IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

BIOLOGICAL ACTIVITY (INVITRO) OF SOME ARYL 1, 4- DI HYDRO PYRIDINE DERIVATIVES

Dr.V. NIRAI MATHI[1]. Dr A.JERAD SURESH[2], S.CHITRA BALA [3]

1. Deputy Controller of Examination, Nursing., Dr.MGR Medical University, Guindy, CHENNAI .600 032.
2. Principal, College of Pharmacy, Madras Medical College, CHENNAI-600 003 (T.N.) INDIA
3. Assistant Professor, Prist University, School of Pharmacy, Mahabalipuram, ECR Campus, CHENNAI-603 102 (T.N.) INDIA

ABSTRACT

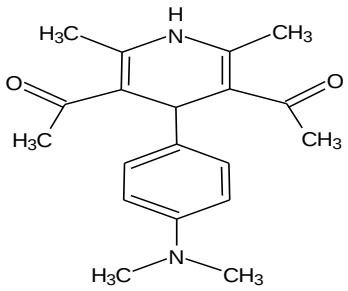
Leptospirosis, a zoonosis with a worldwide distribution is an acute febrile illness caused by spirochetes of the pathogenic *Leptospira interrogans*. The antileptospioral screening of the synthesized 1, 4 –dihydropyridine derivatives against *Leptospiral grippotyphosa organism* were carried out. Motility of the spirochetes were 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 **(CR1 - CR5)** compound **CR5** showed decrease in the motility at higher concentration, where as compound **CR2** was found to inhibit the motility at higher concentration after 4 hrs.

Keywords: 1, 4 - dihydropyridine, leptospirosis, *leptospiral grippotyphosa*, Micro dilution.

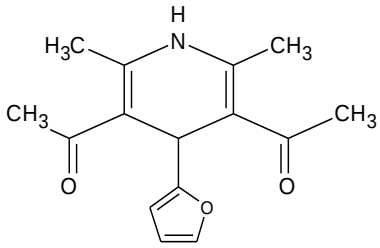
INTRODUCTION

Leptospirosis, an infectious disease that affects human and animals, is considered as the most wide –spread of zoonosis in the world (WHO 1999), which has a large impact on both human and veterinary public health . In developing countries, many cases of leptospirosis in humans come about from activities of daily life, such as walking barefoot in damp conditions or by drinking contaminated water[1] (Levet et al., 2001).Furthermore, there are many stray dogs in most developing countries and they have been classified as a significant reservoir for human infection [2] (Weekes et al., 1997) Human infection occurs as exposure to infected animals, either directly or indirectly through contaminated soil and water. Literature review indicates that 1, 4-dihydro pyridine were reported to possess a number of interesting activities such as antihypertensive, antiarrhythmic [3] (Godfraid et al., 1986), antituberculosis, bronchodilating, antitumour, hepatoprotective, antimutagenic, geroprotective, neuroprotective and platelet anti-aggregation (Cooper et al., 1992), antidiabetic and anti inflammatory activity [4] ,

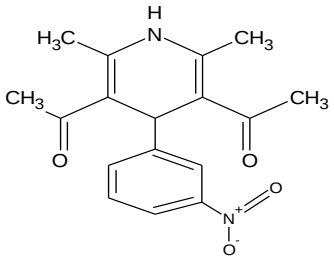
New 1, 4-dihydroderivative **(CR1-CR5)** were synthesized at the institute of Madras Medical College, College of Pharmacy , Chennai and characterized . An economical, solvent free, efficient, eco-friendly synthesis of 1, 4-dihydropyridines based on Hantzsh reaction [5] was adapted. The experimental data and its spectral data of the said derivatives had already published (Niraimathi IJPPS 2012). The 1, 4- dihydropyridines were also known to possess a wide spectrum of antimicrobial activity [6] (Yadav et al., 2001; Sapuluk Prachayasittikul et al., 2008). Keeping these observation in mind the synthesis of a new series of 1, 4 dihydropyridine derivatives and screening their level of antileptospiral activities. The structure of the synthesized compounds is as follows,



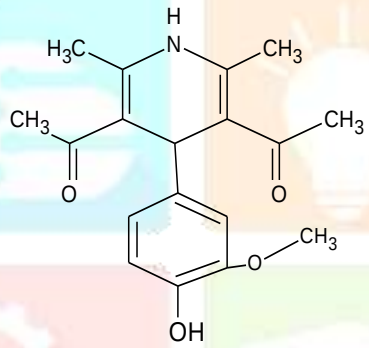
cr1



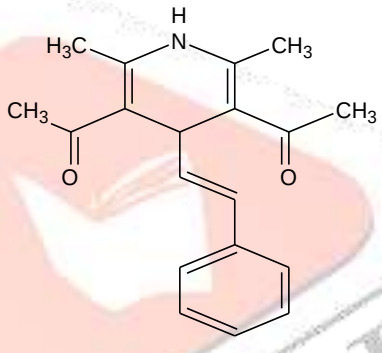
cr2



cr3



cr4



cr5

Where,

CR1-1, 1'-{4-[4-(dimethylamino) phenyl]-2, 6-dimethyl-1, 4-dihydropyridine-3, 5- diyl} diethanone.
CR2-1, 1'-[4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-diyl] diethanone**.**
CR3-1, 1'-[2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-diyl] diethanone - methane (1:1).
CR4-1, 1'-[4-(4-hydroxy-3-methoxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5- diyl] diethanone.
CR5-1, 1'-{2, 6-dimethyl-4-[(E)-2-phenylethenyl]-1, 4-dihydropyridine-3, 5-diyl} diethanone

MATERIALS AND METHODS

Leptospiral activity [7, 8, 9]

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

Medium.

Ellinghauser- Mc McCullough 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
P^H : 7.56 ± 0.2

The P^H of the media was adjusted to 7.5± 0.2 and the base autoclaved at 121˚C for 15 minutes at 15 Ibs pressure and (Hi media along with 2% BSA) filtered through Seitz filter. The test compound at various concentrations ranging from 300µg/mL, 400µg/mL, and 500µgm/mL 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 Ellinghauser- Mc McCullough-Johnson Harris (EMJH) semisolid media by subcultured technique.

Preparation of the test solution

Three different concentrations, 300µg/mL, 400µg/mL, and 500µg/mL and were used for the study. 10 µl each dilution of test were taken in the first series of wells. To all the wells 90µl 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 sulphoxide was added.The test micro titre plate was incubated at room temperature in the dark and then examined for Leptospirocidal effect after 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 and the cover slip is placed then the slides were examined under 20X Dark Field Microscopy for the typical motile *Leptospira*. The motility of the organism was observed. Reduction in number of organism signifies Leptospirocidal effect of the compounds. The results of the study are given in table -1

TABLE 1. Anti leptotospiral Screening (Organism-*leptospiral grippotyphosa*)

S.No	Test compounds (µg/ml)		Mortality After 2 hrs					Mortality After 4 hrs				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1	CR1	300	++	++	++	++	++	++	++	++	++	++
		400	++	++	++	++	++	++	++	++	++	++
		500	++	++	++	++	++	↓	++	++	++	++
2	CR2	300	++	++	++	++	++	++	++	++	++	++
		400	++	++	++	++	++	++	++	++	++	++
		500	↓	↓	++	++	++	-	-	↓	++	++
3	CR3	300	++	++	++	++	++	++	++	++	++	++
		400	++	++	++	++	++	++	++	++	++	++
		500	++	++	++	++	++	↓	↓	++	++	++
4	CR4	300	++	++	++	++	++	++	++	++	++	++
		400	++	++	++	++	++	↓	↓	++	++	++
		500	↓	↓	++	++	++	↓ ↓	↓ ↓	++	++	++
5	CR5	300	↓	↓	↓	++	++	↓ ↓	↓ ↓	++	++	++
		400	++	++	++	++	++	↓ ↓	↓ ↓	++	++	++
		500	++	++	++	++	++	↓ ↓	↓ ↓	++	++	++

NOTE: (++) indicates motility as on initial, ↓ decrease motility,

↓ ↓ Further decrease in motility. (-) indicates no motility

RESULTS AND DISCUSSION

The antileptospiral screening of the synthesized 1, 4–dihydropyridine compounds were determined against *Leptospiral grippotyphosa* organism .The motility of the spirochetes were examined 2 hrs and 4 hrs interval. The motility of the organisms indicates the leptospiral activity of the compounds. Compound CR2 and CR5 showed reduction in motility at higher concentration after 4 hrs intervals. Compound CR2 was found to inhibit the motility against *Leptospira grippotyphosa* at higher concentration at 4 hrs.

ACKNOWLEDGEMENT

The authors are thankful to, Department of Pharmaceutical Chemistry, College of pharmacy, Chennai. Rajiv Gandhi Government hospital, Institute of Microbiology, for extending laboratory facilities to carryout this work.

REFERENCES

Levett, P.N. Leptospirosis. Clinical Microbiology Reviews.2001; 14:296-326.
Weekes, CC., Everard CO. Everard, and. Levett, PN. Seroepidemiology of canine leptospirosis on the island of Barbados. *Veterinary Microbiology.* 1997 ; 51:215-222.
Godfraid T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev. 1986; 38:321.
Cooper. K, Fray.MJ, Parry.M J, Richardson. K, Steele. J. 1ccas antagonists of platelet activating factor.1.Synthesis and structure-activity relationships of 2-(4-heterocyclyl) phenyl derivatives Med Chem. 1992; 3115 – 3129.
Hua- Nan Peng Xiao – Ming peng Da- Gui Zheng P- tolene Sulphonic acid catalysed one – pot synthesis of unsymmetrical 1, 4 – dihydropyridines derivatives via Hantzsch reaction. Asian journal of Chemistry. 2011; 23 (4): 1833-1837
Swarnalatha G. Prasanthi G, Sirisha, Madhusudhana chetty, , 4-Dihydropyridines ; A Multifuntional Molecule – A Review , *International journal of chem*. Tech Research. 2011; 3(1) : 75-89
Ananthanarayana R, Panikar CK, J Textbook of Microbiology. Orient Longman Ltd, Chennai: 2000; p 581
Niraimathi. V, Jerad suresh A, Latha.T, Antileptospiral Screening (*invitro*) Azomethines of Aryl oxazole. International journal of pharmacy and pharmaceutical sciences. 2011; vol.3: suppl 4: P 150-153.
Ramnik sood, "Medical laboratory Technology-Methods and interpretation", Jaypee Brothers and Medical Publishers (p) Ltd., 2011;631