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Synthesis and Anticonvulsant Screening of Some Potentially Active Oximes

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ABSTRACT

Neurological disorders such as epilepsy remain a major concern to public health despite considerable efforts aimed at developing effective medicine. This work reports the synthesis and anticonvulsant screening of four oximes; (E)-N-hydroxy-1-phenylmethanimine (EHPM), (Z)-N-hydroxy-1-phenylmethanimine (ZHPM), (1E,2E)-N,N^{I}-dihydroxy-1,2-diphenylathane-1,2-diimine (EEDDP-E) and (1E,2E)-N,N^{I}-dihydroxy-1,2-diphenylethane-1,2-diimine (ZEDDPE). The compounds were synthesized from the reaction between various carbonyl compounds and hydroxylamine using Beckmann rearrangement reaction. The structures of the synthesized compounds were established using Ultraviolet (UV), Infrared (IR), Proton and Carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The compounds were screened for anticonvulsant activity using maximal electroshock (MES) test in chicks and subcutaneous pentylenetetrazole (scPTZ) seizure test in mice. The neurotoxicity of the compounds was investigated using beam walking assay in mice. In maximal electroshock seizure (MES), model only compound EHPM protected 60% at a dose of 56 mg/kg (0.5%). The other oximes synthesized were generally inactive. However, in subcutaneous pentyleneterazole (scPTZ)-induced seizure test three compounds showed good activity viz: EHPM (83.33%) and ZHPM (66.67%). Compound ZEDDPE provided 100% protection at a dose of 300 mg/kg. The significant activity of the compounds against pentylenetetrazole (scPTZ)-induced seizure suggests that they may be beneficial in absence and complex partial seizures. The moderate activity of EHPM against maximal electroshock is suggestive of potential in generalized tonic-clonic seizure. All the synthesized oximes were found not to produced significant motor coordination deficit at the doses tested indicating that they are not neurotoxic and are therefore recommended for further screening and optimization.

Keywords: Synthesis; imine; maximal electroshock; neurotoxicity; oximes; pentylenetetrazole.

Introduction

Epilepsy is one of the most common chronic disorders of the brain that is characterized by recurrent seizures, which may vary from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions.[1] It has been estimated that 50 million people in the world suffer from epilepsy, of which up to 75% live in resource poor countries with little or no access to medical services or treatment.[2] Currently available antiepileptic drugs are associated with toxicity and serious side effects such as aplastic anaemia, hepatotoxicity, headaches, gingival hyperplasia, drowsiness, and gastrointestinal disturbances. In view of these facts, most epileptopologist agree on the need for the discovery of new anticonvulsant drugs that have higher efficacy and lower toxicity profile.[3,4]

Oximes are chemical compounds belonging to the imines, forming an aldoxime, or another organic group, forming a ketoxime. These compounds did not only represent the series of derivatives of carbonyl compounds but also used as useful intermediate for the important organic synthesis and functional group transformation.[5]

Oximes derivatives of various organic compounds have shown various biological activities such as anticancer,[6] anti-microbial,[7] anti-tumor.[8]

Materials and Methods

Chemicals

All reagents and solvents used for the experiment were of analytical grade and were purchased from Sigma Aldrich. They included hydroxylamine hydrochloride, benzil, carbon disulphide, ethanol, ether, benzene, ethylacetate and n-hexane.

General Experimental Procedure

Boiling points of compounds EHPM and ZHPM were determined using U-shape capillary tube. While the melting point of compounds EEDDPE and ZEDDPE were determined using Gallenklamp melting point apparatus as described by the manufacture's manual. Fourier Transform Infrared (FTIR) spectra were recorded using NICOLET is10 spectrophotometer (Thermo Scientific). ^{1}H-NMR and ^{13}C-NMR spectra were performed using 400 MHz Bruker 400 (Avance 111, Germany), chemical shifts were given in ppm relative to tetramethylsilane.

*Synthesis of (E)-N-hydroxy-1-phenylmethanimine **(1)***

Sodium hydroxide (14 g) was dissolved in 40 mL of distilled water and then mixed with 21 g (20 mL, 0.2 mol) of pure benzaldehyde inside a 250-mL conical flask. 15 g (0.22 mol) of hydroxylamine hydrochloride was added in small portions and the mixture was shaken using mechanical stirrer. Some heat was developed and the

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benzaldehyde eventually disappears. Upon cooling, a crystalline mass of the sodium derivative of the oxime separated out. A sufficient amount of water was added until a clear solution formed, and carbon dioxide was passed into the solution until saturated. A colourless emulsion of the (E)-N-hydroxy-1-phenylmethanimine seperated. The oxime was extracted with ether and then dried using a rotary evaporator. The residue was distilled at reduced pressure (122-124^{0}C/12 mmHg); this gradually solidified on cooling in ice.

*Synthesis of (Z)-N-hydroxy-1-phenylmethanimine (**2**)*
(E)-N-hydroxy-1-phenylmethanimine (10 g) was dissolved in 50 mL of pure anhydrous ether and dry hydrogen chloride was passed over it through a wide delivery tube into the solution with constant shaking. A colourless crystals of the hydrochloride of the (Z)-N- hydroxy-1-phenylmethanimine separated, which was then filtered under vacuum, and washed with dry ether and then transferred to a separating funnel and covered with a layer of ether. A concentrated solution of sodium carbonate was added gradually with constant shaking until effervescence ceases. The ethereal layer separated, which contained the (Z)-N-hydroxy-1-phenylmethanimine, this was then dried over anhydrous sodium sulphate and the ether was removed using a rotary evaporator. The residue crystallizes and the small amount of oily matter was removed by pressing on a porous tile. This was recrystallized by dissolving in the minimum volume of ether and then adding light petroleum.

*Synthesis of (1E, 2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine (**3**)*
Benzil (42 g, 0.2 mol) was grinded to a thin paste with a little ethanol and concentrated aqueous solution of 17.5 g (0.25 mol) of hydroxylamine hydrochloride was added. This was then cooled to -5^{0}C in an ice-salt bath and 30 g of sodium hydroxide as 20% aqueous solution was added dropwise with a rapid mechanical stirring: the temperature was not allowed to rise above 0^{0}C. After 90 minutes the mixture was diluted with water which was then filtered off on a sintered glass funnel. The filtrate was acidified with glacial acetic acid, and allowed to stand for 30 minutes before the crude pinkish (1E, 2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine was filtered off and then recrystallized from aqueous ethanol (60% v/v).

*Synthesis of (1Z,2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine (**4**)*
The (1E, 2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine was converted to (1Z, 2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine after treatment with a solution of hydrogen chloride in ether at room temperature. Solvated crystals inside the ether melted on rapid heating at about 65^{0}C. The ether contained in the crystals was removed in an oven at 50^{0}C and was recrystallized from carbon disulphide to yield pure (1Z,2E)-N,N^I-dihydroxy-1,2-diphenylethanone-1,2-diimine.

Pharmacological Evaluation
Animals
Male albino Swiss mice (18 - 33 g) and white Cockerels (20 - 41 g) were used as experimental animals. Animals were housed under standard laboratory conditions in accordance with the Department of Pharmacological and Therapeutics animal care unit, Ahmadu Bello University, Zaria. The experimental protocol was approved by the institutional Ethical Committee with approval no: COLCAUC/2016/002. The animals were allowed free access to food, water and were acclimatized to room conditions for at least 2 days prior to the experiments. Anticonvulsant evaluations were undertaken using the reported procedure.[9,10] Test compounds and the standard drug (in 2% carboxymethylcellulose) were administered intraperitonally. The mouse beam walking assay was used to evaluate neurotoxicity.[11]

Anticonvulsant Effects in the MES Test
Seizures were induced in mice or chicks with a 100 Hz alternating current of 80 mA intensity. The current was applied via corneal electrode for 0.8 s. Effective protection against the spread of MES-induced seizures was defined as the prevention of the hind leg and tonic maximal extension component of the seizure. 0.5 h after the administration of the compounds, the activity was evaluated using an MES test.[9,10]

The Subcutaneous Pentylenetetrazole Seizure Test (scPTZ)
The test utilized a dose of pentylenetetrazole (85 mg/kg) that produces clonic seizures lasting for a period of at least five seconds in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant was administered subcutaneously. The test compound was administered intraperitoneally in mice and the animals were observed over a 30 min period. Mice were tested at least two different time point (15 min, 30 min and 1 hr) following intraperitoneal administration of 27.75, 55.5 and 111 mg/kg of test compound EHPM, intraperitoneal administration of 28, 56, 100, 112 of test compound ZHPM, and intraperitoneal administration of 30, 100 and 300 mg/kg of both test compounds EEDDPE and ZEDDPE. Absence of clonic spasm indicate a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Neurotoxicity Assay
The neurotoxicity of the compounds was measured in mice using mouse beam walking. The mice were trained to walk from a start platform along ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Thirty (30) minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded.[11]

Results and Discussion

The target compounds EHPM of the desired absolute configurations were synthesized as shown in Scheme 1. The starting materials, pure benzaldehyde and hydroxylamine hydrochloride were heated and the benzaldehyde eventually disappeared to obtain compound **1**. This compound reacted further by passing it over hydrogen chloride through a wide delivery tube to yield compound **2**. The starting materials, benzil and hydroxylamine hydrochloride were cooled to -5^{0}C for 1.5 h to obtain compound **3**. This compound was also reacted further by treatment with a solution of hydrogen chloride to yield compound **4**.
The IR vibration frequency observed at 3316 cm^{-1} and 3385 cm^{-1} indicate the presence of OH group for compounds EHPM and ZHPM, respectively. Also at a frequency 3277cm^{-1}-3261cm^{-1}, a OH group was observed for compounds EEDDPE and ZEDDPE [12]. IR frequency observed at 3064 cm^{-1} and 3065 cm^{-1} indicated the presence of CH stretching vibration for compounds EHPM and ZHPM, respectively. The IR frequency observed at 3061 cm^{-1} and 1458 cm^{-1} indicated the presence of CH stretching vibration for compounds EEDDPE and ZEDDPE, respectively.[12] At frequency 1651 cm^{-1} and 1691 cm^{-1}, a C=N stretching frequency was observed for compounds EHPM and ZHPM. Also at frequency 1647 cm^{-1}- 1651 cm^{-1}, a C=N stretching frequency was observed for compounds EEDDPE and ZEDDPE. At frequency 1444 cm^{-1}- 1493cm^{-1}, N-O stretching frequency was observed for compounds EHPM and ZHPM. At frequency 1377 cm^{-1} and 1376 cm^{-1}, N-O stretching frequency was observed for compounds EEDDPE and ZEDDPE, respectively.[12]

(E)-N-hydroxy-1-phenylmethanimine (1): Yield: 66.7%, bp: 156-158^{0}C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.38 (d, 1H, *J* = 8 Hz) and δH 7.32 (d, 1H, *J* = 8 Hz). ^{13}C-NMR (DMSO, 400 MHz): δC 148.16 (C1), 126.43 (C2 and C6), 128.72 (C3 and C5), 129.27 (C4) and 133.12 (C7).

(Z)-N-hydroxy-1-phenylmethanimine (2): Yield: 70%, bp: 168-170^{0}C. ^{1}H-NMR (DMSO, 400 MHz) δ: 7.58 (d, 1H, *J* = 8Hz) and δH 7.36 (d, 1H, *J* = 8 Hz). ^{13}C-NMR (DMSO, 400 MHz): δC 148.09 (C1), 128.37 (C2 and C6), 128.68 (C3 and C5), 129.22 (C4) and 133.05 (C7).

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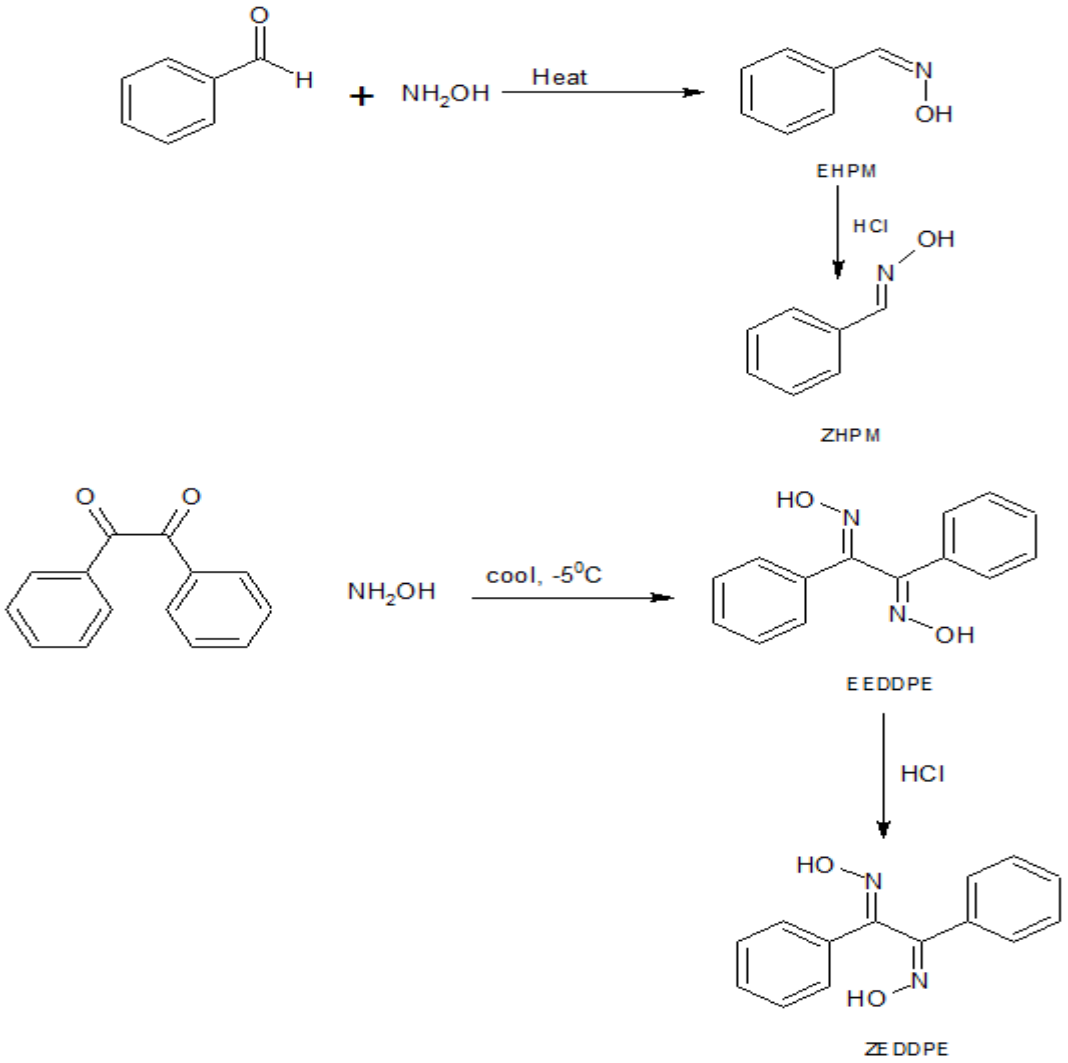
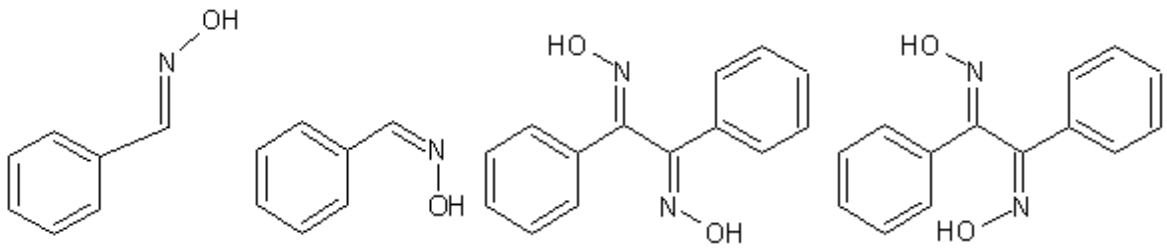


Reagents and conditions; (a) PhCHO, NH_2OH, heated; (b) HCl, wide delivery tube; (c) PhOOPh, NH_2OH, cool, -5^0C; (d) HCl, room temperature.

Scheme 1: Synthetic routes to the target compounds



EHPM ZHPM EEDDPE ZEDDPE

Figure 1: Structures of the target compounds

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Table 1: Effect of Compounds EHPM, ZHPM and Phenytoin on Maximal Electroshock Induced Seizure in Chicks

Treatments(mg/kg)	Quantal Protection	Mean Time of Recovery(mins)
Olive oil (10 ml/kg)	0/10	7.90 ± 1.38
EHPM (112)	1/10	8.78 ± 1.49
EHPM (56	6/10	7.25 ± 0.95
EHPM (28)	0/10	10.30 ± 1.53
ZHPM (111)	2/10	11.38 ± 2.82
ZHPM (55.5)	1/10	6.78 ± 0.62
ZHPM(27.75)	1/10	8.56 ± 0.63
Phenytion (20 mg/kg)	10/10	-

Data are expressed as Mean ± SEM (n = 10) and analysed by one-way ANOVA followed by Dunnett's test, $p<0.05$compared to control (Olive oil, 10ml/kg).

Table 2: Effect of Compounds EEDDPE, ZEDDPE and Phenytoin on Maximal Electroshock Induced Seizure in Chicks

Treatments(mg/kg)	Quantal Protection	Mean Time of Recovery(mins)
Carboxymethylcellulose (2%)	1/10	4.56 ± 0.56
EEDDPE (300)	0/10	5.50 ± 0.52
EEDDPE (100)	0/10	5.80 ± 0.47
EEDDPE (30)	0/10	5.90 ± 0.35
ZEDDPE (300)	0/10	6.50 ± 0.75
ZEDDPE (100)	0/10	6.10 ± 0.50
ZEDDPE (30)	0/10	6.90 ± 0.74*
Phenytion (20 mg/kg)	10/10	-

Data are expressed as Mean ± SEM (n = 10) and analysed by one-way ANOVA followed by Dunnett's test, *$p<0.05$ compared to control (Carboxymethylcellulose, 2%).

Table 3: Effect of EHPM, ZHPM and Sodium Valproate on Subcutaneous Pentylenetetrazole Induced Seizure in Mice

Treatments (mg/kg)	Quantal protection	Mean Onset of seizure(mins)
Olive oil (10 ml/kg)	1/6	8.00 ± 1.82
EHPM (112)	5/6	21.00 ± 0.00
EHPM (56)	5/6	14.00 ± 0.00
EHPM (28)	2/6	17.00 ± 3.46*
ZHPM (111)	4/6	10.50 ± 1.50
ZHPM (55.5)	4/6	20.00 ± 5.00*
ZHPM (27.75)	2/6	18.50 ± 1.94*
Sodium valproate (200 mg/kg)	6/6	-

Data are expressed as Mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's test, *$p<0.05$ compared to control (Olive oil, 10 ml/kg).

Table 4: Effect of EEDDPE, ZEDDPE and Sodium Valproate on Subcutaneous Pentylenetetrazole Induced Seizure in Mice

Treatments (mg/kg)	Quantal Protection	Mean Onset of seizure (mins)
Carboxymethylcellulose (2%)	1/6	9.60 ± 1.75
EEDDPE (300)	1/6	13.20 ± 2.27
EEDDPE (100)	2/6	11.75 ± 3.82
EEDDPE (30)	2/6	13.00 ± 1.47
ZEDDPE (300)	6/6	-
ZEDDPE (100)	2/6	5.50 ± 1.04
ZEDDPE (30)	1/6	14.80 ± 3.12
Sodium valproate (200 mg/kg)	6/6	-

Data are expressed as Mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's test, $p<0.05$ compared to control (Carboxymethylcellulose, 2%).

Table 5: Effect of EHPM, ZHPM and Diazepam on Motor Coordination in Mice

Treatments (mg/kg)	Number of fall (Mean ± SEM)	Time spent on beam (Mean ± SEM)
Olive oil (10 ml/kg)	0.00 ± 0.00	14.60 ± 1.60
EHPM (112)	0.00 ± 0.00	14.60 ± 1.63
EHPM (56)	0.00 ± 0.00	13.60 ± 2.40
EHPM (28)	0.00 ± 0.00	13.40 ± 1.75
ZHPM (111)	0.00 ± 0.00	14.60 ± 1.94
ZHPM (55.5)	0.00 ± 0.00	10.40 ± 0.81
ZHPM (27.75)	0.00 ± 0.00	10.20 ± 1.39
Diazepam (2 mg/kg)	2.40 ± 1.03*	51.40 ± 4.39

Data are expressed as Mean ± SEM (n = 5) and analysed by one-way ANOVA followed by Dunnett's test, *$p<0.05$ compared to control (Olive oil, 10 ml/kg).

Table 6: Effect of EEDDPE, ZEDDPE and Diazepam on Motor Coordination Mice

Treatments (mg/kg)	Number of fall (Mean ± SEM)	Time spent on beam (Mean ± SEM)
Carboxymethylcellulose (2%)	0.00 ± 0.00	14.60 ± 1.60
EEDDPE (300)	0.40 ± 0.25	30.60 ± 12.03
EEDDPE (100)	0.00 ± 0.00	26.40 ± 1.72
EEDDPE (30)	0.00 ± 0.00	22.20 ± 4.28
ZEDDPE (300)	0.40 ± 0.25	14.60 ± 3.34
ZEDDPE (100)	0.00 ± 0.00	26.80 ± 5.86
ZEDDPE (30)	0.00 ± 0.00	20.20 ± 4.35
Diazepam (2 mg/kg)	2.40 ± 1.03*	51.40 ± 4.39

Data are expressed as Mean ± SEM (n = 5) and analysed by one-way ANOVA followed by Dunnett's test, *$p<0.05$ compared to control (Carboxymethylcellulose, 2%).

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(1E,2E)-N,N^{I}-dihydroxy-1,2-diphenylethanone-1,2-diimine (3): Yield: 52.9%, mp: 191-192^{0}C. ^{1}H-NMR (DMSO, 400 MHz) δ: 7.40 (d, 1H, *J* = 8 Hz) and δH 7.32 (d, 1H, *J* = 8 Hz). ^{13}C-NMR (DMSO, 400 MHz): δC 132.85 (C1 and C8), 155.34 (C2), 127.57 (C3, C7, C10 and C14), 127.85 (C4, C6, C11 and C13), 128.93 (C5).

(1Z,2E)-N,N^{I}-dihydroxy-1,2-diphenylethanone-1,2-diimine (4): Yield: 65%, mp: 209-211^{0}C. ^{1}H-NMR (DMSO, 400 MHz) δ: 7.39 (d, 1H, *J* = 4 Hz) and δH 7.35 (d, 1H, J=4Hz). ^{13}C-NMR (DMSO, 400 MHz): δC 132.84 (C1 and C8), 155.36 (C2), 127.59 (C3, C7, C10 and C14), 127.85 (C4, C6, C11 and C13) and 128.92 (C5).

As shown in Table 1-6, compound EHPM protected the chicks against tonic hind limb extension induced by maximal electroshock, it offered 60% protection at a dose of 0.5% (56 mg/kg) while compound ZHPM did not protect the chick against tonic hind limb extension induced by maximal electroshock. The compounds, EEDDPE and ZEDDPE did not protect the chicks against tonic hind limb extension induced by maximal electroshock at a doses of 30, 100, 300 mg/kg, respectively. Both compounds, EHPM and ZHPM showed a good protection against subcutaneous pentylenetetrazole induced seizure in mice at doses of 27.75, 55.5 and 111 mg/kg for EHPM and doses of 28,56 and 112 mg/kg for ZHPM. Compound ZEDDPE displayed an appreciable activity against subcutaneous pentylenetetrazole-induced seizure in mice, it protected 100% at a dose of 300 mg/kg. But compound EEDDPE show know appreciable activity against subcutaneous pentylenetetrazole-induced seizure test model. All the synthesized oximes were found not to produce significant motor coordination deficit at the anticonvulsant doses tested indicating that they are not neurotoxic.

Conclusion

Compounds EHPM, ZHPM, EEDDPE and ZEDDPE were synthesized with appreciable yields. Compound EHPM has shown good activity against both MES and scPTZ seizure tests. ZEDDPE exhibit appreciable activity against scPTZ seizure test. ZHPM has shown good activity against scPTZ seizure test but insignificant activity against MES seizure test. EEDDPE did not show any appreciable activity in the MES and scPTZ test.

EHPM and EEDDPE agree with Shindikar *et al*. pharmacophore model for anticonvulsant activity[13] while ZHPM and ZEDDPE did not agree with the model. But all of them were found not to be neurotoxic.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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