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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, anticonvulsant activity, and pharmacophore study of new 1,5-diaryl-1*H*-1,2,4-triazole-3-carboxamide derivatives

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Abstract 1,5-Diaryl-1*H*-1,2,4-triazole-3-carboxamide derivatives were designed, synthesized, and evaluated for its anticonvulsant activity using maximal electroshock (MES) and chemoshock (scPTZ and Strychnine) animal screen methods. Neurotoxicity was also assessed. In MES model, compound **4f** showed 100% of phenytoin activity after both 0.5 and 4 h. In scPTZ model, compound **4e** showed 100% of sodium valproate activity. In Strychnine model, compound **4e** showed 120% more delay of onset of convulsion and 124% more delay of time of death relative to sodium valproate. Most of the target compounds showed mild neurotoxicity especially compound **4f** which showed excellent activity against electroshock. Pharmacophoric study reveals that the synthesized compounds showed good fitting on the pharmacophoric query with good RMSDX results.

Keywords 1,2,4-Triazoles · Anticonvulsants · Neurotoxicity · Electroshock · Chemoshock

Introduction

Epilepsy is a worldwide disease that affects about 50 million around the world being about 90% of the patients from developing countries. The disease affects people mainly in young age usually before the age of 10. Epilepsy is characterized by a group of chronic neurological disorders as paraoxymal cerebral dysrhythmia that can be manifested as brief episode of disturbance of consciousness with or without convulsions (Wasterlain et al. 1989).

Despite the importance of that disease, the available antiepileptic drugs are limited and until now there is not any complete cure for that disease. The available antiepileptic drugs are capable of suppressing the seizures but they do not affect the full course epileptogenic process (Hassan et al. 2012). Many researchers have been working in the last decades to develop new antiepileptic agents that would be more selective and with lower toxicity (Kulandasamy et al. 2009).

Antiepileptic drugs are a wide range of compounds that mostly act through enhancement of the suppressant neurotransmitter; gamma aminobutyric acid (GABA) or through modulation of voltage-gated ion channel and or through inhibition of synaptic excitation mediated by glutamate receptors (Rho and Sankar 1999; Czapinski et al. 2005; DeLorenzo et al. 2006; De Smedt et al. 2007).

The major difficulty with treatment by antiepileptic drugs arises from the unknown mechanism of resistance (Löscher et al. 2013; Schmidt and Schachter 2014). Moreover, the

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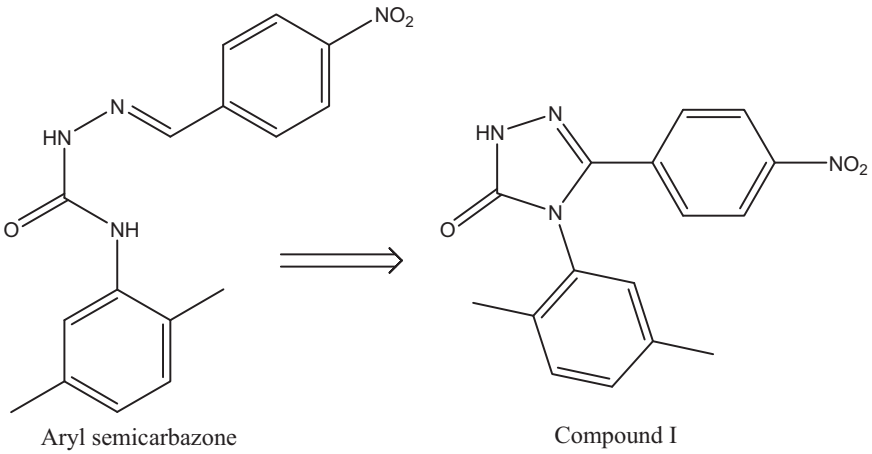


Fig. 1 Structures of the parent semicarbazone and compound **I**

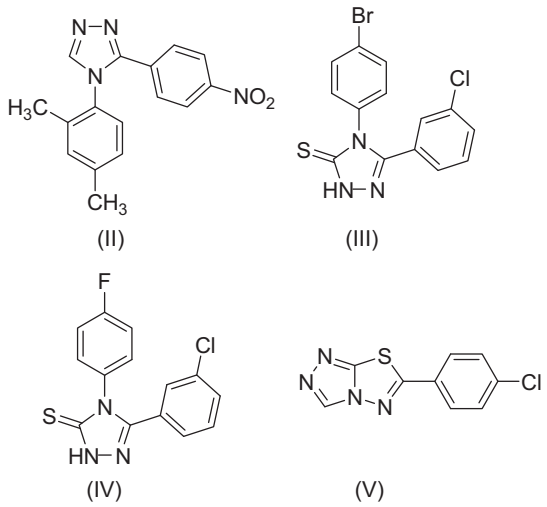


Fig. 2 Chemical structures of some reported anticonvulsant triazole derivatives

effectiveness of the available marketed drugs is largely compromised by notable adverse effects in patients (Lin and Kadaba 1997). Neurotoxicity, impaired memory function, symptoms of depression with life threatening hepatotoxicity, and megaloblastic anemia are examples of such adverse effects (Kwan and Brodie 2001; Perucca and Gilliam 2012; Kamiński et al. 2016).

The literature showed that the effective antiepileptic drugs may have some common features in their structures, which are (i) the hydrogen-bonding domain (usually phenyl rings); and (ii) the electron-donating fragment. Recently, triazole derivatives have been widely used in the last decade as anticonvulsant drugs (Kamboj et al. 2015; Zayed et al. 2017).

Many triazole derivatives have been synthetized and evaluated for their anticonvulsant activities and showed promising results.

Many triazole derivatives have been synthetized and evaluated for their anticonvulsant activities and showed promising results. Shalini et al. synthesized 4,5-diphenyl-2*H*-1,2,4-triazole-3(4 *H*)-one derivatives from aryl semicarbazones, which have been known by its good antiepileptic activity through blocking the voltage-gated sodium ion channels.

They compared the pharmacophore of some 1,2,4-triazole derivatives, e.g. compound **I**, with the parent aryl semicarbazones by merging the energy-minimized structures. The selected 1,2,4-triazoles seem to show a root mean square deviation in the range of 0.228–0.296 A° (Fig. 1) (Shalini et al. 2009).

Also, compound **II** showed protection in maximal electroshock (MES) through GABA mediation. Moreover, compound **II** was subjected to neurochemical estimation in GABA level in rat brain after 100 mg/kg administration that causes GABA level to increase more than 10 times compared to the control (Fig. 2) (Shalini et al. 2009).

Compound **III** was synthesized and evaluated by Luszczki et al. significantly potentiated the anticonvulsant effect of phenobarbital, carbamazepine, and valproate. The mechanism of action had an apparent resemblance to sodium channel antagonists, especially to lamotrigine (Fig. 2) (Luszczki et al. 2012).

Plech et al. synthesized and evaluated the anticonvulsant activity of compound **IV**, whose activity was 5.5 times more potent than valproate (Fig. 2) (Plech et al. 2014a, b).

Moreover, some triazolothiadiazole derivatives were synthesized and evaluated for their anticonvulsant activities. Among which, compound **V** emerged with the most promising anticonvulsant activity (Fig. 2) (Deng et al. 2012).

Based on the above results and in continuation of our efforts (Metwally et al. 2007) directed toward the search for new compounds with anticonvulsant activities getting to be safer, more selective and more effective, a new series of 1,5-diaryl triazoles were designed and synthesized in this study (Fig. 3). The design of the compounds depends on the presence of the triazole ring as the electron-donating fragment with diaryl groups on position no. 1 and 5 presenting

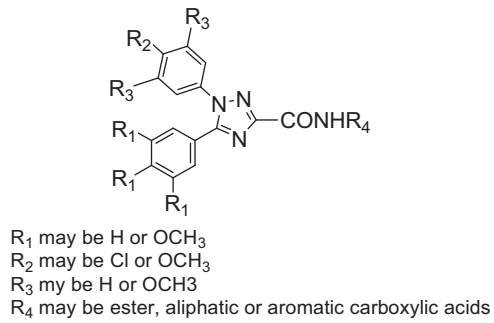
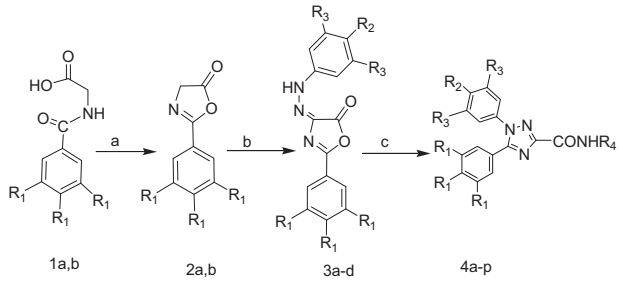


Fig. 3 Design of the synthesized compounds **4a–p**



Scheme 1 Regents and conditions. **a** Ac_2O, 60 °C, 30 min, **b** Diazonium chloride of aniline, 4-chloroaniline or 3,4,5-trimethoxyaniline, NaOAc, 2–8 °C, 2 h, **c** H_2NR_4, AcOH, NaOAc, reflux 2 h

the hydrogen-bonding domain as previously discussed (Kamboj et al. 2015; Zayed et al. 2017). Different substitution on both rings (R_1=H or OCH_3, R_2=Cl or OCH_3, R_3=H or OCH_3; Fig. 3) in order to evaluate the effect of electron-donating and electron-withdrawing groups on the anticonvulsant activity. Different linkers on the triazole ring (R_4=aliphatic or aromatic acids or esters; Fig. 3) were also used in order to evaluate the effect of lipophilicity on the anticonvulsant activity. The compounds were evaluated for their anticonvulsant activity using MES and chemoshock (subcutaneous pentylenetetrazole (scPTZ) and Strychnine) animal screen methods. Also, the neurotoxicity of the synthetized compounds was evaluated using rotarod test. For explaining the possible mechanism of action, the most active compounds were selected for the fitting on the pharmacophoric query.

Results and discussion

Chemistry

The synthetic route used to synthesize 1,5-diphenyl-1*H*-1,2,4-triazole-3-carboxamides **4a–p** is outlined in Scheme 1. Key starting compounds 2-benzamidoacetic acid (hippuric acid) **1a** and 2-3,4,5-trimethoxybenzamidoacetic acid **1b** were prepared in good yield (85%) by the reaction of glycine with benzoyl chloride or 3,4,5-trimethoxybenzoyl chloride in 10% NaOH, respectively. Heating of compounds **1a, b** with acetic anhydride afforded the corresponding compounds **2a, b**, respectively. The synthesis of the key intermediates **3a–d** was carried out using Kuskov-like reaction through coupling of the diazonium salts of aniline, 4-methoxyaniline, or 4-chloroaniline with the active methylene of compounds **2a, b** in presence of sodium acetate. According to Sawdey rearrangement, reaction of compounds **3a–d** with appropriate amino acid in acetic acid in presence of sodium acetate gave the corresponding amides **4a–p** in 55–71% yield (Sawdey 1957; Aly et al. 2014). The structures of synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, and HRMS. The physical and spectral data are listed in the experimental part.

Biological investigations

The anticonvulsant activity of compounds **4a–p** was screened in different animal models like MES, scPT, and i. p. strychnine. Moreover, rotarod test was used to evaluate the neurotoxicity of the selected compounds (Table 1).

Results of the anticonvulsant screening of the target compounds as well as reference standards phenytoin sodium and sodium valproate are summarized in Table 2.

In MES model, tested compounds and phenytoin were injected i.p. at dose of 100 mg/kg 1 h before test. Seizures induced by means of 60 Hz current of 60 mA and the stimulus duration was 0.2 s. Animals in which extensor response was abolished were taken as protected mice.

Most compounds showed parallel activity pattern with phenytoin. Compound **4b** that has an aromatic linker and unsubtituted phenyl ring on triazole showed 78 and 62% of phenytoin activity after 0.5 and 4 h, respectively. Also, other compounds containing aromatic linkers; compounds (**4e**, **4f**, **4n**, and **4o**) showed good anticonvulsant activity. Incorporation of aromatic moiety in (R_4) substitution on C3 of triazole ring in compounds **4b**, **4e**, **4f**, **4n**, and **4o** represent distal hydrophobic domain that enhanced anticonvulsant activity (Prakash and Raja 2011). Compound **4e** showed 89 and 75% of phenytoin activity after 0.5 and 4 h, respectively. Compound **4f** showed 100% of phenytoin activity after both 0.5 and 4 h, respectively. Compound **4n** showed 100 and 88% of phenytoin activity after 0.5 and 4 h, respectively. Compound **4o** showed 67 and 63% of phenytoin activity after 0.5 and 4 h, respectively. Compound **4a** with no substitutions (R_1=H) on phenyl ring on C5 exhibited moderate activity (44% of phenytoin activity), while compound **4c** with chloride substitution (R_2=Cl) on phenyl ring on N1 of triazole showed 89 and 88% of phenytoin activity after 0.5 and 4 h, respectively. Compounds **4g** and **4h** with trimethoxy substitutions (R_1=OCH_3) on phenyl ring on C5 of trizaole showed reasonable activity

Table 1 The prepared compounds and the calculated LogP of the target compounds

Compounds	R_1	R_2	R_3	R_4	cLogP[a]
1a	H	–	–	–	
1b	OCH_3	–	–	–	
2a	H	–	–	–	
2b	OCH_3	–	–	–	
3a	H	H	H	–	
3b	H	Cl	H	–	
3c	H	OCH_3	OCH_3	–	
3d	OCH_3	Cl	H	–	
4a	H	H	H	$-CH_2COOH$	3.10+/− 0.66
4b	H	H	H	$4-C_6H_4-COOH$	5.32+/− 0.65
4c	H	Cl	H	$-CH_2COOH$	3.69+/− 0.67
4d	H	Cl	H	$-(CH_2)_2COOH$	3.86+/− 0.66
4e	H	Cl	H	$-4-C_6H_4-CH_2COOH$	5.09+/− 0.66
4f	H	Cl	H	$-4-C_6H_4-COOEt$	6.75+/− 0.66
4g	H	OCH_3	OCH_3	$-CH_2COOH$	2.54+/− 1.37
4h	H	OCH_3	OCH_3	$-(CH_2)_2COOH$	2.70+/− 1.36
4i	H	OCH_3	OCH_3	$-(CH_2)_3COOH$	2.92+/− 1.36
4j	H	OCH_3	OCH_3	$4-C_6H_4-COOH$	4.76+/− 1.36
4k	H	OCH_3	OCH_3	$4-C_6H_4-CH_2COOH$	3.94+/− 1.36
4l	H	OCH_3	OCH_3	$4-CH_2-C_6H_4-COOH$	4.35+/− 1.36
4m	OCH_3	Cl	H	$-(CH_2)_2COOH$	4.05+/− 0.68
4n	OCH_3	Cl	H	$4-C_6H_4-COOH$	6.11+/− 0.68
4o	OCH_3	Cl	H	$4-CH_2-C_6H_4-COOH$	5.70+/− 0.68
4p	OCH_3	Cl	H	$-4-C_6H_4-COOEt$	6.94+/− 0.68

[a] LogP was calculated using ACD/Labs 2015.2.5 (freeware version)

(89 and 67% of phenytoin activity after 0.5 h, respectively). There is no significant difference in activity between esters and carboxylic acids.

In scPTZ model, tested compounds and sodium valproate were injected i.p. at dose 100 mg/kg. 1 h later, mice were injected with PTZ 70 mg/kg in the scruff of the neck. Animals devoid of generalized convulsions were considered to be protected.

Compound **4b** showed 50% of sodium valproate activity, while compound **4e** showed 100% of sodium valproate activity. Compounds **4f**, **4j**, and **4p** had weak activity and other compounds were inactive. These results revealed that compounds with aromatic substitutions had higher anticonvulsant activity than that with aliphatic substitutions.

In i.p. strychnine model, tested compounds and sodium valproate were injected i.p. at dose of 100 mg/kg. 1 h later, mice were i.p. injected with strychnine 2 mg/kg. The onset of convulsions and time of death was calculated in comparison with control group.

Compound **4e** showed 120% more delay of onset of convulsion and 124% more delay of time of death relative to sodium valproate. Other tested compounds showed weak to moderate protection against strychnine model.

All target compounds showed no or minimal neurotoxicity except compounds **4a**, **4d**, **4k**, and **4m** that had moderate neurotoxicity.

To summarize, compounds with 4-chlorophenyl moiety at N1 of triazole ring (compounds **4c–f** and **4m–p**) showed more protection especially against electroshock than that with unsubstituted phenyl group and this may be attributed to the electron-withdrawing effect of chloro group (Luszczki et al. 2012; Plech et al. 2013; Plech et al. 2014a, b). Trimethoxy aryl compounds were reported to have anticonvulsant activity (Prakash et al. 2010); compound **4n** of the trimethoxy substituted derivatives with phenyl group on C5 of triazole ring showed good protection against electroshock (100 and 88% of phenytoin activity after 0.5 and 4 h, respectively), and with reasonable neurotoxicity as well.

Also compounds with aryl carbamoyl moiety C5 of triazole ring (compounds **4b**, **4e**, **4f**, **4j**, **4k**, **4l**, **4n–p**) showed more protection than those with alkyl carbamoyl group as it represents a distal hydrophobic domain that enhanced the anticonvulsant activity (Prakash and Raja 2011).

Table 2 Anticonvulsant activity of compounds **4a–p** against MES and chemoshock-induced seizures and neurotoxicity in mice with comparison to that of phenytoin and valporoate

Compounds	Anticonvulsant activity					Neurotoxicity[d]	
	MES[a]		Chemoshock				
	0.5 h	4.0 h	scPTZ[b]	i.p. Strychnine[c]		0.5 h	4.0 h
				Onset of convulsions convulsion (h)	Time of death		
Control	0/10	0/10	0	1.12 ± 0.11	3.33 ± 0.20	0/10	0/10
4a	4/10	4/10	0	1.30 ± 0.10**	3.18 ± 0.13**	6/10	5/10
4b	7/10	5/10	40	3.22 ± 0.12*	4.11 ± 0.19*	5/10	3/10
4c	8/10	7/10	0	1.12 ± 0.10**	3.22 ± 0.18**	1/10	0/10
4d	3/10	1/10	0	1.11 ± 0.10**	3.22 ± 0.16**	6/10	5/10
4e	8/10	6/10	80	6.12 ± 0.15*	11.33 ± 0.30*	5/10	4/10
4f	9/10	8/10	20	3.10 ± 0.15*	5.22 ± 0.18*	2/10	1/10
4g	8/10	7/10	20	1.23 ± 0.13**	3.18 ± 0.12**	3/10	2/10
4h	6/10	5/10	0	1.30 ± 0.11**	3.11 ± 0.15**	3/10	3/10
4i	5/10	4/10	0	1.30 ± 0.15**	3.12 ± 0.14**	3/10	3/10
4j	3/10	2/10	20	2.11 ± 0.13*	3.12 ± 0.18**	5/10	4/10
4k	5/10	4/10	0	1.22 ± 0.10**	3.20 ± 0.14**	5/10	5/10
4l	1/10	0/10	0	1.21 ± 0.11**	3.12 ± 0.17**	1/10	1/10
4m	4/10	4/10	20	2.18 ± 0.10*	3.27 ± 0.15**	7/10	7/10
4n	9/10	7/10	0	1.29 ± 0.12**	3.33 ± 0.13**	2/10	2/10
4o	6/10	5/10	0	1.34 ± 0.12**	3.30 ± 0.19**	4/10	3/10
4p	5/10	4/10	20	3.11 ± 0.12*	4.11 ± 0.17*	0/10	0/10
Phenytoin	9/10	8/10	N.D.	N.D.	N.D.	3/10	2/10
Valproate	N.D.	N.D.	80	5.11 ± 0.17*	9.12 ± 0.28*	2/10	1/10

N.D. not determined

[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 in hours)

[d] Rotarod test (number of animals exhibiting toxicity/number of animals tested)

*$P < 0.001$

**not significant

Finally, tested compounds showed more protection against electroshock over chemoshock and this may be due to phenytoin-like action. To get more details about the possible mechanism of action of those compounds, pharmocophoric study was performed.

Pharmacophoric study

A good anticonvulsant drug should show four important pharmacophoric elements. These elements as previously prescribed by Pandeya et al. (2000) are present in many currently used antiepileptic drugs. They include hydrophobic domain (**A**), hydrogen-bonding domain (**HBD**), electron donor moiety (**D**), and distal hydrophobic domain (**R**). A training set composed of 11 different reported compounds that have structure similarity to the synthesized compounds was selected (Supplementary Data). Those drugs are used to obtain the pharmacophore queries. We can notice the presence of the triazole ring that keeps the structure similarity to our compounds.

Table 3 RMSDX values for compounds **4b**, **4c**, **4e–g**, and **4n**

Compounds	4b	4c	4e	4f	4g	4n
RMSDX	0.6307	0.6219	0.6283	0.6247	0.5754	0.6228

Compounds **4b**, **4e**, **4n**, **4f**, **4c**, **4g** with the highest anticonvulsant activity were selected for the pharmacophore study. The pharmacophoric results for the selected compounds are shown in Table 3. All the tested compounds showed good fitting on the pharmacophoric query with good RMSDX results (0.5754–0.6307). Figure 4 showed the pharmacophoric query and distance required for good fitting according to the used training set. Figure 5a showed the fitting of compound **4b** on the pharmacophore query.

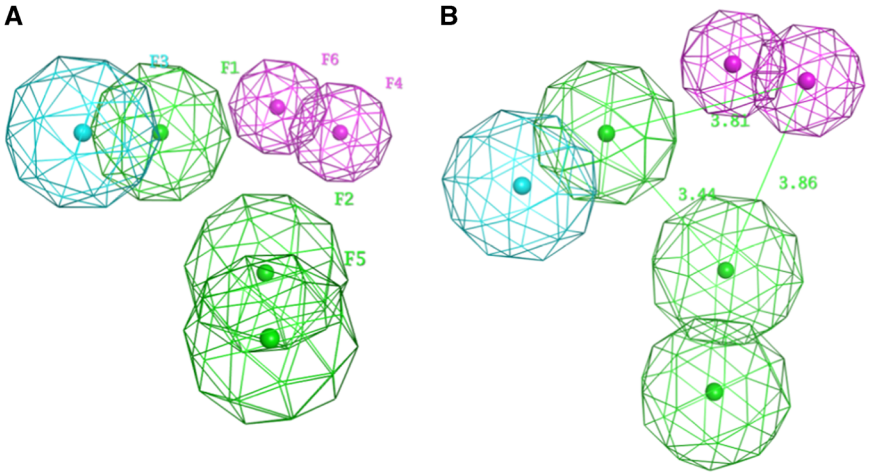


Fig. 4 Pharmacophoric query showing pharmacophoric features. **a** F1 (HydP|HydS), F2 (HydP|ML|HydS), F3 (HydP|ML|HydS|AccP), F4 (ML|AccP|AccS|DonP|DonS), F5 (HydP|HydS), F6 ML&(AccP|AccS|DonS), and required distances (**b**)

Test compounds showed a reasonable fitting on the query as compared with reference drug compound **I** (Fig. 5b; Shalini et al. 2009) that emphasizes the good anticonvulsant activity of the prepared compounds as shown in the previous section.

Conclusion

Sixteen new 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides were prepared and characterized by different spectroscopic and high-resolution mass spectrometry (HRMS) techniques. The anticonvulsant and neurotoxicity of the target compounds in comparison with phenytoin and valproate revealed that compounds with 4-chlorophenyl moiety at N1 (compounds **4c–f** and **4m–p**) and aryl carbamoyl moiety C3 of triazole ring showed more protection specially against electroshock than that with unsubstituted phenyl group like compound **4f**. On the other hand, compound **4e** that of 4-phenyl acetic acid group at N of the carboxamido group showed excellent anticonvulsant activity against both electroshock and chemoshock. In conclusion, the prepared 1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamide derivatives are revealed as promising anticonvulsant agents, stimulating the study of their full structure–activity relationship.

Experimental

Chemistry

Materials and methods

Reactions were routinely monitored by thin-layer chromatography using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) 5×20 cm plates with a layer thickness of 0.2 mm, and spots were visualized by exposure to UV-lamp at $\lambda = 254$ nm. Melting points were determined on Stuart electro-thermal melting point apparatus and are uncorrected.

IR spectra were recorded on Nicolet iS5 FT-IR spectrometer, Faculty of Pharmacy, Minia University. ^{1}H-NMR spectra were carried out using Bruker apparatus 400 MHz spectrometer, Faculty of Pharmacy, BeniSuef University, Egypt, using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) relative to TMS using $CDCl_3$ (7.29 for proton and 76.9 for carbon) or DMSO-d_6 (2.50 for proton and 39.50 for carbon) as a solvent and coupling constants (J) in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; p, pentet; dd, doublet of doublet; m, multiplet. HRMS were obtained on a Thermo Scientific Q Exactive™ Orbitrap mass spectrometer, Faculty of Pharmaceutical Sciences, University of British Columbia, Canada.

*Synthesis of 2-benzamidoacetic acid (hippuric acid) (**1a**) and 2-(3,4,5-trimethoxybenzamido)acetic acid (**1b**)*

To a solution of glycine (0.03 mol, 2.25 g) in distilled water (20 mL), 6 M NaOH (10 mL) was added then benzoyl chloride (0.03 mol, 4.22 g) or trimethoxybenzoyl chloride (0.03 mol, 6.92 g) was added dropwise with stirring till a clear solution was obtained. The solution was acidified by dil.HCl. The formed precipitate was filtered off and dried. Compound **1a** was crystallized from water and compound **1b** was crystallized from CCl_4.

*Synthesis of 4-phenylhydrazono-2-phenyl-4*H*-oxazol-5-one (**3a**), 4-[(4-chlorophenyl)hydrazono]-2-phenyl-4*H*-oxazol-5-one (**3b**), 4-[(3,4,5-trimethoxyphenyl)hydrazono]-2-phenyl-4*H*-oxazol-5-one (**3c**), and 4-[(4-chlorophenyl)*

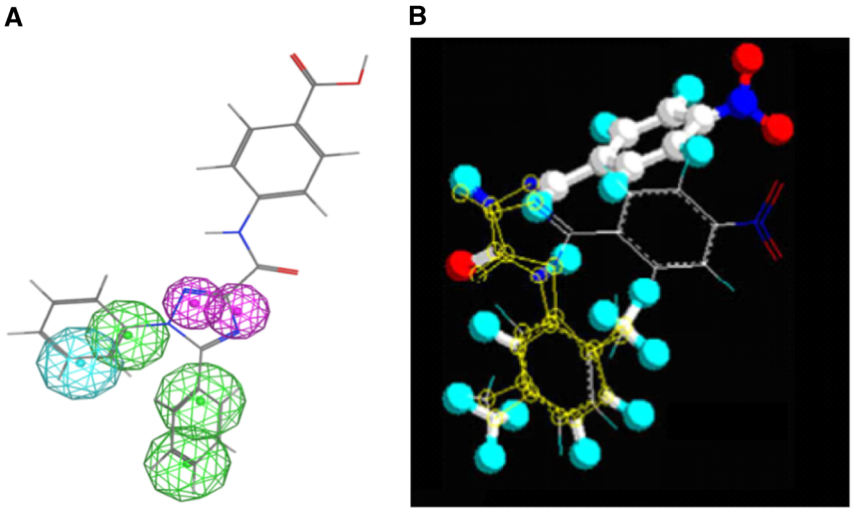


Fig. 5 **a** Fitting of compound **4b** to pharmacophore query, **b** fitting of reference drug compound **I**

*hydrazono]-2-(3,4,5-trimethoxyphenyl)-4*H*-oxazol-5-one (**3d**)*

Hippuric acid **1a** (0.013 mol, 2.33 g) or 2-(3,4,5-trimethoxybenzamido)acetic acid **1b** (0.013 mol, 3.5 g) in acetic anhydride (7.5 mL) was heated until a clear solution of compound **2a** or **2b** was obtained. This solution was cooled to room temperature (solution A). To a cold solution of aniline (0.01 mol, 0.93 g), *p*-chloroaniline (0.01 mol, 1.28 g) or 3,4,5-trimethoxyaniline (0.01 mol, 1.83 g) in 5 N HCl (3.5 mL) in an ice-salt bath 0–5 °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 B). Solution A was added to solution B in presence of anhydrous sodium acetate (0.018 mol, 1.5 g). The reaction mixture was stirred at 0–10 °C for 2 h. The formed precipitate was filtered off and dried (crude yield 70%). The product was crystallized from acetone as yellow crystals; IR (KBr, cm^{-1}): 1795 (C=O), 1630 (C=N), 1520 (C=C), 1230 (C–O–C).

*General procedure for the synthesis of carboxylic acid derivatives of 1,2,4-triazole-3-carboxamides (**4a–p**)*

A mixture of compound **3a** (0.01 mol, 2.65 g), **3b** (0.01 mol, 2.99 g), **3c** (0.01 mol, 3.55 g), or **3d** (0.01 mol, 3.89 g) and appropriate amino acid (0.01 mol) was refluxed in acetic acid (50 mL) in the presence of anhydrous sodium acetate (0.018 mol, 1.5 g) for 2 h. The reaction mixture was cooled and poured into ice water (100 mL). The formed precipitate was filtered off, dried, and crystallized from aqueous methanol.

2-(1,5-Diphenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid (**4a**) Reaction of **3a** (0.01 mol, 2.66 g) with glycine (0.012, 0.90 g) yielded pale yellow crystals (2.41 g, 75%); mp 210–212 °C; IR ν (cm^{-1}): 3675–2595 (OH), 3302 (NH), 1710 (C=O), 1669 (C=O), 1589 (C=N); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 4.34 (d, 2H, $J = 5.5$ Hz, C**H**$_2$), 7.33–7.52 (m, 10H, Ar–**H**), 7.88 (t, 1H, $J = 5.5$ Hz, N**H**); ^{13}C-NMR (125 MHz, $CDCl_3$): 41.16, 125.46, 126.61, 126.78, 128.69, 129.02, 129.55, 130.66, 137.49, 155.30, 155.64, 159.43, 172.55; HRMS: *m/z* calculated for $C_{17}H_{14}N_4O_3[M–H]^-$: 321.09931, found: 321.09933.

4-(1,5-Diphenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (**4b**) Reaction of **3a** (0.01 mol, 2.66 g) with *p*-aminobenzoic acid (0.012 mol, 1.64 g) yielded brown crystals (2.95 g, 77%); mp 279 °C; IR ν (cm^{-1}): 3672–2569 (OH), 3367 (NH), 1722 (C=O), 1689 (C=O), 1594 (C=N); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 7.48–7.58 (m, 10H, Ar–**H**), 7.96 (d, 2H, $J = 8.8$ Hz, **H**$_{2,6}$ of benzoic acid ring), 8.02 (d, 2H, $J = 8.8$ Hz, **H**$_{3,5}$ of benzoic acid ring), 10.87 (s, 1H, N**H**), 12.75 (s, 1H, COO**H**); Anal. calcd for $C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58. Found: C, 68.96; H, 4.27; N, 14.82.

2-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)acetic acid (**4c**) Reaction of **3b** (0.01 mol, 2.99 g) with glycine (0.01 mol, 0.75 g) yielded pale yellow crystals; 45% yield; mp 190 °C; IR (KBr, cm^{-1}): 3345–2550 (OH), 3329 (NH), 1715 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.28 (s, 2H, CH_2), 7.30 (d, 2H, $J = 8.80$ Hz, Ar–H), 7.35 (d, 2H, $J = 8.80$ Hz, Ar–H), 7.41–7.45 (m, 5H, Ar–H), 8.00 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 41.06, 126.54, 126.71, 128.83, 129.12, 129.67, 130.38, 135.44, 136.00, 155.24, 155.78, 159.39, 174.11; HRMS: *m/z* calculated for $C_{17}H_{13}ClN_4O_3[M–H]^-$: 355.06011, found: 355.06070.

3-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid (**4d**) Reaction of **3b** (0.01 mol, 2.99 g) with β-alanine (0.01 mol, 0.89 g) yielded pale yellow crystals; 47% yield; mp 193 °C; IR (KBr, cm^{-1}): 3242–2535 (OH), 3350 (NH), 1722 (carboxylic C=O), 1672 (amidic C=O), 1550 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.66 (t, 2H, $J = 6.00$ Hz, $\underline{CH_2}CO$), 3.79 (t, 2H, $J = 6.00$ Hz, $\underline{CH_2}NH$), 7.33 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.41 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.47–7.61 (m, 5H, Ar–H), 7.98 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 33.69, 50.86, 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.

4-(1-(4-Chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)phenylacetic acid (**4e**) Reaction of **3b** (0.01 mol, 2.99 g) with 2-(4-aminophenyl)acetic acid (0.01 mol, 1.51 g) yielded pale yellow crystals; 74.80% yield; mp 209 °C; IR (KBr, cm^{-1}): 3242–2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.62 (s, 2H, $\underline{CH_2}CO$), 7.32 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.36 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.42–7.49 (m, 5H, Ar–H), 7.55 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.75 (d, 2H, $J = 8.40$ Hz, Ar–H), 9.11 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 40.42, 120.18, 126.56, 126.64, 128.84, 128.95, 129.05, 129.12, 129.72, 129.80, 130.11, 135.53, 136.02, 136.55, 156.54, 156.62, 176.51; HRMS: *m/z* calculated for $C_{23}H_{17}ClN_4O_3[M–H]^-$: 431.09164, found: 431.09183.

Ethyl 4-(1-(4-chlorophenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoate (**4f**) Reaction of **3b** (0.01 mol, 2.99 g) with benzocaine (0.01 mol, 1.65 g) yielded pale yellow crystals; 85.10%; mp 173 °C; IR (KBr, cm^{-1}): 3329 (NH), 1669 (C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (t, 3H, $J = 6.40$ Hz, $CH_2\underline{CH_3}$), 4.39 (q, 2H, $J = 6.40$ Hz, $\underline{CH_2}CH_3$), 7.39 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.56 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.44–7.54 (m, 5H, Ar–H), 7.87 (d, 2H, $J = 8.40$ Hz, Ar–H), 8.10 (d, 2H, $J = 8.40$ Hz, Ar–H), 9.26 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 14.37, 60.92, 119.09, 126.41, 126.46, 126.63, 128.98, 129.03, 129.79, 130.08, 130.90, 135.67, 135.97, 141.37, 155.16, 156.27, 156.56, 166.08; HRMS: *m/z* calculated for $C_{24}H_{19}ClN_4O_3[M–H]^-$: 445.10729, found: 445.10785.

2-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido) acetic acid (**4g**) Reaction of **3c** (0.01 mol, 3.56 g) with glycine (0.012 mol, 2.66 g) yielded white powder (2.91 g, 70%); mp 161–163 °C; IR ν (cm^{-1}): 3668–2620 (OH), 3302 (NH), 1712 (C=O), 1670 (C=O), 1589 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.72 (s, 6H, 2 OC$\underline{H_3}$), 3.87 (s, 3H, OC$\underline{H_3}$), 4.28 (d, 2H, $J = 4.6$ Hz, HN–C$\underline{H_2}$), 6.58 (s, 2H, $H_{2,6}$ of Ar–$\underline{H}$), 7.28–7.53 (m, 5H, Ar–$\underline{H}$), 7.98 (t, 2H, $J = 4.6$ Hz, N$\underline{H}$); HRMS: *m/z* calculated for $C_{20}H_{20}N_4O_6$ $[M–H]^-$: 411.13101, found: 411.13138.

3-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido) propionic acid (**4h**) Reaction of **3c** (0.01 mol, 3.56 g) with β-alanine (0.012 mol, 0.90 g) yielded yellowish white powder (3.1 g, 73%); mp 161–163 °C; IR ν (cm^{-1}): 3670–2644 (OH), 3310 (NH), 1719 (C=O), 1675 (C=O), 1580 (C=N); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.75 (t, 2H, $J = 6.0$ Hz, C$\underline{H_2}$–COOH), 3.75 (s, 6H, 2 OC$\underline{H_3}$), 3.79 (t, 2H, $J = 6.1$ Hz, HN–C$\underline{H_2}$), 3.82 (s, 3H, OC$\underline{H_3}$), 6.57 (s, 2H, $H_{2,6}$ of Ar–$\underline{H}$), 7.39–7.54 (m, 5H, Ar–$\underline{H}$), 7.92 (t, 1H, $J = 6.2$ Hz, N$\underline{H}$); ^{13}C-NMR (125 MHz, $CDCl_3$): 33.73, 34.94, 60.96, 103.09, 126.48, 128.85, 128.93, 130.5, 132.85, 138.56, 153.44, 154.81, 155.90, 159.20, 175.92; HRMS: *m/z* calculated for $C_{21}H_{22}N_4O_6$ $[M–H]^-$: 425.14666, found: 425.14700.

4-((1-(3,4,5-Trimethoxyphenyl)-5-phenyl)-1*H*-1,2,4-triazole-3-carboxamido)butanoic acid (**4i**) Reaction of **3c** (0.01 mol, 3.56 g) with 4-aminobutyric acid (0.012 mol, 1.07 g) yielded yellowish white powder (3.17 g, 73%); mp 135–137 °C; IR ν (cm^{-1}): 3670–2650 (OH), 3310 (NH), 1717 (C=O), 1670 (C=O), 1590 (C=N); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.00 (p, 2H, $J = 7.2$ Hz, HN–CH_2–C$\underline{H_2}$), 2.49 (t, 2H, $J = 7.0$ Hz, C$\underline{H_2}$–COOH), 3.58 (q, 2H, $J = 6.7$ Hz, HN–C$\underline{H_2}$), 3.73 (s, 6H, 2 OC$\underline{H_3}$), 3.87 (s, 3H, OC$\underline{H_3}$), 6.58 (s, 2H, $H_{2,6}$ of Ar–$\underline{H}$), 7.38–7.59 (m, 5H, Ar–$\underline{H}$); ^{13}C-NMR (125 MHz, $CDCl_3$): 24.87, 31.18, 38.62, 56.62, 61.05, 103.04, 126.91, 128.70, 128.94, 130.65, 132.96, 138.70, 153.48, 154.75, 155.00, 159.51, 176.64; HRMS: *m/z* calculated for $C_{22}H_{24}N_4O_6$ $[M–H]^-$: 439.16231, found: 439.16251.

4–(1-(3,4,5-Trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (**4j**) Reaction of **3c** (0.01 mol, 3.56 g) with *p*-aminobenzoic acid (0.012 mol, 1.64 g) afforded a brown colored powder (3.65 g, 77%); mp 269–271 °C; IR ν (cm^{-1}): 3590–2670 (OH), 3300 (NH), 1720 (C=O), 1690 (C=O), 1613 (C=N); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.76 (s, 6H, 2 OC$\underline{H_3}$), 3.71 (s, 3H, OC$\underline{H_3}$), 6.87 (s, 2H, $H_{2,6}$ of Ar–$\underline{H}$), 7.46–7.63 (m, 5H, Ar–$\underline{H}$), 7.93 (d, 2H, $J = 8.8$ Hz, $\underline{H_{2,6}}$ of benzoic acid ring), 8.00 (d, 2H, $J = 8.8$ Hz, $\underline{H_{3,5}}$ of benzoic acid ring), 10.8 (s, 1H, N$\underline{H}$), 12.72 (s, 1H, COO$\underline{H}$); ^{13}C-NMR (125 MHz, DMSO-d_6): 56.68, 60.73, 104.63, 120.33, 129.04, 129.28, 130.60, 130.96, 133.34, 138.66, 142.78, 149.88, 153.50, 155.31, 156.23, 158.00, 167.40; HRMS: *m/z* calculated for $C_{25}H_{22}N_4O_6$ $[M–H]^-$: 473.14666, found: 473.14725.

2-(4–(1-(3,4,5-Trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)phenyl)acetic acid (**4k**) A mixture of compound **3c** (0.01 mol, 2.66 g), 2-(4-aminophenyl)acetic acid (0.012 mol) yielded yellowish white crystals (3.8 g, 78%); IR (cm^{-1}): 3668 (OH), 3302 (NH), 1717 (C=O), 1678 (C=O), 1590 (C=N); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.65 (t, 2H, HOOC–C**H$_2$**), 3.71 (s, 6H, 2 OC**H$_3$**), 3.89 (s, 3H, 2 OC**H$_3$**), 6.61 (s, 2H, 2Ar–H), 7.42–7.60 (m, 5H, Ar–H), 7.93 (d, 2H, $J = 8$ Hz, $H_{2,6}$ of benzoic acid ring), 8.00 (d, 2H, $J = 8$ Hz, $H_{3,5}$ of benzoic acid ring), 9.08 (s, 1H, N**H**); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 40.16, 56.25, 64.05, 103.04, 120.03, 126.75, 126.87, 128.75, 129.57, 130.05, 130.71, 132.49, 136.61, 138.75, 153.52, 154.85, 156.21, 156.62, 175.76; HRMS: *m/z* calculated for $C_{17}H_{14}N_4O_3$ $[M–H]^+$: 487.16231, found: 487.16266.

4–((1-(3,4,5-Trimethoxyphenyl)-5-phenyl-1*H*-1,2,4-triazole-3-carboxamido)methyl) benzoic acid (**4l**) Reaction of **3c** (0.01 mol, 3.56 g) with 4-(aminomethyl)benzoic acid (0.012 mol, 1.81 g) yielded brownish white powder (3.51, 72%); mp 189–191 °C; IR ν (cm^{-1}): 3610–2820 (OH), 3300 (NH), 1720 (C=O), 1670 (C=O), 1602 (C=N); ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 3.67 (s, 6H, 2 OC**H$_3$**), 3.71 (s, 3H, OC**H$_3$**), 4.55 (d, 2H, $J = 6.2$ Hz, NH–C**H$_2$**), 6.81 (s, 2H, $H_{2,6}$ of Ar–**H**), 7.45 (d, 2H, $J = 8.5$ Hz, **H$_{3,5}$** of benzoic acid ring), 7.47–7.56 (m, 5H, Ar–**H**), 7.74 (d, 2H, $J = 8.5$ Hz, **H$_{2,6}$** of benzoic acid ring), 9.35 (t, 1H, $J = 6.2$ Hz, N**H**), 12.85 (s, 1H, COO**H**); ^{13}C-NMR (125 MHz, DMSO-d_6): 42.54, 56.63, 60.71, 104.55, 127.53, 127.73, 129.10, 129.8, 130.81, 133.45, 138.55, 144.94, 149.98, 153.46, 154.99, 156.33, 159.28, 167.61; HRMS: *m/z* calculated for $C_{26}H_{24}N_4O_6$ $[M–H]^-$: 487.16231, found: 487.16269.

3-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)propanoic acid (**4m**) Reaction of **3d** (0.01 mol, 3.89 g) with β-alanine (0.01 mol, 0.89 g) yielded pale yellow crystals; 47% yield; mp 185 °C; IR (KBr, cm^{-1}): 3320–2535 (OH), 3355 (NH), 1715 (carboxylic C=O), 1661 (amidic C=O), 1610 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.77 (t, 2H, $J = 6.40$ Hz, CH_2CO), 3.71 (s, 6H, 2-OCH_3), 3.82 (q, 2H, $J = 6.40$ Hz, CH_2NH), 3.90 (s, 3H, OCH_3), 6.70 (s, 2H, Ar–H), 7.39 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.46 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.90 (t, 1H, $J = 6.40$ Hz, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 33.63, 34.93, 56.13, 60.98, 106.54, 121.24, 126.96, 129.67, 135.59, 136.20, 140.29, 153.33, 155.02, 156.23, 159.10, 175.61; HRMS: *m/z* calculated for $C_{21}H_{21}ClN_4O_6$ $[M–H]^-$: 459.10769, found: 459.10703.

4-(1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)benzoic acid (**4n**) Reaction of **3d** (0.01 mol, 3.89 g) with PABA (0.01 mol, 1.37 g) yielded pale yellow crystals; 77.90% yield; mp 213 °C; IR (KBr, cm^{-1}): 3242–2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1670 (amidic C=O), 1580 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 6H, 2-OCH_3), 3.71 (s, 3H, OCH_3), 6.83 (s, 2H, Ar–H), 7.61 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.68 (d, 2H, $J = 8.40$ Hz, Ar–H), 7.95 (d, 2H, $J = 8.40$ Hz, Ar–H), 8.01 (d, 2H, $J = 8.40$ Hz, Ar–H), 8.69 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 56.32, 60.67, 107.17, 120.41, 122.10, 126.52, 128.61, 130.08, 130.67, 134.87, 136.81, 139.75, 142.80, 153.22, 155.39, 156.33, 157.96, 167.36; HRMS: *m/z* calculated for $C_{25}H_{21}ClN_4O_6$$[M–H]^-$: 507.10769, found: 507.10791.

4-((1-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)methyl)benzoic acid (**4o**) Reaction of **3d** (0.01 mol, 3.89 g) with 4-aminomethylbenzoic acid (0.01 mol, 1.51 g) yielded pale yellow crystals; 73.40% yield; mp 213 °C; IR (KBr, cm^{-1}): 3242–2535 (OH), 3329 (NH), 1718 (carboxylic C=O), 1669 (amidic C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.64 (s, 6H, 2-OCH_3), 3.72 (s, 3H, OCH_3), 4.57 (d, 2H, $J = 4.00$ Hz, **CH_2**NH), 6.79 (s, 2H, Ar–H), 7.46 (d, 2H, $J = 8.00$ Hz, Ar–H), 7.56 (d, 2H, $J = 8.00$ Hz, Ar–H), 7.64 (d, 2H, $J = 8.00$ Hz, Ar–H), 7.92 (d, 2H, $J = 8.00$ Hz, Ar–H), 9.21 (t, 1H, $J = 4.00$ Hz, CONH), 12.70 (s, 1H, COOH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 56.46, 60.67, 66.36, 107.45, 122.26, 127.83, 128.44, 129.81, 130.00, 134.73, 136.99, 139.5, 140.10, 144.85, 153.29, 155.10, 156.74, 159.28, 167.60; HRMS: *m/z* calculated for $C_{26}H_{23}ClN_4O_6$$[M–H]^-$: 521.12334, found: 521.12347.

Ethyl4-(1-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamido)benzoate (**4p**) Reaction of **3d** (0.01 mol, 3.89 g) with benzocaine (0.01 mol, 1.65 g) yielded pale yellow crystals; 87.60% yield; mp 177 °C; IR (KBr, cm^{-1}): 3329 (NH_2), 1669 (C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (t, 3H, $J = 6.40$ Hz, CH_2CH_3), 3.74 (s, 6H, 2-OCH_3), 3.91 (s, 3H, OCH_3), 4.38 (q, 2H, $J = 6.40$ Hz, CH_2CH_3), 6.75 (s, 2H, Ar–H), 7.43 (d, 2H, $J = 8.00$ Hz, Ar–H), 7.50 (d, 2H, $J = 8.00$ Hz, Ar–H), 7.88 (d, 2H, $J = 8.40$ Hz, Ar–H), 8.10 (d, 2H, $J = 8.40$ Hz, Ar–H), 9.27 (s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 14.36, 56.17, 60.94, 61.03, 106.48, 119.12, 120.96, 126.52, 126.97, 129.52, 130.89, 135.83, 136.09, 140.44, 141.33, 153.41, 155.08, 155.94, 156.42, 166.06; HRMS: *m/z* calculated for $C_{27}H_{25}ClN_4O_6$$[M–H]^-$: 535.13899, found: 535.13971.

Biological investigation

MES model

One hundred and eighty male albino mice weighing 25–35 g were divided into 18 equal groups each of 10. The first group was used as a control group administered dimethyl sulfoxide (DMSO) only as a vehicle i.p. The second group was administered phenytoin sodium. The groups from 3–18 were administered test compounds. All the test compounds and phenytoin sodium were dissolved in DMSO and injected i.p. to the animal at dose 100 mg/kg body weight 1 h 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 (Gallagher 1977; Krall et al. 1978).

scPTZ model

Ninety male albino mice weighing 25–35 g were divided into 18 equal groups each of 5 and acclimatized to their environment for at least 1 week before the experiment. The first group was used as a control group administered DMSO only as a vehicle i.p. The second group was administered sodium valproate. The groups from 3–18 were administered test compounds. All the test compounds and sodium valproate were dissolved in DMSO and injected i.p. to the animal at dose 100 mg/kg body weight. 1 h 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 (Krall et al. 1978; Clark et al. 1984).

Intraperitonealy strychnine HCl (S)-induced convulsions

One hundred and eighty male albino mice weighing 25–35 g were divided into 18 equal groups each of 10. The first group was used as a control group administered DMSO only as a vehicle i.p. The second group was administered sodium valproate. The groups from 3–18 were administered test compounds. All the test compounds and sodium valproate were dissolved in DMSO and injected i.p. to the animal at dose 100 mg/kg body weight. 1 h later, mice were injected with strychnine HCl at dose 2 mg/kg body weight i.p. The onset of convulsions and time of death was calculated in comparison with control group (Vogel and Vogel 1997).

Neurotoxicity (rotarod test)

Minimal motor impairment was measured in mice by rotarod test. One hundred and ninety male albino mice weighing 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 19 equal groups each of 10. The first group was used as a control group administered DMSO only as a vehicle i.p. The second group was administered phenytoin sodium. The third group was administered sodium valproate. The groups from 4–19 were administered test compounds. All the test compounds, phenytoin sodium, and sodium valproate were dissolved in DMSO and injected i.p. to the animal at dose 100 mg/kg body weight. Neurotoxicity was indicated by the inability of the animals to maintain equilibrium on the rod for at least 1 min in each of three trials (Dunham and Miya 1957).

Pharmacophoric study

Hardware: Dell Precision™ T3600 Workstation [IntelXeon E5-1660 3.3 GHz, 16 GB 1600 MHz DDR3, ECC RDIMM 1 TB (7200 RPM), 1 GB NVIDIA Quadro 2000, Windows 7 Professional (64 Bit)].

Software: Molecular Operating Environment (MOE) package version 2016.08 (Chemical Computing Group, Inc., Molecular Operating Environment (MOE)). CCG, Montreal, Canada. 2016. http://www.chemcomp.com.

Scheme: PPCH_ALL, Tolerance: 1.0, Threshold: 60%, Consensus score: weighed conformations.

The protocol used is as previously described by Abdel-Aal et al. (2010).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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