



ANTICONVULSANT SCREENING OF THREE NOVEL ISOMERIC FORMS OF 4[(DIMETHYLPHENYL) AMINO]-4-OXOBUT-2-ENOIC ACID IN CHICKS AND MICE

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ABSTRACT

Epilepsy is a disorder of public concern and has been widely treated using various pharmacotherapeutic approaches. Despite many breakthrough in its current management, there is no one drug that is devoid of shortcomings particularly toxicity and cost. Advances in pharmaceutical research have led to the need for a structure guided pharmacologic activity of novel compounds. The aim of this study is to evaluate the anticonvulsant activity of three (3) novel isomeric forms of 4[(dimethylphenyl) amino]-4-oxobut-2-enoic acid in chicks and mice. The 3 isomeric forms i.e. {4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid (A), 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid (B) and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid (C)} were subjected to acute toxicity studies using Lorke's method of 1983 and anticonvulsant screening using Maximum Electro-Shock Test (MEST) and Pentylenetetrazole test (PTZ). The Median lethal doses (LD50) of compounds A, B, and C were estimated to be 775, 1131, and 1131 mg/kg respectively. In the MEST, compound A (50, 100 and 200 mg/kg) and B (75, 150 and 300 mg/kg) did not show protection at all the doses tested, while compound C (75, 150 and 300 mg/kg) showed a 20% protection across all the doses tested. In the PTZ, compound A, B and C showed no protection. In conclusion, all the 3 isomeric forms of 4[(dimethylphenyl) amino]-4-oxobut-2-enoic acid though possessed some level of protection but not significant against MEST and PTZ models.

Keywords: Anticonvulsant, Epilepsy, Isomers, MEST, PTZ

INTRODUCTION

Epilepsy is a chronic disorder of the brain characterized by recurrent seizures from episodic neuronal discharge. Seizures are brief episodes of involuntary shaking which may involve a part of the body (partial) or the entire body (generalized) and sometimes accompanied by loss of consciousness (WHO, 2016). There is no recognized cause of epilepsy, although it may develop following a brain damage caused by infection, tumor, trauma or other kinds of neurological defects and diseases (Rang *et al.*, 2007). Over 50 million people have been estimated to have epilepsy in the world of which about 75% or more are in developing countries and underdeveloped countries with little or no access to proper medical services (Meinardi *et al.*, 2001; Ngugi *et al.*, 2010; WHO 2016).

Researchers have continue to search for more effective, selective and less toxic antiepileptic drugs considering the various challenges associated with antiepileptic drugs such as; cost, toxicity, side effects, continuous medication and poor response to therapy. As pharmaceutical researches advance, the need for a structure guided pharmacologic activity of

a compound through the identification of pharmacophore is required. Historically, pharmacophores were established by Lemont Kier, who first mentioned the concept in 1967 and used the term in his publication (Kier 1971). The following structural features of an anticonvulsant were also proposed: aryl binding site responsible for hydrophobic interaction; electron donor system (C=N); and most importantly a hydrogen bonding area as represented by carboxamide CONH₂ function (Dimmock *et al.*, 1995).

Unverferth *et al.* (1998) employed five well known but structurally different anticonvulsants with sodium channel blockade activity carbamazepine, phenytoin, lamotrigine, Zonisamide, and Rufinamide to propose a 3-point pharmacophore for sodium blockade activity. The model comprises an electron donor, aryl ring or other hydrophobic units and a hydrogen bond acceptor/donor unit. Shindikar *et al.*, (2006) proposed a two-point pharmacophore, consisting of an aromatic center separated from an H-Bonding acceptor/donor group within a distance range of 4.18-6.88 Å for anticonvulsants active at the sodium channels.

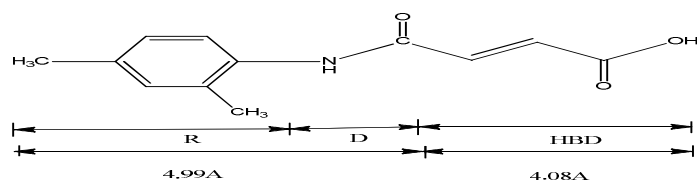


Figure 1: Distances between the various groups essential for anticonvulsant activity

The dicloro isomeric forms of the compound in figure 1 containing 4-[(2, 4- dichlorophenyl) amino]-4-oxobutenoic acid, and its 2, 5- isomeric forms have been synthesized and found to have proconvulsive activities (Abdullahi, 2013). This could be due to a decrease in lipophilicity of the dichloro substitute on the aromatic ring thus, reducing the ability of the drug to cross the blood-brain barrier that is necessary for activity. They further suggested that there is a need to structurally modify the compounds to a more lipophylic state. This led to the replacement of the dichloro- substitute with dimethyl substituent on the aryl moiety as done in this current work.

This study was aimed at screening for anticonvulsant activity of 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid in chicks and mice.

MATERIALS AND METHODS

Chemicals/Drugs

Compound A {4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid}, compound B {4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid} and compound C {4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid}, Phenytoin sodium (Hospira, UK Limited), Sodium valproate (Sanofi Aventis, U.S), Pentylenetetrazole (Sigma chemical Co. St Louis, USA), Ethanol and Distilled water.

Laboratory Animals

Albino mice (20 to 25g) and day old chicks were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and National Animal Production and Research Institute (NAPRI), Shika, Zaria respectively. The animals were maintained in a well-ventilated room, fed on standard animal feed and water provided *adlibitum* under standard laboratory conditions in accordance with the Ahmadu Bello University research policy, ethics and regulation governing the care and use of experimental animals.

Route of Drug Administration

All treatments were administered intraperitoneally (IP) except for pentylenetetrazole that was administered

subcutaneously (SC). Also all solvents used were of analytical grades.

Acute Toxicity Study

The median lethal dose (LD₅₀) was conducted using the method of Lorke, (1983). In the first phase, nine (9) mice were divided into three (3) groups of three (3) mice each and were treated with graded doses of 10, 100 and 1000mg/kg body weight each of compound A, B and C. The mice were observed for any sign of toxicity and death within 24 hours. In the second phase, four (4) mice were administered graded doses of 1200, 1600, 2900 and 5000 mg/kg body weight respectively intraperitoneally with each of compound A, B and C. The mice were observed for any sign of toxicity and death within 24 hours. The LD₅₀ value was calculated as the geometric mean of the highest non-lethal dose and lowest lethal dose.

Maximal Electroshock-Induced Convulsion Test in Chicks

The method of Swinyard and Kupferberg (1985) was employed. Ugobasile electro-convulsive machine (model 7801) connected to a stabilizer with corneal electrodes placed on the upper eyelids of the chicks after dipping them in normal saline. A current (80 mA) which induced tonic seizures in 90% of the control groups of chicks. The shock duration, frequency and pulse width was set and maintained at 0.8 sec, 100 pulse/sec and 0.6 ms respectively were used throughout the study. Seizures were manifested as hind limb tonic extension (HLTE). One hundred and ten (110) day-old chicks were randomly divided into eleven (11) groups of ten (10) chicks each. Group I were administered distilled water (10 ml/kg), group II were administered Phenytoin (20 mg/kg), groups III-V were administered graded doses of compound A (50, 100 and 200 mg/kg) respectively, groups VI-VIII were given graded doses of compound B (75, 150 and 300 mg/kg) respectively and groups IX-XI were administered graded doses of compound C (75, 150 and 300 mg/kg) respectively.

Thirty minutes later, maximum electroshock was administered to induce convulsion in the chicks. The ability of the extract to prevent this feature or reduce the mean recovery time of convulsion was considered as an indication of anticonvulsant activity.

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Pentylenetetrazole (PTZ)-Induced Convulsion in Mice

The method of Swinyard and Goodwill (1989) was employed. Fifty five (55) mice were divided into eleven (11) groups of five (5) mice each. Group I received distilled water (10 ml/kg), group II received Sodium valproate (200 mg/kg), groups III-V received graded doses of compound A (50, 100, and 200 mg/kg) respectively, groups VI-VIII were administered graded doses of compound B (75, 150 and 300 mg/kg) respectively and groups IX-XI were given graded doses of compound C (75, 150 and 300 mg/kg) respectively.

After 30 minutes, all the groups were administered freshly prepared PTZ (100 mg/kg) subcutaneously. All the mice were observed for a period of 30 minutes after scPTZ. The

absence of tonic extension of limbs for at least 5 seconds duration indicates a compound's ability to abolish the effect of PTZ on seizure threshold.

Data Presentation and Analysis

Data were presented as mean \pm standard error of mean (SEM) and as percentages in tables and figures. Statistical analysis was carried-out using One-way ANOVA followed by Dunnett's post hoc test using SPSS software version 23.0. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Acute Toxicity Studies

The median lethal doses (LD₅₀) of compound A, B, and C were estimated to be 775, 1131, and 1131 mg/kg respectively.

Table 1: Effect of Compounds A, B and C on Maximal Electroshock Test (MEST) in Chicks

Treatments (mg/kg)	Mean Time of Recovery (min)	Quantal protection	Percentage protection (%)
Distilled water (ml/kg)	8.30 \pm 0.99	0/10	0.00
Compound A:			
200	14.70 \pm 2.53	0/10	0.00
100	10.20 \pm 1.30	0/10	0.00
50	10.40 \pm 1.50	0/10	0.00
Compound B:			
300	5.90 \pm 0.43	0/10	0.00
150	5.20 \pm 0.36	0/10	0.00
75	6.50 \pm 0.92	0/10	0.00
Compound C:			
300	7.7 \pm 1.51	2/10	20.00
150	5.80 \pm 1.01	2/10	20.00
75	5.80 \pm 1.08	2/10	20.00
Phenytyion 20	2.00 \pm 1.37*	8.0/10	80.00

n=10; Values are presented as Mean \pm SEM and percentages. Mean recovery time of seizure compared to normal saline group using One way ANOVA followed by Dunnett's post hoc test; Compound A {4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid}, compound B {4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid} and compound C {4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid}; * = $p < 0.05$.

Table 2: Effect of Compounds A, B and C on Pentylenetetrazole (PTZ) - Induced Seizure in Mice

Treatments (mg/kg)	Mean Onset of seizure (min)	Quantal Protection	Percentage protection (%)
Distilled water	5.40 \pm 0.51	0/5	0.00
Compound A:			
200	8.80 \pm 1.07	0/5	0.00
100	8.80 \pm 1.71	0/5	0.00
50	6.40 \pm 2.30	0/5	0.00
Compound B:			
300	5.6 \pm 1.63	0/5	20.00
150	6.0 \pm 0.89	0/5	0.00
75	5.20 \pm 0.80	0/5	0.00
Compound C:			
300	10.80 \pm 2.10	0/5	0.00
150	4.80 \pm 0.66	0/5	0.00
75	3.20 \pm 0.49	0/5	0.00
Sodium valproate 200	27.00 \pm 2.80*	4/5	80.00

n=5; Values are presented as Mean \pm SEM and percentages. Mean onset of seizure compared to normal saline group using One way ANOVA followed by Dunnett's post hoc test; Compound A {4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid}, compound B {4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid} and compound C {4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid}; * = $p < 0.05$.

DISCUSSION

The present study attempts to investigate the anticonvulsant potentials of 3 novel synthesized compound A {4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid}, compound B {4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid} and compound C {4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid} in animal models. The median Lethal Dose (LD₅₀) obtained for compounds A, B and C falls within the range of drugs classified as slightly toxic, as all the LD₅₀ values fall between 500-5000 mg/kg (Hollinger, 2003; Corbett *et al.*, 1984; Matsumura, 1975). During the MEST, there was no significant effect of compounds A, B and C on the mean recovery time at all the doses tested, indicating the inability of the compounds to protect the chicks against seizures. While, compound A and B did not show any protection against death, compound C showed 20% protection against death at all the doses tested. Meanwhile, Phenytoin which is a standard anticonvulsant drug significantly decreased the mean time of recovery and produced 80% protection against death. In contrast to our study, there was a significant decrease in both the onset and mean time of recovery for some dichloro- substitutes compounds (Abdullahi, 2013).

MEST is a standard AED test that evaluates the testing material's ability to protect against HLTE (DeLorenzo *et al.*, 2001). It is a model for generalized tonic clonic seizure, which is highly reproducible with a consistent end point. Protection against HLTE also indicates the ability of a testing material to inhibit or

prevent seizure discharge within the brainstem seizure substrate (Browning, 1992).

In the present study using PTZ test, there was no significant effect of compounds A, B and C on the mean onset of seizures at all the doses tested, indicating the inability of the compounds to protect the chicks against seizures. So also, the compounds did not show any protection against death at all the doses tested. However, sodium valproate, which is a standard anticonvulsant drug significantly increase the mean onset of seizures and produced 80% protection against death. Anticonvulsant activity in PTZ test identifies compounds that can raise the seizure threshold in the brain (Raza *et al.*, 2001). Antiepileptic drugs effective in the therapy of generalized seizure of *petit-mal* type exhibit dose dependent suppression of PTZ induced seizure e.g. phenobarbitone, valproate, ethosuximide and benzodiazepines. PTZ has been shown to interfere with GABA neurotransmitter and the GABAA receptor complex (DeDeyn *et al.*, 1992). Antagonism of PTZ-induced seizure suggests potentiating effect on GABAergic neurotransmission.

CONCLUSION

In conclusion, all the 3 isomeric forms of 4[(dimethylphenyl) amino]-4-oxobut-2-enoic acid though possessed some level of protection but did not show significant anticonvulsants activity against MEST and PTZ models. Further studies using different anticonvulsant models are recommended to confirm our findings.

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