SYNTHESIS AND ANTIMICROBIAL EVALUATION OF OXAZOLIDINONES DERIVATIVES

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ABSTRACT
In order to develop relatively small molecules as pharmacologically active molecules, a series of novel oxazolidinones having benzothiazinen and their derivatives were synthesized, and characterized by IR, 1H NMR and Mass spectral studies. Oxazolidinones were prepared from R-glycidylbutarate and Para bromo aniline. Various substituted oxazolidinones benzothiazinen were prepared by simple reflux in the presence of acetonitrile. Treatment of these oxazolidinones benzo thiazinen deravatives with methanesulfonyl gives its sulphonates derivatives on further treatment with sodium azide and tri phenyl phosphine in acetic anhydride to give its acetamide derivatives. Further the synthesized compounds were evaluated for antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and antifungal activity against, Candida albicans and Aspergillus niger.

Keywords: Oxazolidinones, Benzothiaizinen, Antibacterial, Antifungal, Antimicrobial.

INTRODUCTION
Oxazolidinone are well known five membered nitrogen and oxygen containing compounds. These have been reported to possess biological activities such as antibacterial activity1. The emergence of bacterial resistance to the antibiotics poses a serious concern for medical professionals during the last decade 2. In particular multi-drug-resistant Gram-positive bacteria including methicillin-resistant Staphylococcus aureus (MRSA) 3 and Staphylococcus epidermidis (MRSE), and vancomycin-resistant Enterococci (VRE) are of major concern.4
Oxazolidinones, a new class of synthetic antibacterial agents, exhibit activity against a large number of Gram-positive organisms. Many oxazolidinone derivatives are in clinical use such as linezolid, eperezolid as antimicrobial agent \(^5\) Linezolid is the first oxazolidinone approved for the treatment of Gram-positive bacterial infections in humans \(^6\). Since Linezolid, the many attractive traits of oxazolidinone series have encouraged further work in this area, and also the literature reveals extensive chemical programs exist \(^7\). At present, most efforts are focused on substituted phenyl oxazolidinones. Benzothiazinen are associated with diverse biological and pharmacological activities like antimicrobial \(^8\), anti-inflammatory \(^9\).

By considering the above facts and their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some new chemical entities incorporating the two active pharmacophores in a single molecular framework and to evaluate their biological activities.

The incorporation of two moieties increases biological activity of both and thus it was of value to synthesize some new heterocyclic derivatives having two moieties in the same molecules.

The synthesized compounds were screened for, antibacterial and antifungal activities

**MATERIAL AND METHODS**

All the chemicals were analytical grade; all substituted Bezo thazine ,Triethylamine,Methane sulfonate,Dichloro methane, Oxazolidene , Hydrochloric acid, Glacial acetic acid ,Tri phenyl phosphine. Sodiumazide General procedure to synthesis of oxazolidinones having benzo thiazinen moieties and its derivatives

**The synthesis consists of the four major steps which are as follows:**

1. Synthesis of 5-(hydroxymethyl)-3-(4-(4-methyl-3,4dihydro-2H-benzo[b][1,4]thiazin-3-ylamino) phenyl) oxazolidin-2-one derivatives from benthiazine amines derivatives and (3-(4-fluorophenyl)methylene oxazolidine-5yl by simple reflux for three hours using acetonitrile solvent. \(^10\)

2. Conversion of 5-(hydroxymethyl)-3-(4-(4-methyl-3,4dihydro-2H-benzo[b][1,4]thiazin-3-ylamino) phenyl) oxazolidin-2-one derivatives to its methane sulfonate derivatives by using triethylamine in DCM later methanesulfonyl chloride added drop wise under vigorous stirring. Stirring for an additional 10–15 min completed the reaction\(^11\)
3.3-(4-(3,4dihydro-2h-benzo[b] [1,4] thiazin-3-ylamino) phenyl)oxazolidin-5-yl) methyl methane sulfonate derivatives was convertated to azido derivatives by treating with sodiumazide in N,N-dimethyl formamide (DMF).^1^n

4.5-(Azidomethyl)-3-(4-(4-Methyl-3,4dihydro-2h-Benzof][1,4]Thiazin3ylamino)Phenyl) Oxazolidin -2-one was convertated to its acetamide derivatives by treating with tri phenyl phosphine and hydrochloric acid later extracted with AcOEt.^1^n

![General Scheme of synthesis](image)

R= p-BrC₆H₅, p-ClC₆H₅, p-FC₆H₅, p-OCH₃C₆H₅, p-CH₃C₆H₅, C₆H₅, o-BrC₆H₅, o-ClC₆H₅, p-FC₆H₅, o- OCH₃C₆H₅

All reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystallization.

All the synthesised compound was purified by TLC method and characterised by IR, ^1^H NMR and mass spectral method. IR was recorded in bruker alpha model using ATR. ^1^H NMR data were recorded in (DMSO) on a Avance 400MHZ spectrophotometer using TMS as an
internal standard. The mass spectra were recorded using LC-MS (SHIMADZU 2010-AT) under electro spray ionisation (ESI) technique.

**Compound Code PKSN4C:** N-((3-(4-((3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)(4-fluorophenyl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide

IR (KBr) cm\(^{-1}\): 3297 (N-H stretching), 3163 (aromatic C-H stretching), 2550 (S-H stretching), 1616 (aromatic C=C stretching), 824 (C-H deformation), 1124 (C-N stretching), 1698 (C=O stretching in Oxazolidine ring)

\(^1\)H NMR (\(\delta\)) in ppm: 8.08 (1H, s, NH), 7.38-8.08 (8H, d, Ar-H), 7.83-7.40 (4H, d, Ar-H in Benzothiazine ring), 3.31 (1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxaazolidine ring), 3.49 (1H, s, O-CH\(_3\)),

MS m/z (M\(^+\)) 493.

**Compound Code PKSN4E:** N-((3-(4-((3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)(p-tolyl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide

IR (KBr) cm\(^{-1}\): 3120 (N-H), 3046 (aromatic C-H stretching), 2650 (S-H stretching), 1583 (aromatic C=C stretching), 838 (aromatic C-H deformation), 1189 (C-N stretching), 1647 (C=O stretching in Oxazolidine),

\(^1\)H NMR (\(\delta\)) in ppm: 8.4 (1H, s, NH), 7.8-7.21 (8H, d, Ar-H), 7.66-7.11 (4H, d, Ar-H in Benzothiazine ring), 3.31 (1H, d, N-C-H in Benzothiazine ring), 4.55 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxaazolidine ring), 3.83 (1H, s, O-CH\(_3\)),

MS m/z (M\(^+\)) 489.

**Compound Code PKSN4J:** N-((3-(4-((4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)(pentan-2-yl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide

IR (KBr) cm\(^{-1}\): 3300 (N-H), 3160 (aromatic C-H stretching), 838 (aromatic C-H deformation), 614 (C-Cl stretching), 1433 (C-N stretching), 1676 (C=O stretching in Oxaozolidine),

\(^1\)H NMR (\(\delta\)) in ppm: 10.44 (1H, s, NH), 6.71-7.16 (9H, d, Ar-H), 6.84-7.14 (4H, d, Ar-H in Benzothiazine ring), 3.18 (1H, d, N-C-H in Benzothiazine ring), 3.55 (1H, d, S-C-H in Benzothiazine), 3.35 (1H, d, Oxaazolidine ring), 3.03 (1H, d, N-CH\(_3\) in Benzothiazine ring),

MS m/z (M\(^+\)) 505.
Anti-microbiological Evaluation

Antibacterial Activity and Antifungal Activity Studies

All the synthesized compounds were evaluated for the antimicrobial activity by cup-plate method. The following micro organisms were used to study the antibacterial activity of synthesized compound *B.subtilis, S.aureus, E.coli, P.aeruginosa* where as antifungal activities of synthesized compounds were studied against *Candida albicans* and *A.niger*. Amoxicillin and Fluconazole was taken as standard drug for the comparison of the activity of the synthesized compound for antibacterial and anti-fungal activity respectively.

RESULTS AND DISCUSSION

Table 2: (PHYSICAL DATA OF SYNTHESIZED COMPOUND)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Comp. Code</th>
<th>Mol. Formula</th>
<th>Mol.Wt</th>
<th>M.P°C</th>
<th>Rf value (solvent system)</th>
<th>Physical Nature</th>
<th>% Yield</th>
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<tbody>
<tr>
<td>1</td>
<td>PKSN4A</td>
<td>C_{26}H_{25}BrN_{4}O_{3}S</td>
<td>552</td>
<td>193-196</td>
<td>0.34 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>Brown Crystal</td>
<td>69</td>
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<tr>
<td>2</td>
<td>PKSN4B</td>
<td>C_{26}H_{25}ClN_{4}O_{3}S</td>
<td>508</td>
<td>186-189</td>
<td>0.40 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>Yellow Crystal</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>PKSN4C</td>
<td>C_{26}H_{25}FN_{4}O_{3}S</td>
<td>492</td>
<td>168-171</td>
<td>0.46 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>White Crystal</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>PKSN4D</td>
<td>C_{27}H_{28}N_{4}O_{4}S</td>
<td>504</td>
<td>174-177</td>
<td>0.25 C_{6}H_{5}CH_{3};CH_{3}OH (95:5)</td>
<td>Violet Crystal</td>
<td>74</td>
</tr>
<tr>
<td>5</td>
<td>PKSN4E</td>
<td>C_{27}H_{28}N_{4}O_{3}S</td>
<td>508</td>
<td>166-169</td>
<td>0.35 C_{6}H_{5}CH_{3};CH_{3}OH (95:5)</td>
<td>Pale Yellow Crystal</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>PKSN4F</td>
<td>C_{26}H_{26}N_{4}O_{3}S</td>
<td>474</td>
<td>156-159</td>
<td>0.31 C_{6}H_{5}CH_{3};CH_{3}OH (95:5)</td>
<td>Pale Yellow Crystal</td>
<td>66</td>
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<td>7</td>
<td>PKSN4G</td>
<td>C_{26}H_{25}BrN_{4}O_{3}S</td>
<td>552</td>
<td>194-197</td>
<td>0.48 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>Brown Crystal</td>
<td>75</td>
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<tr>
<td>8</td>
<td>PKSN4H</td>
<td>C_{26}H_{25}ClN_{4}O_{3}S</td>
<td>508</td>
<td>187-190</td>
<td>0.50 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>Yellow Crystal</td>
<td>81</td>
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<tr>
<td>9</td>
<td>PKSN4I</td>
<td>C_{26}H_{25}FN_{4}O_{3}S</td>
<td>492</td>
<td>186-189</td>
<td>0.50 C_{2}H_{5}COO:C_{6}H_{4} 20:80</td>
<td>White Crystal</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>PKSN4J</td>
<td>C_{27}H_{28}N_{4}O_{4}S</td>
<td>504</td>
<td>172-175</td>
<td>0.26 C_{6}H_{5}CH_{3};CH_{3}OH (95:5)</td>
<td>Violet Crystal</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 2: Antimicrobial Data Activity of Oxazolidinones having benzo thiazinen moieties

<table>
<thead>
<tr>
<th>Comp Code</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>C. albicans</th>
<th>A. niger</th>
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</thead>
<tbody>
<tr>
<td>PKSN4 A</td>
<td>5.3</td>
<td>6.3</td>
<td>5.3</td>
<td>5.4</td>
<td>21.3</td>
<td>40.1</td>
</tr>
<tr>
<td>PKSN4 B</td>
<td>16.1</td>
<td>9.2</td>
<td>10.3</td>
<td>11.2</td>
<td>23.3</td>
<td>21.5</td>
</tr>
<tr>
<td>PKSN4 C</td>
<td>13.3</td>
<td>10.1</td>
<td>11.8</td>
<td>19.6</td>
<td>21.2</td>
<td>19.4</td>
</tr>
<tr>
<td>PKSN4 D</td>
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<td>6.3</td>
<td>5.7</td>
<td>5.2</td>
<td>17.6</td>
<td>10.4</td>
</tr>
<tr>
<td>PKSN4 E</td>
<td>11.6</td>
<td>4.9</td>
<td>11.6</td>
<td>5.1</td>
<td>22.4</td>
<td>23.1</td>
</tr>
<tr>
<td>PKSN4 F</td>
<td>5.3</td>
<td>4.3</td>
<td>4.7</td>
<td>5.0</td>
<td>21.3</td>
<td>11.3</td>
</tr>
<tr>
<td>PKSN4 G</td>
<td>23.3</td>
<td>5.2</td>
<td>12.2</td>
<td>10.9</td>
<td>22.4</td>
<td>16.1</td>
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<tr>
<td>PKSN4 H</td>
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<td>42.3</td>
<td>41.2</td>
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<tr>
<td>PKSN4 I</td>
<td>10.9</td>
<td>5.6</td>
<td>5.4</td>
<td>5.1</td>
<td>22.1</td>
<td>23.4</td>
</tr>
<tr>
<td>PKSN4 J</td>
<td>12.3</td>
<td>11.4</td>
<td>11.2</td>
<td>14.4</td>
<td>17.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.6</td>
<td>8.3</td>
</tr>
</tbody>
</table>

The effect of synthesized Oxazolidinones having benzo thiazinen moieties has shown antibacterial and antifungal activity to certain extent. The results of these synthesized compounds are summarized in table 2. Among the screened compounds, PKSN4A, PKSN4D & PKSN4F have shown good antibacterial activity against gram +ve and gram -ve bacteria compared to the standard drug amoxicillin. Whereas PKSN4D and PKSN4J have shown significant antifungal activity against both C.albicans and A.niger compared to the standard drug Fluconazole.

CONCLUSION

Results of present study demonstrate that a new class of different Oxazolidinones having benzothiazinen moieties were synthesized and evaluated for anti-microbial activities. Among tested compounds PKSN4A, PKSN4D & PKSN4F moiety showed better antibacterial activity where as PKSN4D and PKSN4J moiety showed better anti-fungal activity. It can be concluded that Oxazolidinones having benzothiazinen moieties class of compounds certainly holds great promise towards the good activity leads in medicinal chemistry. A further study require more information concerning pharmacological activity is in progress.

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REFERENCES


