Research Article

Design and Synthesis of Novel Vicinal Diaryl ١,٢,٤triazole Derivatives with Potential Antiepileptic Activity

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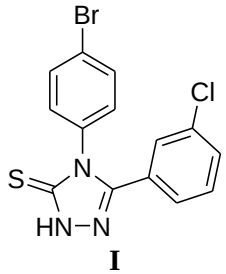
Abstract
A vicinal diaryl ١,٢,٤triazole derivativethat hasthe structure feature of antiepileptic drugs has been prepared and testedusingmaximal electroshock test(MES).The target compound gives ٥/١٠ versus ٩/١٠ protection ratio for phenytoin.
Key words: Antiepileptic drugs, vicinal diaryl,١,٢,٤triazole.

Introduction
Epilepsy is a serious neurological disease in the brain. As reported by WHO, more than ٥٠ million were affected by epilepsy worldwide. Epilepsy is characterized by recurrent seizures due to abnormal excessive discharge of cerebral neurons.[١] Epileptic seizures appear as involuntary movement, sensation, or thoughts. The disease is caused by various factors as cerebral organic lesion, head trauma, neurological disease, metabolic disorders, alcohol abuse, drug overdose or toxicities. Although antiepileptic mechanism is complex, there is some common chemical features between antiepileptic drugs as Hydrophobic domain (A), Electron donor atom (D), and Hydrogen acceptor /donor unit (HAD).[٢]

Hydrophobic domain is either substituted or unsubstituted aromatic ring, electron rich atom and hydrogen acceptor /donor unit and distal hydrophobic domain but not essential. Anticonvulsant activity is one of the most important biological activities of vicinal diphenyltriazoles. Luszczki et al., synthesized and evaluated anticonvulsant activity of ٤-(٤-bromophenyl)-٥-(٣-chlorophenyl)-٢,٤-dihydro-٣H-١,٢,٤-triazole-٣-thione(I), which significantly potentiated the anticonvulsant effect of phenobarbital, carbamazepine and sodium valproate. The mechanism of action had an apparent resemblance to sodium channel antagonists, especially to lamotrigine.



I

The targeted compound ٤ has these features; carboxylic group acts as (HAD), vicinal diaryl rings act as hydrophobic grouphave two proximal, and both amide nitrogen atom and $N_٢$ of triazole ring can act as electron rich atom.

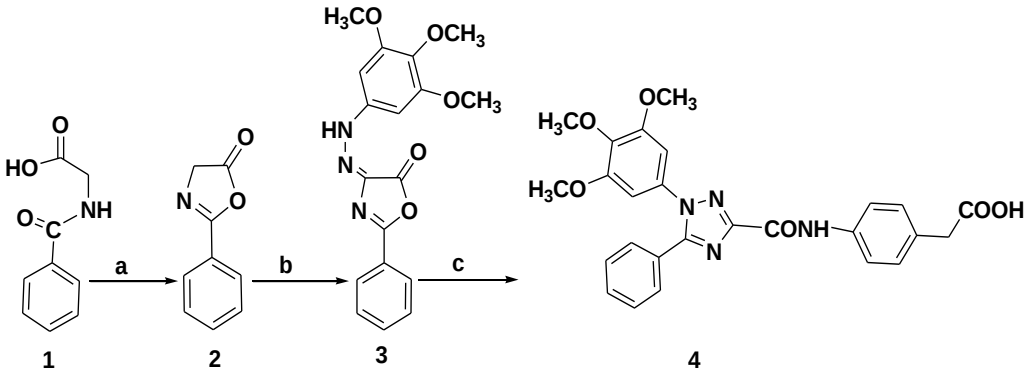
According to the above found results, the present work involves design, synthesis and evaluation of novel vicinal diaryl ١,٢,٤triazole derivativefor its anticonvulsant activity in maximal electroshock (MES)

Result and discussion

Chemistry

Key starting compound, ٢-(benzamido) acetic acid ١ was prepared in high yield (٨٥٪) by the reaction of glycine with benzoyl chloride in ١٠٪ NaOH. Heating of compound ١ with acetic anhydride afforded the corresponding lactone. The methylene group of lactonepossessesnucleophilic character and the degree of nucleophilicity depends on the electron withdrawing effect of the adjacent carbonyl group.

The synthesis of the key intermediate compound ٣ was carried out using Kuskov like reaction through coupling of the diazonium salt of ٣,٤,٥-trimethoxyaniline with the active methylene of lactone in presence of sodium acetate. The diazonium salt acts as an electrophile which was attacked by the carbanion derived from the active methylene group in lactone. This reaction is usually carried out in cold aqueous solution buffered with sodium acetate. The reaction yields an unstable azo compound, which spontaneously tauto-merizes to hydrazone derivative compound ٣ in ٧٠٪ yields. The target compound ٤ was obtained in a good yield by Sawdey rearrangement.[٣] The reaction involves attack of a nucleophile, ٢-(٤-aminophenyl) acetic acid with opening of the oxazoline ring to form acyclic carboxamide derivatives, which then recyclized via loss of water molecule to afford ١,٢,٤-triazole-٣-carboxamide derivative ٤ as shown in scheme ١.



Regents and conditions:a) Ac_2O, ٦٠°C, ٤٠ min. b) ٤-chloroaniline, HCl, NaOAc, ٢-٨°C, ٢ hr. c) ٢-(٤-aminophenyl)acetic acid, AcOH, NaOAc, reflux ٢ hr

Scheme ١. Synthesis of compound ٤

Biological evaluation (*in vivo*antiepileptic activity)

The resulted anticonvulsant activity of compounds ٤ was performed using phenytoin and sodium valproate as reference. Compound ٤ showed the same protection activities at ٠.٥ hr and ٤hr (٥/١٠ protection rate versus ٩/١٠ for phenytoin) (Table ١). It was observed that compound ٤ showed no activity against seizures for subcutaneous pentylenenetetrazole (scPTZ) preatreated mice while it had a weak activity against seizures for strychnine induced seizues.

Table (١): Anticonvulsant activities of compound٤

Compound	Anticonvulsant Activity					Neurotoxicity[d]	
	MES[a]		scPTZ[b]	Strychnine[c]			
	٠.٥ (hr)	٤.٠ (hr)		Onset of convulsion	Time of death	٠.٥ (h)	٤.٠ (h)
Control	٠/١٠	٠/١٠	٠%	١.١٤±٠.١٢	٣.١١±٠.٢٠	٠/١٠	٠/١٠
Compound٤	٥/١٠	٥/١٠	٠%	١.٢٢±٠.١٠	٤.١١±٠.١٧	٥/١٠	٥/١٠
Phenytoin sodium	٩/١٠	٨/١٠	N.D.	N.D.	N.D.	٣/١٠	٢/١٠
Sodium valproate	N.D.[e]	N.D.	٨٠	٥.٤٨±٠.١٢	٨.١٢±٠.٢٨	١/١٠	٠/١٠

[a]Maximal electroshock test (number of animals protected / number of animals tested)
[b] Subcutaneous pentylenetetrazole test (% protection)
[c]Intrapertonial strychnine test (onset of convulsions and time to death)
[d]Rotarod test (number of animals exhibiting toxicity / number of animals tested)
[e] Not Determined

Experimental

Chemistry

Materials and methods

Melting points were determined on Stuart electro-thermal melting point apparatus and are uncorrected.IR spectra were recorded on Nicolet iS٥ FT-IR spectrometer at Minia University. ^{١}H NMR spectra were carried out using Bruker apparatus ٤٠٠ MHz spectrometer, using TMS as internal reference.High resolution mass spectra (HRMS) were obtained on a Thermo Scientific Q Exactive™ Orbitrap mass spectrometer at The University of British Columbia Canada.Reactions were routinely monitored by thin-layer chromatography (TLC) using Merck ٩٣٨٥ pre-coated aluminum plate silica gel (Kieselgel ٦٠) ٥ x ٢٠ cm plates with a layer thickness of ٠.٢ mm, and spots were visualized by exposure to UV-lamp at λ = ٢٥٤ nm.

Materials: Chemicals and solvents used in the preparation of the target compounds are of commercial grade, and purchased from Aldrich, Merck, Fluka, Cambrian chemicals, and El-Nasr pharmaceutical Chemicals Companies.

Synthesis of ٢-phenyl-٤*H*-oxazol-٥-one(٢)

Hippuric acid ١ (٢.٣٣ g, ٠.٠١٣ mol) in acetic anhydride (٧.٥ ml) was heated until a clear solution was obtained then this solution was cooled to room temperature.

Synthesis of ٤-(٣,٤,٥ trimethoxyphenyl)-hydrazono-٢-phenyl-٢-oxazolin-٥-one (٣)

Acetic anhydride (٧ mL) was added to hippuric acid ٦ (٠.٠١٣ mol, ٢.٣٣ g). The mixture was heated until a clear yellow solution was obtained. The solution was cooled to room temperature to form solution A. A mixture of ٣,٤,٥ trimethoxyaniline (٠.٠١٣ mol, ٢.٣٨g), and HCl (٥N, ٣ mL) was kept in an ice-salt bath. A solution of sodium nitrite (٠.٠١٣ mol, ٠.٨٩٧ g) in water (١ mL) was added in a drop wise manner, and the mixture was stirred for ١٠ min. Anhydrous sodium acetate (٠.٠١٨mol, ١.٥g) was added to the mixture to form solution B. Solution A was added to solution B in a drop wise manner and stirring was continued at ٠-١٠°C for ٢ hr. The formed precipitate was filtered off, washed with distilled water and dried to give compound ٣ as red precipitate (yield ٧٠٪); IR (KBr, cm-١): ١٧٨٤ (C=O),١٦٣٠ (C=N), ١٥٧٧ (C=C), ١٢٣٠ (C-O-C)

Synthesis ٢-(٤ –(١-(٣,٤,٥ trimetho xyphenyl)-٥-phenyl-١*H*-١,٢,٤-triazole-٣-carboxamido)phenyl) acetic acid (٤)

A mixture of compound٢ (٠.٠١ mol, ٢.٦٦ g), ٢-(٤-aminophenyl) acetic acid (٠.٠١٢ mol), and anhydrous sodium acetate (٠.٧٥ g) were dissolved in glacial acetic acid (٢٥ mL). The reaction mixture was stirred under reflux for ٢ hr, and was left to cool, and was poured to ice water. The precipitate formed was filtered off, washed with water, and recrystalized by reaction with saturated solution of sodium bicarbonate and warmed gently the resulted solution was filtered and other organic impurities with organic solvent, the aqueous solution was acidified with dil. HCl then filtered under vacuum and collected. Yellowish white crystals (٣.٨g,٧٨٪); IR ν (cm^{-1}): ٣٦٦٨(OH), ٣٣٠٢ (NH), ١٧١٧ (C=O), ١٦٧٨ (C=O), ١٥٩٠ (C=N); ^{1}H-NMR (٤٠٠ MHz, DMSO-d_6) δ (ppm): ٣.٦٥ (t, ٢H, HOOC-C**H**$_2$), ٣.٧١ (s,٦H, ٢ OC**H**$_2$), ٣.٨٩ (s,٣H, ٢ OC**H**$_2$), ٦.٦١ (s, ٢H, ٢Ar-H), ٧.٤٢-٧.٦٠(m, ١٠H, ١٠ Ar-H), ٧.٩٣(d,٢H,J = ٨ Hz, $H_{2',6'}$ of benzoic acid ring), ٨.٠٠(d, ٢H, J = ٨ Hz, $H_{3',5'}$ of benzoic acid ring), ٩.٠٨ (s, ١H, N**H**); ^{13}C-NMR (١٠٠ MHz, DMSO-d_6) δ (ppm):٤٠.١٦, ٥٦.٢٥, ٦٤.٠٥, ١٠٣.٠٤, ١٢٠.٠٣,١٢٦.٧٥, ١٢٦.٨٧, ١٢٨.٧٥, ١٢٩.٥٧, ١٣٠.٠٥, ١٣٠.٧١, ١٣٢.٤٩, ١٣٦.٦١, ١٣٨.٧٥, ١٥٣.٥٢, ١٥٤.٨٥, ١٥٦.٢١, ١٥٦.٦٢, ١٧٥.٧٦ HRMS: m/z calculated for $C_{١٧}H_{١٤}N_٤O_٢$ $[M-H]^+$: ٤٨٧.١٦٢٣١, found: ٤٨٧.١٦٢٦٦

Biological evaluation

Maximal electroshock (MES) model

Thirty male albino mice weighting ٢٥-٣٥ g were divided into three equal groups each of ten. The firstgroup was used as a control group administered dimethyl sulfoxide (DMSO) only as a vehicle intraperitonealy. The second group was administered phenytoin sodium. The third was administered test compound. Compound ٤ and phenytoin sodium were dissolved in DMSO and injected intraperitonealy to the animal at dose ١٠٠ mg/kg body weight one hour before MES test. Seizures were induced by means of ٦٠ Hz current of ٦٠ mA delivered through ear electrodes [Hugo Basile-Italy]. The stimulus duration was ٠.٢ s and pulse width was ٠.٤. The criterion to indicate the convulsion response was the hind limb tonic extension (HLTE). The presence or absence of HLTE was noted. Animal in which extensor response was abolished were taken as protected mice.[٤-٥]

Evaluation of *in vivo* (antiepileptic activity Subcutaneous pentylenetetrazole (scPTZ) model)

Thirty male albino mice weighting ٢٥-٣٥ g were divided into three equal groups each of five and acclimatized to their environment for at least one week before the experiment. The first group was used as a control group administered DMSOonly as a vehicle intraperitonealy. The second group was administered sodium valproate. The third group was administered test compounds.

The test compound and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose ١٠٠ mg/kg body weight. One hour later, mice were injected with PTZ ٧٠ mg/kg body weight in scruff of the neck.

The dose of PTZ was selected by preliminary screening as lower dose failed to induce typical seizures while higher doses only increased the mortality. Animals devoid of generalized convulsions were considered to be protected and the results were represented as protection percent.[٦]

Intraperitonealy strychnine HCl induced convulsions

Thirty male albino mice weighting ٢٥-٣٥ g were divided into three equal groups each of ten. The first group was used as a control group administered DMSO only as a vehicle intraperitonealy. The second group was administered sodium valproate.

The third group was administered test compound. Compound ٤ and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose ١٠٠ mg/kg body weight. One hour later, mice were injected with strychnine HCl at dose ٢ mg/kg body weight intraperitonealy. The onset of convulsions and time of death was calculated in comparison with control group.[٧]

Neurotoxicity (Rotarod test)

Minimal motor impairment was measured in mice by rotarod test. Thirty male albino mice weighting ٢٥-٣٥ g were trained to stay

on accelerating rotarod that rotates at ١٠ r.p.m., the rod diameter was ٣.٢ cm. and trained animals were divided into four equal groups each of ten. The first group was used as a control group administered DMSO only as a vehicle intraperitonealy. The second group was administered phenytoin sodium. The third group was administered sodium valproate. The fourth group was administered test compound. Compound ٤, phenytoin sodium and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose ١٠٠ mg/kg body pweight. Neurotoxicity was indicated by the inability of the animals to maintain equilibrium on the rod for at least ١ minute in each of three trials.[٨]

Conclusion

The similarities of the selected compound to phenytoin not only on the structural features required for anticonvulsant activity, but also it resembles phenytoin on its mechanism of anticonvulsant activity. Compound ٤ have MES induced seizures inhibition less than phenytoin. It also has minimal activity against strychnine and scPTZ induced convu-lsions. Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission.[٩] Compound ٤ failed to protect animals from seizure induced by Strychnine at the dose of ٣٠٠ mg/kg. It is known that Strychnine directly antagonizes the inhibitory spinal reflexes of glycine,[١٠] so the results suggest that compound ٤ could neither influence glycine system nor gabergic neuro-transmission.

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