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Research Article

ANTILEPTOSPIRAL SCREENING (*INVITRO)* OF AZOMETHINES OF ARYL OXAZOLE

DR. MRS. V. NIRAIMATHI*, DR. A. JERAD SURESH, T.LATHA

Department of Pharmaceutical Chemistry, College of pharmacy, Madras Medical College, Chennai, India. Email: vnm_anr@yahoo.co.in

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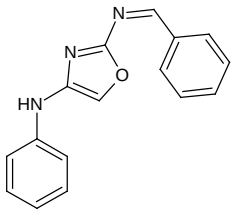
ABSTRACT

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 antileptospioral screening of the synthesised 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 synthesised compounds (7a-e) , compound **7c** showed decrease in the motility at lower concentration and inhibited the motility at higher concentration.

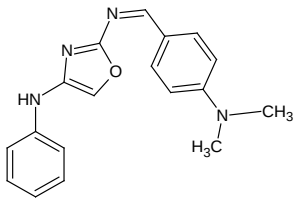
Keywords: Leptospirosis, *Leptospiral grippotyphosa*, Aryl oxazoles, Micro dilution

INTRODUCTION

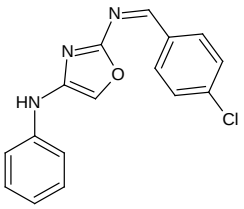
Heterocyclic analogues of oxazole scaffolds possess diversified biological activities such as antibacterial[1, 2], antifungal[3], antitubercular [4], antihyperglycemic[5] anti-inflammatory[6], anti-proliferate[7].Five novel azomethines of aryl oxazoles (7a- 7e) were synthesised and characterised. The 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, conjuctival-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



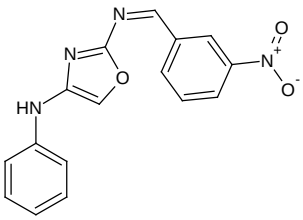
7a



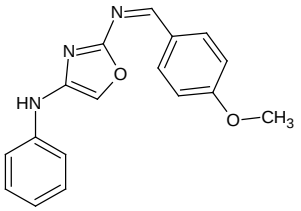
7b



7c



7d



7e

7a- N^4-phenyl-N^2-[(*Z*)-phenylmethylidene]-1, 3-oxazole-2,4-diamine.
7b - N^2-{(*Z*)-[4-(dimethylamino) phenyl] methylidene}-N^4-phenyl-1,3- oxazole-2,4-diamine.
7c- *N2*-[(*Z*)-(4-chlorophenyl) methylidene]-N^4-phenyl-1,3-oxazole-2,4 diamine .
7d-N^2-[(*Z*)-(3-nitrophenyl) methylidene]-N^4-phenyl-1,3-oxazole-2,4-diamine .
7e - N^2-[(*Z*)-(4-methoxyphenyl) methylidene]-N^4-phenyl-1,3-oxazole-2,4-diamine .

MATERIALS AND METHODS

Antileptospiral activity

Microplate- based microbial assay for leptospiral activity[8, 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 Seitz filter. The test compound at various concentration ranging from 300µg/mL, 400µg/mL 500 µg/mL and were tested for leptospiral activity against actively motile fresh culture *of Leptospira grippotyphosa.*

Motility Testing

Actively motile one week old cultures of *Leptospira grippotyphosa* obtained from Leptospira Reference Laboratory, Amsterdam, and Netherlands were used in the study. The strains were maintained in Ellinghausen – Mc McCullough –Johnson Harris (EMJH) semisolid media by subculturing. They were subcultured in (EMJH) liquid medium at an interval of one week time.

Preparation of test solution

Three different concentrations, 300µg/mL, 400 µg/mL and 500 µg/mL were used for the test.10µl from each dilution under test were taken in the first wells respectively. To all the wells, 90 µl of distilled water was added. From the first well (10^{-1} dilution), 10 µl was transferred to the second well to make a dilution of $10^{-2.}$ In this way, each concentration under test was made up to 10^{-5}.

Inoculation & Incubation

In micro dilution procedure, 90 µl culture of *Leptospiral grippotyphosa* and 10 µl test drug in concentration, from 10^{-1} to 10^{-5} were added to duplicate wells in a 96 wells micro titre plate. Two wells were kept as controls with 90 µl culture to which 10 µl of dimethyl formamide was added. The test micro titre 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 micro titre 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 organism 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 synthesised compounds were determined against *Leptospiral grippotyphosa* organism. Motility of the spirochetes were examined at two hours interval. The motility of the organisms indicates the leptospiral 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 1: Antileptospiral activity of azomethines of aryl oxazole

Sample code		After 2hrs					Sample code	After 4hrs				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	A	+	+	+	+	+	7a	↓	↓	+	+	+
7a	B	↓	+	+	+	+		↓	↓	+	+	+
	C	↓	+	+	+	+		↓	↓	↓	+	+
7b	A	+	+	+	+	+	7b	↓	+	+	+	+
	B	↓	+	+	+	+		↓	↓	+	+	+
	C	↓	+	+	+	+		↓	↓	+	+	+
7c	A	↓	↓	↓	+	+	7c	↓	↓	↓	+	+
	B	↓	↓	↓	↓	+		↓	↓	↓	↓	+
	C	↓	↓	↓	↓	↓		↓	-	↓	↓	↓
7d	A	↓	+	+	+	+	7d	↓	↓	+	+	+
	B	↓	+	+	+	+		↓	↓	+	+	+
	C	↓	+	+	+	+		↓	↓	↓	+	+
7e	A	+	+	+	+	+	7e	+	+	+	+	+
	B	+	+	+	+	+		↓	+	+	+	+
	C	↓	+	+	+	+		↓	+	+	+	+

\+ Motility as on initials; ↓ Decrease motility; --- no motility; A -*300µg/mL;* B -400µg/mL; C- 500µg/Ml

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 500µgm/mL.

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