Research Article

Design, synthesis and biological evaluation of novel 1-(4-chlorophenyl)-5-phenyl-*1H*-1,2,4-triazole-3-carboxamide derivative with potential anticonvulsant activity

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Abstract

3-(1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido) propanoic acid was synthesized and evaluated for its anticonvulsant activity using maximal elecroshoch (MES) and chemoshock (scPTZ and Strychnine) animal screen methods. Neurotoxicity was also assessed. In MES model, it showed more protection than phenytoin after 4 hr. In Strychnine model, it showed moderate activityin comparing with sodium valproate. And it showed no neurotoxicity.

Key words: 1,2,4-Triazoles, anticonvulsants, neurotoxicity, electroshock, chemoshock.

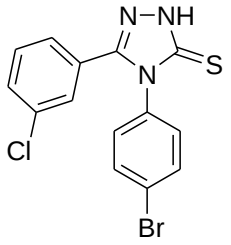
Introduction

Epilepsy is one of the most widespread neurological diseases, itaffects about 65 million people worldwide, and one third of them are uncontrolled by currently used anticonvulsants.[(1)]Approximately 95% of currently usedmedications were approved before 1985 and they cause undesirable effects such as drowsiness, ataxia, GI disturbance, hepatotoxicity, megaloblastic anemia and even life-threatening conditions.[(2)]

Phenytoin is a traditional anticonvulsant drug which has also other medical uses in cardiac arrhythmia, Digoxin toxicity[(3)], Trigeminal neuralgia[(4)] and topically in wound healing[(5)]. However, clinical use of phenytoin is limited due to its CNS adverse effects and its interaction with metabolism of several drugs.[(6)]

There are four pharmacophoric elements that are necessary for good anticonvulsant activity as suggested by Pandeya et al., These elements are present in many currently used antiepileptic drugs and include hydrophobic domain (A), hydrogen bonding domain (HBD), electron donor moiety (D), and distal hydrophobic domain (R).[(7)]

Anticonvulsant activity is one of the most important biological activities of vicinal diphenyltriazoles. Luszczki et al., synthesized and evaluated anticonvulsant of 4-(4-bromophenyl)-5-(3-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-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.[(8)]



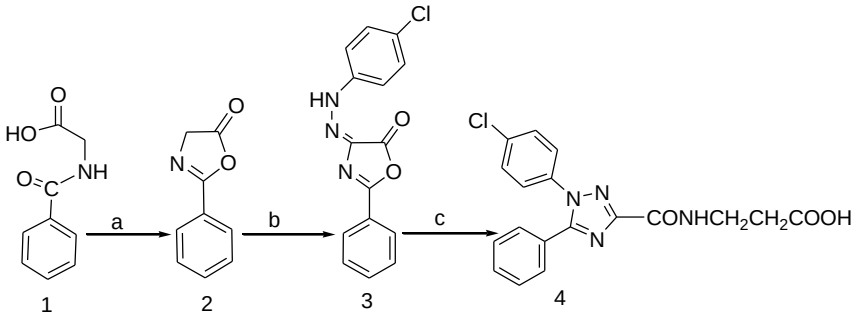
(I)

According to the above found results, we design, synthesize and evaluate 1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamide derivative for its anticonvulsant activity in both maximal electroshock (MES) and chemoshock (scPTZ and Strychnine models).

Results and discussion

Chemistry

The synthetic route used to prepare 1-(4-chlorophenyl)-5-phenyl-1H-1,2,4-triazole-3-carboxamides is outlined in Scheme 1. Key starting compound**1**, hippuric acidwas prepared in good yield (85%) by the reaction of glycine with benzoyl chloride in 10% NaOH. Heating of compound **1** in acetic anhydride afforded compound **2**. The synthesis of the key intermediate **3** was carried out using Kuskov like reaction through coupling of the diazonium salt of 4-chloroaniline with the active methylene of compound **2** in presence of sodium acetate. According to Sawdey rearrangement[9], reaction of compound **3** with β-alanine in glacial acetic acid was carried out in presence of sodium acetate to give the target compound **4**.



Scheme 1: Regents and conditions:a) Ac_2O, 60ºc, 40 min. b) 4-chloroaniline, HCl, NaOAc, 2-8ºc, 2 hr. c) $H_2NCH_2CH_2COOH$, AcOH, NaOAc, reflux 2 hr.

Biology

Results of the anticonvulsant screening for the target compound **4** as well as reference standards phenytoin sodium and sodium valproate are summarized in Table1.

In MES model, Compound **4** showed delayed onset of action but after 4 hr showed more protection than phenytoin. And it was inactive in scPTZ model. In Strychnine model, it showed moderate anticonvulsant activity in comparing with sodium valproate. And it showed no neurotoxicity.

Table (1)

Compound	Anticonvulsant Activity					Neurotoxicity[d]	
	MES[a]		scPTZ[b]	Strychnine[c]			
	0.5 (hr)	4.0 (hr)		Onset of convulsion	Time of death	0.5 (h)	4.0 (h)
Control	0/8	0/8	0	1.12±0.11	3.33±0.20	0/10	0/10
4	3/10	10/10	0	3.11±0.12	4.11±0.17	0/10	0/10
Phenytoin sodium	9/10	8/10	N.D.	N.D.	N.D.	3/10	2/10
Sodium valproate	N.D.[e]	N.D.	80	5.11±0.17	9.12±0.28	2/10	1/10

[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

Melting point was determined on Stuart electro-thermal melting point apparatus and is uncorrected.IR spectrum was recorded on Nicolet iS5 FT-IR spectrometer at Minia University.^{1}H-NMR spectrum was carried out using Bruker apparatus 400 MHz spectrometer, using TMS as internal reference at Beni-Suif University.High resolution mass spectrum (HRMS) was obtained on a Thermo Scientific Q Exactive™ Orbitrap mass spectrometer. Reactions were routinely monitored by thin-layer chromatography (TLC) using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) 5 x 20 cm plates with a layer thickness of 0.2 mm, and spots were visualized by exposure to UV-lamp at $\lambda = 254$ 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 2-phenyl-4*H*-oxazol-5-one *2*

Hippuricacid**1** (0.013 mol, 2.33 g) in acetic anhydride (7.5 ml) was heated until a clear solutionwas obtained; this solution was cooled to room temperature.

Synthesis of 4-[(4-chlorophenyl) hydrazono]-2-phenyl-4*H*-oxazol-5-one *3*

To a cold solution of 4-chloroaniline (0.01 mol, 1.275 g) in 5 N HCl (3.5 ml) in an ice-salt bath 0-5^{o}C, a solution of sodium nitrite (0.013 mol, 0.897 g) in water (5 ml) was added in a dropwise manner. The reaction mixture was left for 10 min. Solution of compound **2** was added in presence of anhydrous sodium acetate (0.018 mol, 1.5 g). The reaction mixture was stirred at 0-10^{o}C for 2 h, the formed precipitate was filtered off and dried (crude yield 70%). the product was crystallized from acetone; IR (KBr, cm^{-1}): 1795 (C=O), 1630 (C=N), 1520 (C=C), 1230 (C-O-C).

Synthesis of 3-(1-(4-chlorophenyl)-5-phenyl-4*H*-1,2,4-triazole-3-carboxamido) propanoic acid *4*

A mixture of compound **3** (0.01 mol, 2.995 g) and β-alanine (0.01 mol, 0.89 g)was refluxed in acetic acid (50 ml) in the presence of anhydrous sodium acetate (0.018 mol, 1.5 g) for 2 hours. The reaction mixture was cooled and poured into ice water (50 ml). The formed precipitate was filtered off, dried and crystallized from aqueous methanol.

Pale yellow crystals (1.74 g; 47% yield); mp 193°C; IR (KBr, cm^{-1}):3301 (OH),1669 (C=O) 1588 (C=N); ^{1}H-NMR (400 MHz,

$CDCl_3$) δ (ppm): 2.66 (t,2H, aliphatic CH_2), 3.79 (t, 2H, aliphatic CH_2), 7.33 (d, 2H, *J* = 8.40 Hz, Ar-H), 7.41 (d, 2H, *J* = 8.40 Hz, Ar-H) , 7.49-7.58 (m, 5H, Ar-H), 7.98 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 33.69, 34.95, 126.27, 126.63, 128.91, 129.08, 129.71, 130.99, 135.51, 135.90, 155.00, 156.18, 158.95, 176.02; HRMS: m/z calculated for $C_{18}H_{15}ClN_4O_3$ $[M-H]^+$: 369.07599, found: 369.07507.

Biology

Anticonvulsant activity

Maximal electroshock (MES) model

One hundred male albino mice weighting 25-35 g were divided into ten equal groups each of ten. The first group was used as a control group administered dimethyl sulfoxide (DMSO) only as a vehicle intraperitonealy. The second group was administered phenytoin sodium. The groups from 3-10 was administered test compounds. All the test compounds and phenytoin sodium were dissolved in DMSO and injected intraperitonealy to the animal at dose 100 mg/kg body weight one hour before MES test. Seizures were induced by means of 60 Hz current of 60 mA delivered through ear electrodes [Hugo Basile-Italy]. The stimulus duration was 0.2 s and pulse width was 0.4. 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.[10-11]

Subcutaneous pentylenetetrazole (scPTZ) model

Fifty male albino mice weighting 25-35 g were divided into ten 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 DMSO only as a vehicle intraperitonealy. The second group was administered sodium valproate. The groups from 3-10 was administered test compounds. All the test compounds and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose 100 mg/kg body weight. One hour later, mice were injected with PTZ 70 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.[11-12]

Intraperitonealy strychnine HCl induced convulsions

One hundred male albino mice weighting 25-35 g were divided into ten 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 groups from 3-10 was administered test compounds. All the test compounds and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose 100 mg/kg body weight. One hour later, mice were injected with strychnine HCl at dose 2 mg/kg body weight intraperitonealy. The onset of convulsions and time of death was calculated in comparison with control group.[13]

Neurotoxicity (Rotarod test)

Minimal motor impairment was measured in mice by rotarod test. One hundred and ten male albino mice weighting 25-35 g were trained to stay on accelerating rotarod that rotates at 10 r.p.m. the rod diameter was 3.2 cm. trained animals were divided into eleven 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 groups from 4-11 was administered test compounds. All the test compounds, phenytoin sodium and sodium valproate were dissolved in DMSO and injected intraperitonealy to the animal at dose 100 mg/kg body pweight. Neurotoxicity was indicated by the inability of the animals to maintain equilibrium on the rod for at least 1 minute in each of three trials.[14]

Conclusion

A novel 3-(1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acidwas prepared and characterized by different spectroscopic and Mass analysis

techniques. The synthesized compound showed significant anticonvulsant activity against both electroshock and chemoshock patterns with acceptable safety profile on CNS.

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