ANDILEPTOSPIRAL SCREENING (INVITRO) OF AZOMETHINES OF ARYL OXAZOLE

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ABSTRACT
Leptospirosis is now acknowledged as the most widespread zoonoses in the world. Several studies have highlighted on the epidemiology, pathology and variable clinical features of this condition. The present study involves the antileptospiral screening of the synthesized compounds against Leptospiral grippotyphosa organism. Motility of the spirochetes was examined by micro dilution method under 20X dark field microscopy with two hours interval. The reduction in the motility of the organisms and the arrest of the growth of the organism indicates the leptospiral activity of the compounds. Among the synthesized compounds (7a-e), compound 7c showed decrease in the motility at lower concentration and inhibited the motility at higher concentration.

Keywords: Leptospirosis, Leptospiral grippotyphosa, Aryl oxazoles, Micro dilution

INTRODUCTION
Heterocyclic analogues of oxazole scaffolds possess diversified biological activities such as antibacterial1,2, antifungal3, antitubercular4, antihyperglycemic5, anti-inflammatory6, anti-proliferate7. Five novel azomethines of aryl oxazoles (7a-7e) were synthesised and characterised. The experimental data and its spectral data and other biological activities of the said derivatives had already been sent for publication. All the synthesised compounds were screened for antileptospiral activity. Leptospirosis is a contemporary, ubiquitous, zoonotic disease, worldwide in distribution which affects the internal organs producing multiple organ dysfunction (MOD) to multiple organ failure (MOF), which is basically an occupational disease; man gets the infection by virtue of his occupation. The disease is transmitted through the direct contact with urine of infected animals, particularly rats or indirectly by contact with water contaminated with urine of infected animals. It affects man, pets, cattle, rodents, and wild animals. The symptoms include fever, severe headache, chills, myalgia, pain, conjunctival-suffusion/ red eyes, vomiting and diarrhoea. The aryl oxazoles are known to possess antimicrobial activity; hence it prompted us to perform antileptospiral screening activity of . The structures of the synthesised compounds are as follows.

\[
\begin{align*}
7a & : N^4-\text{phenyl}-N^2-\{\text{(Z)-phenylmethylidene}\}-1,3-\text{oxazole-2,4-diamine}. \\
7b & : N^2\{\{\text{(Z)-4-(dimethylamino) phenyl} \text{ methylidene}\}-N^4-\text{phenyl}-1,3-\text{oxazole-2,4-diamine}. \\
7c & : N^2-\{\{\text{(Z)-4-chlorophenyl} \text{ methylidene}\}-N^4-\text{phenyl-1,3-oxazole-2,4-diamine}. \\
7d & : N^2-\{\text{(Z)-3-nitrophenyl} \text{ methylidene}\}-N^4-\text{phenyl-1,3-oxazole-2,4-diamine}. \\
7e & : N^2-\{\{\text{(Z)-4-methoxyphenyl} \text{ methylidene}\}-N^4-\text{phenyl-1,3-oxazole-2,4-diamine}. \\
\end{align*}
\]

MATERIALS AND METHODS
Antileptospiral activity
Microtitre-based microbial assay for leptospiral activity8,9

Leptospiral activity (invitro) was determined using a sensitive and quick micro plate method with three different concentrations of tested compounds. For assay, 96 well bottomed micro titre plates of polystyrene were used. The plates were sterilized and examined for absence of polystyrene. The plates were sterilized and examined for absence of any external contaminating microbial growth in the wells.

Medium
Ellinghausen – Mc McCullough –Johnson Harris (EMJH) medium
- Sodium phosphate Dibasic : 1.0g/litre
- Potassium Phosphate Monobasic : 0.3g/litre
- Sodium Chloride : 1.0g/litre
- Ammonium chloride : 0.25g/litre
- Thiamine : 0.005g/litre
- pH : 7.56± 0.2

The pH was adjusted to 7.5± 0.2 and the base was autoclaved at 121°C for 15 minutes at 15 lbs pressure and the enrichment (Hi
media along with 2% BSA was filtered through a 0.22 μm filter. The test compound at various concentrations ranging from 300 μg/mL, 400 μg/mL, 500 μg/mL and 1000 μg/mL were added to double wells in a 96 wells microtitre plate. Two wells were kept as controls with 90 μl of medium and 10 μl of dimethyl formamide was added. The test microtitre plate were incubated at room temperature in the dark and then examined for leptospirocidal effect at 2 hrs and 4 hrs.

**Examination of Leptospirocidal Effect**

After incubation using a micropipette, 10 μl from each well of the microtitre plate was taken at 2 hrs and kept on a clean glass slide, cover slip is placed, then the slides were examined under 20X with Dark Field microscopy for the typical motile leptospira. The motility of the organisms was observed. Reduction in number of organism signifies leptospirostatic effect of the compounds and the arrest of the growth of the organisms indicates leptospirocidal effect of the compounds.

**RESULT AND DISCUSSION**

The antileptospiral screening of the synthesized compounds were determined against Leptospira grippotyphosa organism. Motility of the spirochetes were examined at two hours interval. The motility of the organisms indicates the antileptospiral activity of the compounds. Compound 7c decreased the motility at lower concentration and inhibited the growth at higher concentrations. Compound 7a, 7c, 7d, 7e decreased the motility at lower concentration. The results of the study are presented in Table 1

<table>
<thead>
<tr>
<th>Sample code</th>
<th>After 2hrs</th>
<th>After 4hrs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10⁻¹</td>
<td>10⁻²</td>
</tr>
<tr>
<td>7a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7b</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7c</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7d</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7e</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Among the tested compounds 7c exhibited good antileptospiral activity than all other compounds. It showed arrest in the motility of the organism at the concentration of 500 μg/mL.

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**REFERENCES**


