 1H, ArH), 7.50 (s, 1H, =CH), 7.42 (m, 1H, ArH), 7.17 (m, 1H, ArH), 7.10 (d, 1H, ArH), 5.44 (s, 1H, OH), 3.68 (s, 2H, CH\(_2\)); MS: m/z 348.07, 349.08 (M+1), 350.08 (M+2).

Assessment of structural similarity of test compounds with standard drugs

Assessment of structural similarity of target compounds to standard drugs involves the study of physico-chemical and steric similarity between the standard drugs and new analogues for effective binding with receptors. The usual approach to assess similarity is to examine resemblance between molecular properties of target compounds with standard drugs. Therefore, we calculated a number of parameters for test compounds (4a-4h) using Chem3D and compared them to the values obtained for target compounds (Chem3D, version 10). Cefixime and tosufloxacin tosylate were used as the standard drugs for assessment of structural similarity.

Various set of parameters were used for calculations, given in Table 2. The distance \(d_i\) of a particular target compound \(i\) can be presented as:

\[
d_i = \sum_{j=1}^{N} (1 - X_{ij}/X_{i,\text{standard}})^2 / n
\]

Where, 
\(X_{ij}\) is value of molecular parameters \(i\) for compound \(j\).
\(X_{i,\text{standard}}\) is the value of the same molecular parameter \(i\) for standard drug.
\(n\) is the total number of considered molecular parameters.

The similarity of the compounds can be calculated as:

Percentage similarity\(= (1-R) \times 100\)

Where,
\(R\) is quadratic mean also known as the root mean square and \(R\) can be calculated as:

\(R = \sqrt{\sum d_i^2}\)

All the synthesized compounds showed good percentage similarity when compared with standard drugs (Table 3).
**Table 3. Assessment of structural similarities of tested compounds 4a-4h with standard drugs**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Percent similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cefixime</td>
</tr>
<tr>
<td>1.</td>
<td>4a</td>
<td>95.74</td>
</tr>
<tr>
<td>2.</td>
<td>4b</td>
<td>96.72</td>
</tr>
<tr>
<td>3.</td>
<td>4c</td>
<td>81.08</td>
</tr>
<tr>
<td>4.</td>
<td>4d</td>
<td>99.7</td>
</tr>
<tr>
<td>5.</td>
<td>4e</td>
<td>98.73</td>
</tr>
<tr>
<td>6.</td>
<td>4f</td>
<td>75.37</td>
</tr>
<tr>
<td>7.</td>
<td>4g</td>
<td>73.96</td>
</tr>
<tr>
<td>8.</td>
<td>4h</td>
<td>80.79</td>
</tr>
</tbody>
</table>

**Pharmacological activity**

**Procedure for determination of antibacterial activity**

The newly-synthesized oxazolone compounds (4a-4h) were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis* and *Pseudomonas aeruginosa* by cup-plate method. Nutrient agar media was prepared by melting agar on water bath and then cooled it to 45°C with gentle shaking, to bring about uniform cooling. Nutrient agar media was inoculated with fresh prepared culture media and mixed by gentle shaking before pouring on a sterilized petri dish. Poured the inoculated media into petri dish and allowed to set for some time. Cups were made by punching the agar surface with a sterile cork bore (8 mm) and the punched part of the agar media was removed by scooping. Solutions containing 12.5, 25, 50, 100, 200, 400, 800 and 1600 μg/mL of the test compound, were added to each cup. Dimethyl formamide (DMF) was used as solvent, to prepare the stock solution. Amoxicillin and cefixime were taken as positive control and DMF was taken as blank (did not show any activity against test organism). The plates were incubated at 37°C for 24 h and the results were recorded. The zones of inhibition of the microbial growth produced by different concentration of test compounds (50 μl/disc) were measured in millimetres (mm) [18].

**Procedure for the determination of the antifungal activity**

The *in vitro* antifungal activity of test compounds was evaluated using *Candida albicans* and *Aspergillus niger* strains, by cup plate technique, in Saboraud’s dextrose broth culture media. The stock solution of test compounds were prepared in dimethyl formamide (DMF) and the serial dilution of test compounds were carried out for obtaining the concentration, ranging from 12.5, 25, 50, 100, 200, 400, 800 and 1600 μg/mL. Fluconazole was taken as positive control and DMF was taken as blank (did not show any activity against test organism). The test compounds at various concentrations were added to the cup made by puncturing the agar dextrose media by sterilised cork bore. The plates were incubated at 37°C for 48 h. The zones of inhibition of the microbial growth (50 μl/disc) produced by different concentration of test compounds were measured in millimetres (mm) [17-19]. The results of minimum inhibitory concentration of the compounds against various pathogenic microorganisms were recorded after incubation at 37°C for 48 h as listed in Table 4. It was determined that solvent had no antimicrobial activity against any of the test microorganisms.

**RESULTS AND DISCUSSION**

In this work, total eight derivatives of oxazolone containing 4-substituted benzylidene (4a-4h) were prepared by base-induced cycloaddition of phthaloylglycylglycine [2] to aldehyde in a solvent such as acetic anhydride with anhydrous sodium acetate in good yield (Scheme 1). The structures of the synthesized compounds were supported using different spectroscopic methods like UV, IR, ^1^H NMR, mass and...
 elemental analysis. All the synthesized compounds were also evaluated for their antimicrobial activity. Antibacterial activity of test compounds (4a-4h) were determined using four different strains *Staphylococcus aureus* (Gram positive), *Staphylococcus epidermidis* (Gram positive), *Proteus mirabilis* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative), by cup-plate method. The antifungal activity was evaluated using *Candida albicans* and *Aspergillus niger* strains, by broth dilution method. Stock solutions of test compounds were prepared in dimethyl formamide solution. Antimicrobial activity was carried out at eight different concentrations (12.5, 25, 50, 100, 200, 400, 800 and 1600 μg/mL). Antibacterial activity of test compounds was compared with two different standard compounds (amoxicillin and cefixime) and the antifungal activity were compared with standard (fluconazole). Assessment of structural similarities of all the synthesized compounds showed good percentage similarity when compared with standard drugs (Table 3). The antimicrobial activity has been shown in Table 4 and represented in terms of minimum inhibitory concentrations (MICs, μg/mL). From all the compounds 4b, 4c, 4d, 4g and 4h have shown significant antibacterial activity against *Staphylococcus epidermidis* and compound 4d has shown highest activity against *Staphylococcus aureus* (Fig 1). The entire newly synthesized compound (4a-4h) exhibited considerable activity against *Pseudomonas aeruginosa* and compound 4c showed highest activity against *Proteus mirabilis*. Compound 4c was the most potent antibacterial and antifungal activities among the compounds. The entire screened compound (4a-4h) exhibited excellent antifungal activity against *Aspergillus niger* (Fig 2), when compared to standard drug (fluconazole).

Overall, a series of oxazolone derivatives were synthesized for their antimicrobial activity. Minimum inhibitory concentrations (MICs, μg/mL) of synthesized compounds were determined on different
microorganisms using amoxicillin, cefixime and fluconazole as reference drugs. From the activity (MICs) data it was concluded that all the compounds (4a-4h) showed antimicrobial activity against bacteria including Staphylococcus aureus, Staphylococcus epidermidis, Proteus mirabilis, Pseudomonas aeruginosa and fungus including Candida albicans, Aspergillus niger. Amongst all compounds 4b, 4c, 4d, 4g and 4h have shown moderate antibacterial activity against Staphylococcus epidermidis. Compound 4c and compound 4d showed highest activity against Proteus mirabilis and Staphylococcus aureus respectively. All the tested compounds (4a-4h) showed significant antibacterial activity against Pseudomonas aeruginosa and potent antifungal activity against Aspergillus niger respectively, when compared to the reference drugs. So, the significant antimicrobial activity of compound may be due to the presence of oxazolone moiety in addition to benzylidene nucleus.

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