Synthesis and biological investigation of isoxazolo[4,5-e][1,2,4]triazine derivatives

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ABSTRACT

In present report we have successfully synthesized isoxazolo[4,5-e][1,2,4]triazine derivatives in quantitative yields using PEG-400 as green reaction medium. The synthesized compounds were screened for antimicrobial against selected bacterial strains. The results of anti-bacterial activity revealed that some of the compounds were found to be active antibacterial agent.

Keywords: isoxazolo[4,5-e][1,2,4]triazine, PEG-400, Antibacterial activity

INTRODUCTION

Heterocyclic chemistry acquired a unique place in the field of medicine and pharmaceutical, which comprises wide number of organic compounds containing one or more heterocyclic ring imparting the various biological activities. There are hardly very few numbers of drugs which does not contain the heterocyclic compounds.

The triazine is a six-membered heterocyclic ring, analogous to the benzene ring but with three carbons replaced by nitrogens. The three isomers of triazine are distinguished from each other by the positions of their nitrogen atoms, and are referred to 1,2,3-triazine, 1,2,4-triazine and 1,3,5-triazine [1] Figure 1.

![Figure 1: Isomeric forms of Triazine](image)

Among the isomeric forms of the triazine, 1,2,4-triazine occupied unique position in the field of medicinal chemistry due to their biological interest [2]. 1,2,4-Triazines and their derivatives have been widely studied in terms of their synthetic methodologies and reactivity since some of these derivatives were reported to have promising biological activities [3-5]. 1,2,4-Triazine derivatives have been reported to possess a broad spectrum of biological activities, including antifungal [6, 7], anti-HIV [8], anticancer [9], antianxiety [10] anti-inflammatory [11], analgesic [12] and...
antihypertensive activities [13]. Besides this, triazines were used as herbicides, pesticides and dyes [14,15]. Lamotrigine [16] (Fig 1d), is an anticonvulsant drug used in the treatment of epilepsy [17] and bipolar disorder contains 1,2,4-triazine scaffold.

These above observations prompted us to synthesize isoxazole derivatives of triazine derivatives as antimicrobial agents.

MATERIALS AND METHODS

Chemistry
All reagents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored through thin layer chromatography (TLC) on pre-coated Merck alu-foil plate (silica gel 60F-254, 0.25 mm thickness) visualized by iodine vapors. Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded (in KBr pellets) on SCHIMADZU spectrophotometer. ¹H NMR spectra were recorded on an Avance/Bruker 300/400 MHz spectrophotometer using TMS as an internal standard. All NMR spectra were obtained in DMSO d₆/deuterated chloroform (CDCl₃); chemical shifts are reported in parts per million, and coupling constant in hertz (Hz). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet). The mass spectra were recorded on GC–MS SHIMDZU (Q2010 PLUS) in Elmode spectrometer and mass values are reported in m/z.

General Procedure for preparation of 5a-j
A mixture of 4a-j (1 mmol) and hydroxylamine hydrochloride (1.2 mmol) was refluxed in PEG-400as green reaction solvent (10 mL) in the presence of NaOH for 3 hours. After completion (TLC), the reaction mixture was poured in ice cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol to afford the pure product (5a-j). The remaining derivatives were also prepared by the same procedure.

General Procedure for preparation of 6a-j
A mixture of 5a-j (1 mmol) secondary amines and paraformaldehyde (excess) was refluxed in ethanol (10 mL) for 3 hours. After completion (TLC), the reaction mixture was poured in ice cold water, solid separated out. The separated solid was filtered; the crude product was recrystallized from ethanol-chloroform mixture to afford the pure product (6a-l). The remaining derivatives were also prepared by the same procedure.

Spectral data of some selected compounds
3-[3-(4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5a) IR (KBr, v max cm⁻¹); 3321, 3034, 1607, 754; ¹H NMR (DMSO d₆, 400 Hz) δ 8.45 (s, 1H, thiazole), 8.39 (s, 1H, NH), 7.23-7.57 (m, 19 H, Ar-H); ESMS for Molecular Formula C₇₁H₅₁ClN₉O₅: 528.

3-[3-(4-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5b) IR (KBr, v max cm⁻¹); 3331, 3031, 1607, 1532; ¹H NMR (DMSO d₆, 400 Hz) δ 8.47 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.20-7.66 (m, 19 H, Ar-H); ESMS for Molecular Formula C₇₁H₅₁N₉O₅: 539.

3-[3-(4-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5c) IR (KBr, v max cm⁻¹); 3312, 3042, 1610, 1530; ¹H NMR (DMSO d₆, 400 Hz) δ 8.42 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.31-7.79 (m, 19 H, Ar-H); ESMS for Molecular Formula C₇₁H₅₁N₉O₅: 539.

3-[3-(4-Methyl-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5d) IR (KBr, v max cm⁻¹); 3321, 3034, 2922, 1608; ¹H NMR (DMSO d₆, 400 Hz) δ 8.48 (s, 1H, thiazole), 8.40 (s, 1H, NH), 7.21-7.59 (m, 18 H, Ar-H), 2.31 (s, 3H, CH₃); ESMS for Molecular Formula C₇₃H₅₇N₉O₅: 539.

3-[3-(4-Methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5e) IR (KBr, v max cm⁻¹); 3314, 3022, 2954, 1607, 1178; ¹H NMR (DMSO d₆, 400 Hz) δ 8.46 (s, 1H, thiazole), 8.41 (s, 1H, NH), 7.13-7.53 (m, 18 H, Ar-H), 4.13 (s 2H, CH₂), 2.24 (t, 4H, CH₂), 1.50 (m, 6H, CH₂); ESMS for Molecular Formula C₇₅H₆₀N₉O₅: 626.

3-[3-(4-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (5f) IR (KBr, v max cm⁻¹); 3029, 2930, 1605, 1530; ¹H NMR (DMSO d₆, 400 Hz) δ 8.45 (s, 1H, 2.24
incubated at 37±0.5˚C for 24 h. Zone of inhibition and minimum inhibitory concentrations (MICs) were noted. The target compound dilution ranging from 25 to 250 mg/mL separately for each bacterial strain. All the plates were prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of

Table-1

| 3-[3-(3-Nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-5,6-diphenyl-7-piperidin-1-ylmethyl-6,7-dihydro-isoxazolo[4,3-e][1,2,4]triazine (6d) IR (KBr, vmax cm⁻¹); 3032, 2943, 1609, 1132. ¹HNMR (DMSO d⁶, 400 Hz) δ 8.46 (s, 1H, thiazole), 7.25-7.72 (m, 19 H, Ar-H), 4.23 (s 2H, CH₃), 2.30 (s, 3H, CH₃), 2.27 (t 4H, CH₂), 1.48 (m, 6H, CH₂); ESMS for Molecular Formula; C₃₅H₃₂N₈O₇, 621.

Biology

The antimicrobial activities of the synthesized compounds (IIIa–p) were determined by agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards, (NCCLS)[18–20]. The compounds were evaluated for antimicrobial activity against bacteria viz. Escherichia coli (MTCC 2939), Salmonella typhi (MTCC 98), Staphylococcus aureus (MTCC 96) and Bacillus subtilis (MTCC 441). The antibiotic Tetracycline (25 mg/mL) is used as reference antibacterial drug for comparison. Dimethylsulphoxide (1%, DMSO) was used as a control.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5˚C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of target compound dilution ranging from 25 to 250 mg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5˚C for 24 h. Zone of inhibition and minimum inhibitory concentrations (MICs) were noted. The results of antibacterial studies are given in Table 2.

RESULTS AND DISCUSSION

Chemistry

As part of our research programme, and in continuation of our work on the development of environmentally friendly methodologies for the preparation of biologically active compounds [21-24], herein we report an efficient synthesis of isoxazolo[4,5-e][1,2,4]triazine derivatives. The title compounds were synthesized in three steps with good yield (Table-1). Initially, substituted heteroaldehydes (1a–j) were condensed with hippuric acid (2) in the presence of sodium acetate in acetic anhydride. The reaction follows the Knowenegal condensation followed by acid catalysed cyclization in the presence of acetic anhydride by the loss of the water molecule to afford 3a–j in quantitative yields. Furthermore, the formation of 4a–j was proceeding through the nucleophilic attack of phenyl hydrazine on 3a–j which undergoes acid catalysed ring expansion to give 4a–j. This 4a–j was used as precursor for the synthesis of title compound which was achieved by the reaction of 4a–j with hydroxylamine hydrochloride under neutral condition.

The structures of synthesized compounds were established on satisfactory spectral analysis. The formation of 4a–j was confirmed by the IR, NMR and mass spectral analysis. The IR spectra of 4a indicate presence of a sharp peak at 3342 cm⁻¹ due to presence of –NH group, the peak at 1678 cm⁻¹ was attributed for the presence of C=O group. Furthermore, ¹HNMR shows a down field peak at 8.43 δ ppm due to presence of –NH proton, a presence of sharp singlet at 7.12 δ ppm confirms the presence of C=C-H proton and all other protons are appeared at their respected region. Mass spectrum revealed that the molecular weight of the compound was corresponds to the molecular ion peaks. The formation of 5a–j was confirmed by the IR spectra, the disappearance of peak from 1678 cm⁻¹ confirms the formation of 5a–j. The disintegration of singlet from 7.12 in ¹HNMR spectrum further supports formation of 5a–j. Mass spectra were in accordance with molecular weight of the compounds.

Molecular Formula; C₃₅H₃₂N₈O₇, 621.
Scheme 1: Synthesis of isoxazolo[4,5-e][1,2,4]triazine derivatives; a) NaOAc/Ac₂O, 2-3 hrs; b) Phenyl hydrazine/ NaOAc/AcOH, 3 hrs, reflux; c) NH₂OH.HCl/NaOH/ PEG-400, 3 hrs, reflux. d) Pipyridine/ 1,2,4-triazine/ Morpholine, paraformaldehyde /EtOH, Reflux.

Table 1: Physical data of isoxazolo [4,5-e][1,2,4]triazine derivatives

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Product</th>
<th>Ar/Het</th>
<th>Yields in %</th>
<th>MP in °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>Br</td>
<td>82</td>
<td>168-171</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>Cl</td>
<td>84</td>
<td>155-158</td>
</tr>
<tr>
<td>3</td>
<td>6c</td>
<td>O₂N</td>
<td>80</td>
<td>175-178</td>
</tr>
<tr>
<td>4</td>
<td>6d</td>
<td>O₂N</td>
<td>88</td>
<td>169-171</td>
</tr>
<tr>
<td>5</td>
<td>6e</td>
<td>O₂N</td>
<td>79</td>
<td>140-143</td>
</tr>
</tbody>
</table>
The IR spectrum of 6a-j reveals the absence of peak at from 3342 cm\(^{-1}\) and singlet from 8.43 \(\delta\) ppm in the \(^1\)HNMR and the presence of singlet at 1.45 \(\delta\) ppm in \(^1\)HNMR for six proton of two \(-\text{CH}_3\) group and singlet at 4.21 \(\delta\) ppm confirms the presence of \(-\text{CH}_2\)– proton. The mass spectra of the compounds were in accordance with molecular weight of the compounds.

**Biology**

The synthesized compounds were screened for their antimicrobial potency against selected microbial strains. The results of *in vitro* antibacterial activities of compounds(6a-j) against various bacterial strains are summarized in Table 2. It has been observed that some of compounds exhibited interesting antibacterial activities. Compounds 6a, 6b, 6f, 6g, 6i and 6j showed effective activity against *E. coli*, and compounds 6a, 6f, 6g and 6i were displayed a good zone of inhibition against *B. subtilis*. Compounds 6b, 6g and 6j displayed a slightly active towards *S. typhi* and *S. aureus*. Compounds 6c, 6e, and 6h were displayed less active against all tested bacteria. On the other hand, it was found that compounds 6a, 6d, 6g and 6j were showed stronger inhibitory activity against all bacteria than other compounds.

**Table 2. Antibacterial Activity of isoxazolo[4,5-e][1,2,4]triazine derivatives**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>EC</th>
<th>St</th>
<th>Sa</th>
<th>Bs</th>
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<tbody>
<tr>
<td>6a</td>
<td>18</td>
<td>12</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>6b</td>
<td>16</td>
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<td>14</td>
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<td>6c</td>
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<td>18</td>
<td>21</td>
</tr>
<tr>
<td>6d</td>
<td>21</td>
<td>18</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>6e</td>
<td>14</td>
<td>09</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>6f</td>
<td>18</td>
<td>16</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>6g</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>6h</td>
<td>12</td>
<td>-</td>
<td>10</td>
<td>08</td>
</tr>
<tr>
<td>6i</td>
<td>16</td>
<td>14</td>
<td>18</td>
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<tr>
<td>6j</td>
<td>14</td>
<td>15</td>
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<td>12</td>
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<tr>
<td>Tetracyclin</td>
<td>30</td>
<td>28</td>
<td>30</td>
<td>32</td>
</tr>
</tbody>
</table>

*Zone of inhibition measured in mm; Ec-Escherichia coli; St-Salmonella typhi; Sa-Staphylococcus aureus; Bs-Bacillus subtilis; ‘-‘ Indicates the concentration >100 mg/mL.*

**CONCLUSION**

In summary, we have synthesized isoxazolo[4,5-e][1,2,4]triazine derivatives in quantitative yields. Furthermore, the antibacterial activity of compounds revealed that the compounds 6a, 6c, 6d, 6f, and 6g found to active against all selected bacterial strains. A cursory look at structure activity relationship revealed that for the manifestation of
antibacterial activity the groups like Cl, NO₂, methyl and methoxy have to incorporated with isoxazolo[4,5-e][1,2,4]triazine scaffold.

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REFERENCES