Int J Pharma Res Health Sci. 2016; 4 (6): 1474-1477
DOI:10.21276/ijprhs.2016. 06.08
Joseph *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
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International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

# Antiepileptic Activity of Novel Isoxazole Derivatives

Lincy Joseph *, Mathew George
Pushpagiri College of Pharmacy, Tiruvalla, Kerala – 689107, India.

ARTICLE INFO

Received: 21 Nov 2016
Accepted: 17 Dec 2016

ABSTRACT

To synthesize novel Isoxazole derivatives, characterize them and subject for screening anticonvulsant activity. Current therapeutic treatment for convulsion is associated with a wild variety of side effects. The bicyclic isoxazole analogues in health care can indicate an important source of new pharmaceuticals. In the present study, the antiepileptic activity of bicyclic isoxazole analogues was evaluated against electrically and chemically induced seizures. The seizures were induced in rat by maximal electric shock and Pentylenetetrazole models. Drugs were administered orally 1hr prior to induction of seizures.The antiepileptic activity of *bicyclic isoxazole analogues* was compared with the standard anticonvulsive agents phenytoin and diazepam. The data was analysed by one way ANOVA followed by Dunnett's test.

**Keywords:** Epilepsy, Bicyclic isoxazole analogues, Maximal electro shock, Pentylenetetrazole.

## 1. INTRODUCTION

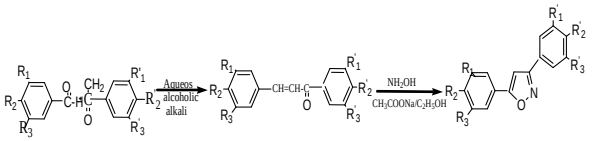
Epilepsy is the fourth most common neurological disorder and affects people of all ages Epilepsy means the same thing as "seizure disorders" It is characterized by unpredictable seizures and can cause other health problems and is a spectrum condition with a wide range of seizure types and control varying from person-to-person. The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valproate carry with several serious side effects notably neurotoxicity. [1-3] The major treatment of epilepsy is anticonvulsant medications, possibly for the person's entire life. However, newer antiepileptic's like gabapentin, vigabatrin, lamotrigine etc. are used supplemental to the

**Corresponding author ***
Prof Dr Lincy Joseph
Pushpagiri College of Pharmacy, Tiruvalla, Kerala – 689107, India
Email: mathewlincg@yahoo.com

conventional agents. The search for antiepileptic compounds with more selectivity and lower toxicity is the main area of investigation in medicinal chemistry. Many patients with antiepileptic therapy fail to experience adequate control of their seizures. Other patients do so only at the expense of significant toxic side effects. In a continuing study of potential anticonvulsants, we have extended the initial evaluation with isoxazole derivatives. The maximum electroshock method (MES) is the best qualitative preclinical test to test effectiveness of a drug or compound against generalized tonic-clonic seizure. In the MES test, mice or rats receive an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs, with tonic extension as the endpoint of the test. The test is easily conducted, requires a minimal investment in equipment and technical expertise, and is well standardized. Pentylenetetrazole clinically used as a circulatory and respiratory stimulant and at high doses cause convulsions. One study assessed the effect of cAMP, its analogs and dependent protein kinase on pentylenetetrazole-induced seizure in vivo. The convulsive dose of PTZ inducing a clonic seizure of at least 5 sec duration in 97% animal studies.[7]The study of anticonvulsant isoxazoles has been reported in a number of articles. Thus it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. In this light hereby prepared many Isoxazole derivatives which screened for anticonvulsant activity by chemically induced method and maximal electroshock seizure (MES) method.

## 2. MATERIALS AND METHODS

When aromatic aldehydes react with aromatic ketones in aqueous alcoholic alkaline medium chalcones are formed. Thenthese are made to react with hydroxylamine hydrochloride and sodium acetate to prepare title compounds. The prepared isoxazole compounds are subjected to *in vivo* anticonvulsant screening by chemical induced convulsion method using Pentylenetetrazole and by MES
method.




**EXPERIMENTAL ANIMALS:**

*Wistar albino* rats of either sex 150-250 gm of body weight obtained from animal house, Department of Pharmacology, PCP, Tiruvalla. Rat were housed in groups of 6 per cage. All the animals were maintained under standard conditions, that is room temperature 26 ±1°C, relative humidity 45-55% and 12:12 h light-dark cycle. The cages were maintained clean and all experiments were conducted between 9am to 4pm.

Acute toxicity study[5]

Wistar albino ratsof either sex (150 - 250 g weight) were used for acute oral toxicity study as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes [5.]

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Pushpagiri College of Pharmacy, Tiruvalla and all the experiments were conductedaccording to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

*DRUGS AND CHEMICALS*

Ethanol (Spectrum Chemicals), Carboxy Methyl Cellulose (India Sea Foods), Tab. Diazepam (Abbott Laboratory limited.), Tab.Phenytoin (Abbott Laboratory limited.), Pentylenetetrazol.

**PHARMACOLOGICAL SCREENING:**

ANTICONVULSANT ACTIVITY OF ISOXAZOLE SERIES

PENTYLENE TETRAZOLE INDUCED SEIZURE

The model used for the evaluation is PTZ induced convulsion in rats. PTZ (70mg/Kg i.p.) was used to induce conculsionto *Wistar albino rats.* Rats were divided in to 3 groups. The 1st group of animals were administered 1% CMC (1 ml / 100 g) orally. Group 2 animals were treated with diazepam (4mg/Kg i.p). Group 3 animals were treated with different compounds of isoxazole series. Drug pretreatment was given 1hr prior to the administration of PTZ and immediately after PTZ administration rats were observed for onset of convulsion.

*MES INDUCED SEIZURE*

*Wistar albino rats* of either sex with a body weight between 150 -250g were divided into three groups of six animals each, Group A served as control and was administered With 1% sodium CMC, Group B with Diazepam (4mg/kg i.p) and served as standard. Group C treated with different compounds of isoxazole series (100mg/kg).MES seizures were induced by electro -convulsiometer. The electroshock was applied via ear clip electrodes separately to each rat. The stimulus duration was 0.2sec and the current frequency 150 mA. The rats were placed in a rectangular plastic cage with an open top, permitting full view of animal's Motor responses to seizure in the study of convulsions. Duration of seizure was observed in seconds. [4, 5]

**STATISTICAL ANALYSIS:**

All data were represented as mean ±SEM values. Data were analyzed by one-way ANOVA. Whenever ANOVA was significant, further comparison was made against the vehicle treated groups were performed using the Dunnett's - tests. The level of statistical significance adopted was $P < 0.05$.

## 3. RESULTS

*Acute toxicity*

Acute toxicity study was performed according to OECD guidelines 423 using Female *Wistar albino rat.* At

300mg/kg, the extract was neither produced mortality nor the signs of morbidity. Hence the dose 60mg/kg (1/5th of 300mg/kg) was selected for further studies.

**Table 1: Effect of isoxazole analogues and Diazepam in PTZ Model**

| Compound | Onset of Convulsion in Min |
|---|---|
| $L_1$ | 12 |
| $L_2$ | 15 |
| $L_3$ | 2.5 |
| $L_4$ | 3 |
| $L_5$ | 12 |
| $L_6$ | 15 |
| $L_7$ | 2 |
| $L_8$ | 2 |
| $L_9$ | 4 |
| $L_{10}$ | 12 |
| $L_{11}$ | 15 |
| $L_{12}$ | 10 |
| $L_{13}$ | 5 |
| $L_{14}$ | 8 |
| $L_{15}$ | 15 |
| $L_{16}$ | 3 |
| $L_{17}$ | 7 |
| $L_{18}$ | 11 |
| $L_{19}$ | 9 |
| $L_{20}$ | 7 |
| $L_{21}$ | 4 |
| $L_{22}$ | 3 |
| $L_{23}$ | 4 |
| $L_{24}$ | 14 |
| $L_{25}$ | 15 |
| standard | 17 |

Values are expressed as Mean ± SEM for 6 animals, *P<0.05, **P<0.01, significantly when compared with control group.

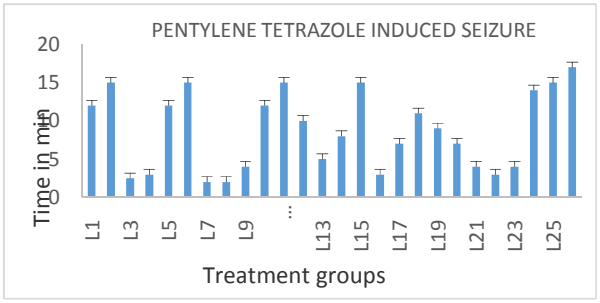



**Fig 1: Effect of Isoxazole analogues and standard (diazepam 4 mg/kg) compared with vehicle treated control (1% CMC).**

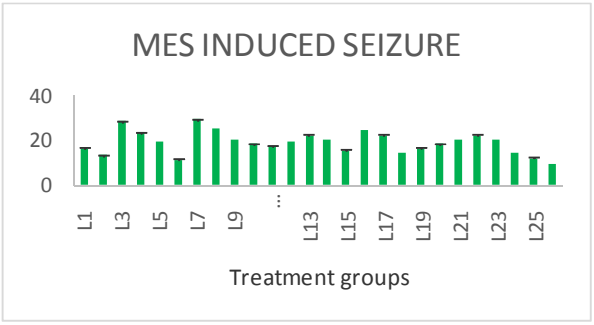



**Fig 2: Effect of Isoxazole analogues and standard (diazepam 4 mg/kg) compared with vehicle treated control (1% CMC).**

**Table 2: Effect of Isoxazole Analogues and Diazepam In Mes model**

| Compound | Duration of Convulsion in Sec |
|---|---|
| $L_1$ | 16 |
| $L_2$ | 13 |
| $L_3$ | 28 |
| $L_4$ | 23 |
| $L_5$ | 19 |
| $L_6$ | 11 |
| $L_7$ | 29 |
| $L_8$ | 25 |
| $L_9$ | 20 |
| $L_{10}$ | 18 |
| $L_{11}$ | 17 |
| $L_{12}$ | 19 |
| $L_{13}$ | 22 |
| $L_{14}$ | 20 |
| $L_{15}$ | 15 |
| $L_{16}$ | 24 |
| $L_{17}$ | 22 |
| $L_{18}$ | 14 |
| $L_{19}$ | 16 |
| $L_{20}$ | 18 |
| $L_{21}$ | 20 |
| $L_{22}$ | 22 |
| $L_{23}$ | 20 |
| $L_{24}$ | 14 |
| $L_{25}$ | 12 |
| standard | 9 |

Values are expressed as Mean ± SEM for 6 animals, *P<0.05, **P<0.01, significantly when compared with control group.

## 4. DISCUSSION

Isoxazole exhibits broad spectrum of biological activity and also forms a part of various biodynamic agents. The substituted isoxazoles are also considered to be important due to their versatility towards chemical transformations to useful synthetic intermediates.

We synthesise 25 novel isoxazole derivatives and acute toxicity study shows that synthesized 25 novel isoxazole derivatives are devoid of any serious toxic effect at the dose of 60 mg/Kg and no mortality was found. Based on this data the 60 mg/ Kg was selected as test dose for pharmacological screening. Grandma type of epilepsy is induced by MES method. Many literature study state that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread and can be prevented either by drugs that inhibit voltage-dependent Na+ channels or by drugs that block glutaminergic excitation mediated by NMDA receptor. The test group and the drug diazepam (4mg/kg) when compared to control treated group for the significant decrease in duration of seizure (secs) in rat. In PTZ-induced seizure model, the glutamatergic system, especially NMDA receptors, has also play an important role.

None of the tested compounds are superior to Diazepam which was the standard. Two compounds have given protection against convulsion for 25 minutes; In those compounds Phenyl ring at fifth position of hetero ring carried $NO_2$ group and phenyl attached at third position of hetero ring possessed OH as $R_2$' substitution. An another compound also showed equal action where first phenyl ring at $5^{th}$ position had CH3 substitution and $NH_2$ in second phenyl ring attached at third position of hetero ring. Moderate protection have been given in another set of compounds where Bromine substituted in Phenyl ring group. Presence of nitrated ($R_1$/$R_2$) aromatic ring at 5-C and hydroxyl substituted phenyl ring at 3-Cof Isoxazole exhibited maximum protection against convulsion.

## 5. CONCLUSION

All the compounds exhibited anticonvulsant activity among tested 25 isoxazole derivatives. From these L1, L2, L5, L6, L10, L11, L12, L15, L18, L19, L24 and L25 shows significant anticonvulsant activity compared other compounds in both PTZ induced model and MES model.

## 6. ACKNOWLEDGEMENT

This study is funded by SERB of DST-Govt. of India. Spectral Characterization have been done STIC-cochin university of Science &Technology, Kerala,India.

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**Conflict of Interest: None**

**Source of Funding: Nil**