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Synthesis and anticonvulsant evaluation of 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazoles as open-chain analogues of 7-alkoxyl-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinolines

Jing Chen,[a,b] Xian-Yu Sun,[b] Kyu-Yun Chai,[c] Jin-Seok Lee,[c] Mi-Sun Song[c] and Zhe-Shan Quan[a,b,*]

[a]*Key Laboratory of Organism Functional Factors of the Changbai Mountain, Yanbian University, Ministry of Education, Yanji, Jilin 133002, China*
[b]*College of Pharmacy, Yanbian University, Yanji, Jilin 133000, China*
[c]*Department of Chemistry, Wonkwang University, Iksan 570-749, Republic of Korea*

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Abstract—A series of 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazole derivatives was synthesized as open-chain analogues of 7-alkoxyl-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinolines. Their anticonvulsant activities were evaluated by the maximal electroshock test (MES test) and their neurotoxicity was evaluated by the rotarod neurotoxicity test (Tox). MES test showed that 3-ethyl-4-(4-octyloxyphenyl)-4*H*-1,2,4-triazole **3q** was found to be the most potent with ED_{50} value of 8.3 mg/kg and protective index ($PI = TD_{50}/ED_{50}$) value of 5.5, but compound **3r**, 3-ethyl-4-(4-octyloxyphenyl)-4*H*-1,2,4-triazole, exhibited better PI value of 9.3, which was much greater than PI value of the prototype drug phenytoin. For explanation of the possible mechanism of action, the compound **3r** was tested in pentylenetetrazole test, isoniazid test, thiosemicarbazide test, 3-mercaptopropionic acid and strychnine test.

1. Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than 60 million people worldwide according to epidemiological studies.[1] For epilepsy treatment, nearly 95% of clinically available drugs were approved before 1985 and they could provide satisfactory seizure control for 60–70% of patients. These drugs, however, also cause notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anaemia,[2–4] and even life-threatening conditions.[5] Research to find more effective and safer antiepileptic drugs is, therefore, imperative and challenging in medicinal chemistry.

In our previous work, a series of derivatives of 6-alkoxy-3,4-dihydro-1*H*-quinoline-2-ones were first found to have anticonvulsant activities, among which 6-benzyloxy-3,4-dihydro-1*H*-quinoline-2-one showed the strongest activity with an ED_{50} value of 29.6 mg/kg in the maximal electroshock test (MES) and a TD_{50} value greater than 300 mg/kg.[6] Introduction of triazole ring to the first and second position of this 6-benzyloxy-3,4-dihydro-1*H*-quinoline-2-one caused a remarkable increase in the anticonvulsant activity, such as 7-benzyloxyl-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinoline (compound **1a**), which showed ED_{50} values of 17.3 and 24 mg/kg in the MES and the sc-PTZ tests, respectively.[7] Another derivative in the group of 7-alkoxy-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinoline, 7-(4-fluorobenzyloxyl)-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinoline (compound **1b**), showed ED_{50} values of 11.1 and 6.7 mg/kg, protective index ($PI = TD_{50}/ED_{50}$) values of 4.6 and 8.1 in the MES and the pentylenetetrazole tests, respectively, and thus demonstrated comparable anticonvulsant potency to that of phenobarbital in the corresponding tests.[8]

Keywords: 1,2,4-Triazole; Anticonvulsant activity; Open-chain analogue; Maximal electroshock test; Neurotoxicity.
* Corresponding author. Tel.: +86 433 2660606; fax: +86 433 2660568; e-mail: zsquan@ybu.edu.cn

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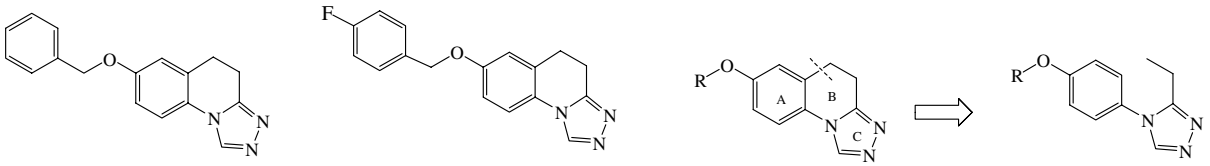


Analyzing the relationship of anticonvulsant activity and the structure of 7-alkoxyl-4,5-dihydro[1,2,4] triazolo[4,3-*a*]quinoline (**1**), it was found that triazole ring C may be the main structure combined with receptor, aromatic ring A and 7-alkoxyl enhanced the hydrophobic ability of target compounds, thus make them more permeable to the blood–brain barrier and enhance anticonvulsant activity. So we thought that the presence of A and C ring was essential structure for the anticonvulsant activity. In this paper, we designed and prepared a series of 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazole derivatives with ring B opened and pseudocycles' structure existing as open-chain analogue of 7-alkoxy-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinoline. The hypothesis was that a rotatable triazole ring may have higher affinity for the receptor and enhance their anticonvulsant activity. The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models, i.e., maximal electroshock (MES) induced seizure in mice. The rotarod assay was performed in mice to evaluate the neurotoxicity of the compounds. For explaining the possible mechanism of action, the compound 3-ethyl-4-(4-octyloxyphenyl)-4*H*-1,2,4-triazole **3q** was tested in pentylenetetrazole, isoniazid, thiosemicarbazide, 3-Mercaptopropionic Acid and strychnine induced test.

2. Results and discussion

2.1. Synthesis

There are many methods reported to synthesize triazole derivatives. An efficient one-pot, three-component synthesis of substituted-1,2,4-triazoles has been developed by Michael,[9] utilizing a wide range of substituted primary amines, acyl hydrazines and dimethylformamide dimethyl acetal. In this paper, intermediate compound **2** was prepared by the Michael method, and then compound **2** reacted with appropriate alkyl halide to produce target compounds 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazoles (**3a**–**s**) (Scheme 1).

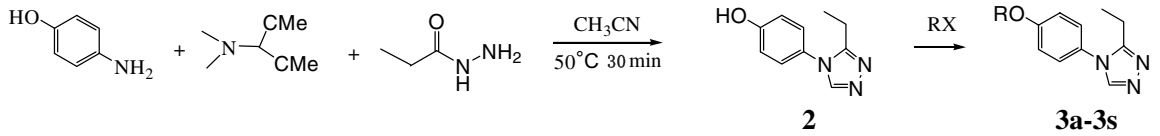
2.2. Pharmacology

The anticonvulsant activity against MES-induced seizure and neurotoxicity TD_{50} of target compounds are shown in Table 1, and also the anticonvulsant activities and neurotoxicity of reference compounds **1a**–**e** are list to show the activity transformation tendency. As shown in Table 1, all open-chain compounds exhibited strong potency at a dose of 30 mg/kg against MES induced seizure, but in the former paper,[8] only five target compounds exhibited anticonvulsant activity ED_{50} at a dose of 30 mg/kg.

First, comparing the anticonvulsant activity of open-chain analogues and the leading triazolo[4,3-*a*] quinoline compounds, among these substituted-phenoxyl derivatives we could see the following activity order, **1a** > **3a, 1b** > **3b** and **1c** > **3j**; although the anticonvulsant activity is comparatively weaker than the leading compound, the open-chain analogues **3a**–**l** possess lower neurotoxicity ranging from 61.5 to 189.3 mg/kg than leading compounds **1a**–**d** ranging from 54.5 to 65.8 mg/kg. Among these alkoxyl derivatives, the most potent compound **3q** (3-ethyl-4-(4-octyloxyphenyl)-4*H*-1,2,4-triazoles) exhibited significantly enhanced activity with ED_{50} value of 8.3 mg/kg than compound **1d** with ED_{50} value of 13.5 mg/kg, especially, the alkoxyl open-chain derivatives also possess remarkable lower neurotoxicity than the leading compound.

Analyzing the activities of synthesized compounds **3a**–**s**, the following SAR was gained. The pharmacology result revealed that atom Cl gave more contribution to the anticonvulsant activity than atom F, and the position of atom Cl on the phenyl ring greatly influenced the anticonvulsant activity, the activity order being 2-Cl > 2,6-Cl_2 > 4-Cl > 3-Cl. The 4-Cl derivative **3f** exhibited weaker activity with ED_{50} value of 19.7 mg/kg than 2-Cl derivative **3 g** with ED_{50} value of 13.2 mg/kg, but compound **3f** exhibited the lowest neurotoxicity with TD_{50} value of 136.9 mg/kg, which was the safest among all target compounds with PI value of 10.4 and better than the reference drug phenytoin. But no visible difference was found between compounds with atom F substituted on different position of the phenyl ring. Comparing the influence of electron–donor group to anticonvulsant activity, their contribution order is 4-OCH_3 > 3,4-OCH_2O– > 4-CH_3.

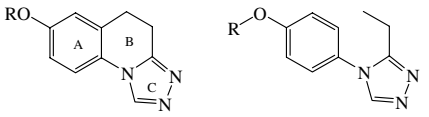
Length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 4-alkyloxyl derivatives. As shown in Table 1, derivative **3q** was the most active compound in seven alkoxyl-substituted compounds, suggesting that appropriate length of the alkyl chain at C-7 position, or appropriate lipophilic property, was essential to the anticonvulsant activity of these compounds. Compound **3q**, with ED_{50} value of 8.3 mg/kg, was better than Phenytoin in anti-MES activity. In addition, compound **3r**, with ED_{50} of 8.8 mg/kg and lower neurotoxicity and thus a high PI value of 9.3, could also be considered a potentially useful and safe therapeutic. And the anticonvulsant activity decreased obviously when alkyl chain number



2 3a-3s

Scheme 1. The synthesis route of compounds **3a**–**s**.

Table 1. Quantitative anticonvulsant data in mice (test drug administered ip)



1a-1e **3a-3s**

Compound	R	MES, ED_{50}[a]	TD_{50}[b]	PI (TD_{50}/ED_{50})
1a	$-CH_2C_6H_5$	17.3 (14.8–20.4)[c]	61.4 (51.4–73.3)	3.5
1b	$-CH_2C_6H_5$(*p*-F)	11.8 (10.9–12.9)	54.5 (46.1–64.5)	4.6
1c	$-CH_2C_6H_5$(*p*-CH_3)	20.5 (17.0–24.5)	65.8 (55.6–77.7)	3.2
1d	–*n*-C_6H_{13}	13.5 (11.5–15.8)	30.3 (25.5–36.0)	2.2
3a	$-CH_2C_6H_5$	20.8 (16.6–26.0)	101.8 (81.5–127.2)	4.9
3b	$-CH_2C_6H_5$(*p*-F)	22.8 (18.2–28.5)	91.3 (73.1–114.1)	4.0
3c	$-CH_2C_6H_5$(*o*-F)	21.2 (17.0–26.5)	61.5 (49.2–76.9)	2.9
3d	$-CH_2C_6H_5$(*m*-F)	22.8 (18.2–28.5)	91.2 (73.0–114.0)	4.0
3e	$-CH_2C_6H_5$(2,6-F_2)	15.8 (12.6–19.7)	109.0 (87.2–136.2)	6.9
3f	$-CH_2C_6H_5$(*p*-Cl)	19.7 (15.8–24.6)	136.9 (109.6–171.1)	10.4
3g	$-CH_2C_6H_5$(*o*-Cl)	13.2 (10.6–16.5)	76.1 (60.9–95.1)	5.8
3h	$-CH_2C_6H_5$(*m*-Cl)	27.4 (21.9–34.2)	109.5 (87.6–136.8)	4.0
3i	$-CH_2C_6H_5$(2,6-Cl_2)	15.8 (12.6–19.7)	131.5 (105.2–164.3)	8.3
3j	$-CH_2C_6H_5$(*p*-CH_3)	25.5 (20.4–31.9)	101.0 (80.8–126.2)	4.0
3k	$-CH_2C_6H_5$(*p*-OCH_3)	15.8 (12.6–19.7)	131.5 (105.2–164.3)	8.3
3l	$-CH_2C_6H_5$(3,4-OCH_2O–)	20.1 (16.1–25.1)	189.3 (151.4–236.4)	9.4
3m	$-C_2H_5$	>100	—[d]	—
3n	–*n*-C_3H_7	>100	—	—
3o	–*n*-C_4H_9	>100	—	—
3p	–*n*-C_6H_{13}	13.2 (10.6–16.5)	62.1 (49.7–77.6)	4.7
3q	–*n*-C_7H_{15}	8.3 (6.6–10.4)	45.6 (36.5–57.0)	5.5
3r	–*n*-C_8H_{17}	8.8 (7.04–11.0)	82.2 (65.8–102.7)	9.3
3s	–*n*-$C_{12}H_{25}$	>100	—	—
Phenytoin		9.5 (8.1–10.4)	65.5	6.9

[a] The dose measured in mg/kg.
[b] Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administrated.
[c] The 95% confidence limits.
[d] Not tested.

lengthened to 12, even showing no activity at a dose of 100 mg/kg.

As a result of first test, compound **3r** showed strong anticonvulsant activity and the best PI value in MES test, so it was then selected for further investigations against seizures induced by pentylenetetrazole, 3-mercaptopropionic acid, thiosemicarbazide, isoniazid and strychnine to prove its anticonvulsant activity and speculate about the possible mechanism of anticonvulsant action. As shown in Table 2, compound **3r** was effective against the seizures induced by pentylenetetrazole, isoniazid, 3-mercaptopropionic acid and thiosemicarbazide with ED_{50} values of 26.4, 38.1, 31.7 and 22.8 mg/kg, respectively.

Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission.[10,11] GABA is the main inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to

Table 2. Anticonvulsant activity of compound **3r** in chemically induced seizures tests

Compound	Pentylenetetrazole	Isoniazid	Thiosemicarbazide	3-Mercaptopropionic acid	Strychnine
3r	26.4	38.1	31.7	22.8	—[a]

[a] Compound **3r** failed to control the seizure induced by Strychnine at the dose of 300 mg/kg.

promote and facilitate seizures,[12] while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study tend to suggest that the derivatives in this study might have inhibited or attenuated pentylenetetrazole-induced and isoniazid-induced seizures in mice by enhancing GABAergic neurotransmission.

3-Mercaptopropionic acid and thiosemicarbazide were seen as the competitive inhibitor of GABA synthesis enzyme glutamate decarboxylase (GAD), inhibiting the synthesis of GABA to decrease the GABA level in the brain.[13,17] The moderate antagonism of 3-mercaptopropionic acid-induced and thiosemicarbazide-induced seizures suggests that the compound **3r** might activate GAD or inhibit aminotransferase (GABA-T) in the brain.

As shown in Table 2, compound **3r** failed to protect animals from seizure induced by Strychnine at the dose of 300 mg/kg. It is known that Strychnine directly antagonizes the inhibitory spinal reflexes of glycine,[14] so the result suggesting that compound **3r** could not influence glycine system.

3. Conclusions

In conclusion, 4-alkoxyl-3-ethyl-4-*H*-1,2,4-triazoles, the open-chain analogues of 7-alkoxyl-4,5-dihydro-[1,2,4]-triazolo[4,3-*a*]quinolines, exhibited remarkable anticonvulsant activity and lower neurotoxicity. Especially, compound **3r** produced significant antagonism activity against seizures induced by pentylenetetrazole, 3-mercaptopropionic acid, thiosemicarbazide and Isoniazid, suggested that the compound **3r** might have effects on GABAergic neurotransmission and activate glutamate decarboxylase (GAD) or inhibit (GABA)–α-oxoglutarate aminotransferase (GABA-T) in the brain.

4. Experimental

4.1. Chemistry

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730. ^{1}H NMR spectra were measured on AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (Perkin-Elmer, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade.

4.1.1. Synthesis of 4-(4-hydroxyphenyl)-3-ethyl-4 H-1,2,4-triazole (2). Dimethoxy-*N*,*N*-dimethylmethanaminel 6.5 g (55 mmol) was added to a solution of propionohydrazide 4.8 g (55 mmol) in acetonitrile (20 ml) in a 100-ml round-bottomed flask equipped with a reflux condenser. The reaction mixture was warmed to 50 °C for 30 min and then *p*-aminophenol 5.45 g (50 mmol) in acetonitrile (10 ml) was added and also added acetic acid (3 ml) as a catalyst at the same time. The reaction temperature was raised to 120 °C for 3 h. After being cooled and concentrated, the product was recrystallized in ice-water. The precipitate was collected through filtration and dried in a vacuum to produce the crude product with a moderate yield and pure enough for the next stage.

Yield, 93%, mp 206–208 °C. ^{1}H NMR ($CDCl_3$) δ: 1.27 (t, J = 7.5 Hz, 3H, –CH_3), 2.74 (q, J = 7.5 Hz, 2H, –CH_2), 7.00 (d, 2H, J = 8.5 Hz, Ar-H),7.10 (d, 2H, J = 8.5 Hz, Ar-H), 8.40 (s, 1H, triazole-H). IR (KBr) cm^{-1}: 3405 (OH), 1593 (C═N), 1286 (C—N), 1190 (N—N). MS *m*/*z*: 190 (M+1). Anal. Calcd for $C_{17}H_{17}N_3O$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.27; H, 6.08; N, 21.95.

4.1.2. General procedure for the synthesis of 4-(4-alkoxylphenyl)-3-ethyl-4*H*-1,2,4-triazoles (3a–s). A mixture of compound **2** 1.9 g (10 mmol) and alkyl halide (11 mmol) in 50 ml anhydrous ethanol was refluxed for 4 h in the presence of NaOH 0.4 g (10 mmol). After the solvent was removed, the residue was dissolved in dichloromethane (60 ml) and washed with water (2× 30 ml), the dichloromethane layer was dried over $MgSO_4$, and then the solvent was removed under reduced pressure. A pale yellow solid or oil was obtained after being purified by silica gel chromatography (ethyl acetate/methanol, 10:1). The yield, melting point and spectral data of each compound are given below.

4.1.2.1. 3-Ethyl-4-(4-benzyloxyphenyl)-4*H*-1,2,4-triazole (3a). Yield = 78.8%, mp 102–105 °C. ^{1}H NMR ($CDCl_3$) δ: 1.19 (t, J = 7.5 HZ, 3H, –CH_3), 2.67 (q, J = 7.5 Hz, 2H, –CH_2), 5.11 (s, 2H, OCH_2), 7.15 (d, 2H, J = 8.3 Hz, Ar-H), 7.29–7.37 (m, 5H, Ar-H), 7.43 (d, 2H, J = 8.3 Hz, Ar-H), 8.45 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1606 (C═N), 1292 (C—N), 1242, 1031 (C—O—C), 1176 (N—N). MS *m*/*z* 280: (M+1); Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.07; H, 6.08; N, 14.95.

4.1.2.2. 3-Ethyl-4-(4-fluorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3b). Yield = 66.3%, mp 95–98 °C. ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, J = 7.5 Hz, 3H, – CH_3), 2.71 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.14 (s, 2H, –OCH_2–), 6.90 (d, 2H, J = 8.7 Hz, Ar-H), 7.24 (d, 2H, J = 8.7 Hz, Ar-H), 7.35–7.50 (m, 4H, Ar-H), 8.50 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1602 (C═N), 1282 (C—N), 1238, 1072 (C—O—C), 1170 (N—N). MS: *m*/*z* 298 (M+1); Anal. Calcd for $C_{17}H_{16}FN_3O$: C, 68.67; H, 5.42; N, 14.13. Found: C, 68.42; H, 5.25; N, 14.05.

4.1.2.3. 3-Ethyl-4-(2-fluorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3c). Yield = 73.5%, mp 104–105 °C. ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, J = 7.5 Hz, 3H, –CH_3), 2.73 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.21 (s, 2H, –OCH_2–), 7.19 (d, 2H, J = 8.64 Hz, Ar-H), 7.51–7.56 (m, 4H, Ar-H), 7.38 (d, 2H, J = 8.64 Hz, Ar-H), 8.52 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1604 (C═N), 1297 (C—N),

1240, 1061(C—O—C), 1147 (N—N). MS *m/z*: 298 (M+1); Anal. Calcd for $C_{17}H_{16}FN_3O$: C, 68.67; H, 5.42; N, 14.13. Found: C, 68.53; H, 5.36; N, 13.82.

4.1.2.4. 3-Ethyl-4-(3-fluorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3d). Yield = 75.7%, mp 76–78 °C. ^{1}H NMR ($CDCl_3$) δ: 1.27 (t, J = 7.5 Hz, 3H, –CH_3), 2.71 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.12 (s, 2H, –OCH_2–), 7.08 (d, 2H, J = 7.38 Hz, Ar-H), 7.38–7.69 (m, 4H, Ar-H), 7.35 (d, 2H, J = 7.38 Hz, Ar-H), 8.16 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1608 (C═N), 1287 (C—N), 1237, 1071 (C—O—C), 1175 (N—N). MS: *m/z* 298 (M+1); Anal. Calcd for $C_{17}H_{16}FN_3O$: C, 68.67; H, 5.42; N, 14.13. Found: C, 68.51; H, 5.38; N, 13.81.

4.1.2.5. 3-Ethyl-4-(2,6-difluorobenzyloxy)phenyl-4*H*-1,2,4-triazole (3e). Yield = 82.5%, mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ: 1.23 (t, J = 7.5 Hz, 3H, –CH_3), 2.74 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.22 (s, 2H, –OCH_2–), 7.21 (d, 2H, J = 8.70 Hz, Ar-H), 7.42 (d, 2H, J = 8.70 Hz, Ar-H), 7.01–7.51 (m, 3H, Ar-H), 8.53 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1608 (C═N), 1285 (C—N), 1248, 1072 (C—O—C), 1168 (N—N). MS: *m/z* 316(M+1); Anal. Calcd for $C_{17}H_{15}F_2N_3O$: C, 64.75; H, 4.79; N, 13.33. Found: C, 64.51; H, 4.65; N, 13.15.

4.1.2.6. 3-Ethyl-4-(4-chlorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3f). Yield = 75.7%, mp 104–105 °C. ^{1}H NMR ($CDCl_3$) δ: 1.18 (t, J = 7.5 Hz, 3H, –CH_3), 2.45 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.02 (s, 2H, –OCH_2–), 7.01 (d, 2H, J = 7.82 Hz, Ar-H), 7.15 (d, 2H, J = 8.67 Hz, Ar-H), 7.26–7.32 (m, 4H, Ar-H), 8.11 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1604 (C═N), 1247 (C—N), 1242, 1012 (C—O—C), 1180 (N—N). MS: *m/z* 314 (M+1); Anal. Calcd for $C_{17}H_{16}ClN_3O$: C, 65.07; H, 5.14; N, 13.39. Found: C, 64.96; H, 5.08; N, 13.18.

4.1.2.7. 3-Ethyl-4-(2-chlorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3g). Yield = 81.0%, mp 118–120 °C. ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, J = 7.5 Hz, 3H, –CH_3), 2.75 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.26 (s, 2H, –OCH_2–), 7.36 (d, 2H, J = 3.57 Hz, Ar-H), 7.47 (d, 2H, J = 3.57 Hz, Ar-H), 7.57–7.60 (m, 4H, Ar-H), 8.54 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1605 (C═N), 1252 (C—N), 1242, 1008 (C—O—C), 1176 (N—N). MS: *m/z* 314 (M+1); Anal. Calcd for $C_{17}H_{16}ClN_3O$: C, 65.07; H, 5.14; N, 13.39. Found: C, 64.89; H, 5.07; N, 13.16.

4.1.2.8. 3-Ethyl-4-(3-chlorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3h). Yield = 79.8%, mp 102–104 °C. ^{1}H NMR ($CDCl_3$) δ: 1.28 (t, J = 7.5 Hz, 3H, –CH_3), 2.71 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.10 (s, 2H, –OCH_2–), 7.10 (d, 2H, J = 7.92 Hz, Ar-H), 7.21 (d, 2H, J = 7.92 Hz, Ar-H), 7.27–7.54 (m, 4H, Ar-H), 8.16 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1607 (C═N), 1272 (C—N), 1232, 1028 (C—O—C), 1175 (N—N). MS: *m/z* 314 (M+1); Anal. Calcd for $C_{17}H_{16}ClN_3O$: C,65.07; H, 5.14; N, 13.39. Found: C, 64.86; H, 5.06; N, 13.17.

4.1.2.9. 3-Ethyl-4-(2,6-dichlorobenzyloxyphenyl)-4*H*-1,2,4-triazole (3i). Yield = 82.2%, mp 141–142 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, J = 7.5 Hz, 3H, –CH_3), 2.76 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.38 (s, 2H, –OCH_2–), 7.24 (d, 2H, J = 8.76 Hz, Ar-H), 7.37 (d, 2H, J = 8.76 Hz, Ar-H), 7.40–7.49 (m, 3H, Ar-H), 8.55 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1608 (C═N), 1287 (C—N), 1229, 1018 (C—O—C), 1183 (N—N). MS: *m/z* 348 (M+1); Anal. Calcd for $C_{17}H_{15}Cl_2N_3O$: C, 58.63; H, 4.34; N, 12.07. Found: C, 58.42; H, 4.16; N, 11.91.

4.1.2.10. 3-Ethyl-4-(4-methanebenzyloxyphenyl)-4*H*-1,2,4-triazole (3j). Yield = 93.8%, mp 113–114 °C. ^{1}H NMR ($CDCl_3$) δ: 1.22 (t, J = 7.5 Hz, 3H, –CH_3), 2.33 (s, 3H, Ar-CH_3), 2.70 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.10 (s, 2H, –OCH_2–), 7.15 (d, 2H, J = 8.52 Hz, Ar-H), 7.34 (d, 2H, J = 8.52 Hz, Ar-H), 7.12-7.31 (m, 4H, Ar-H), 8.49 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1604 (C═N), 1247 (C—N), 1242, 1012(C—O—C), 1180 (N—N). MS: *m/z* 294 (M+1); Anal. Calcd for $C_{18}H_{19}N_3O$: C,73.69; H, 6.53; N, 14.32. Found: C, 73.65; H, 6.41; N, 14.05.

4.1.2.11. 3-Ethyl-4-(4-methoxybenzyloxyphenyl)-4*H*-1,2,4-triazole (3k). Yield = 76.9%, mp 93–95 °C. **^{1}H NMR** ($CDCl_3$) δ: 1.22 (t, J = 7.5 Hz, 3H, –CH_3), 2.72 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 3.33 (s, 3H, CH_3O–), 5.08 (s, 2H, –OCH_2–), 7.16 (d, 2H, J = 2.22 Hz, Ar-H), 7.35 (d, 2H, J = 2.22 Hz, Ar-H), 7.37–7.39 (m, 4H, Ar-H), 8.52 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1610 (C═N), 1296 (C—N), 1246, 1020 (C—O—C), 1168 (N—N). MS: *m/z* 310 (M+1); Anal. Calcd for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.64; H, 6.02; N, 13.35.

4.1.2.12. 3-Ethyl-4-(3,4-methylenedioxybenzyloxyphenzol)-4*H*-1,2,4-triazole (3l). Yield = 78.8%, mp 89–90 °C. ^{1}H NMR ($CDCl_3$) δ: 1.21 (t, J = 7.5 Hz, 3H, –CH_3), 2.71 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 5.05 (s, 2H, –OCH_2–), 5.93 (s, 2H, –OCH_2–O–), 6.98–6.95 (m, 3H, Ar-H), 7.15 (d, 2H, J = 8.78 Hz, Ar-H), 7.34 (d, 2H, J = 8.78 Hz, Ar-H), 8.48 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1606 (C═N), 1288 (C—N), 1246, 1031 (C—O—C), 1176 (N—N). MS: *m/z* 324 (M+1); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.65; H, 5.16; N, 12.88.

4.1.2.13. 3-Ethyl-4-(4-ethoxyphenyl)-4*H*-1,2,4-triazole (3m). Yield = 77.5%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.80 (t, J = 6.8 Hz, 3H, –CH_3), 1.19 (t, J = 7.5 Hz, 3H, –CH_3), 2.66 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 3.89 (t, J = 6.4 Hz, 2H, –CH–O–), 7.06 (d, 2H, J = 8.4 Hz, Ar-H), 7.53 (d, 2H, J = 8.4 Hz, Ar-H), 8.18 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1610 (C═N), 1290 (C—N), 1256, 1036 (C—O—C), 1174 (N—N). MS: *m/z* 218 (M+1); Anal. Calcd for $C_{12}H_{15}N_3O$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.12; H, 6.75; N, 19.14.

4.1.2.14. 3-Ethyl-4-(4-propoxyphenyl)-4*H*-1,2,4-triazole (3n). Yield = 77.5%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.80 (t, J = 6.8 Hz, 3H, –CH_3), 1.00 (t, J = 7.5 Hz, 3H, –CH_3), 1.39 (m, 2H, –CH_2–), 2.45 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 3.72 (t, J = 6.4 Hz, 2H, –CH–O–), 6.78 (d, 2H, J = 3.46 Hz, Ar-H), 6.98 (d, 2H, J = 3.46 Hz, Ar-H), 7.91 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1611 (C═N), 1292 (C—N), 1246, 1038 (C—O—C), 1176

(N—N). MS: *m/z* 232 (M+1); Anal. Calcd for $C_{13}H_{17}N_3O$: C, 67.51; H, 7.41; N, 18.17. Found: C, 67.31; H, 7.35; N, 18.02.

4.1.2.15. 3-Ethyl-4-(4-butoxyphenyl)-4*H*-1,2,4-triazole (3o). Yield = 79.3%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.84 (t, J = 6.8 Hz, 3H, –CH_3), 1.12 (t, J = 7.5 Hz, 3H, –CH_3), 1.37 (m, 2H, –CH_2–), 1.66 (m, 2H, –CH_2–), 2.57 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 3.87 (t, J = 6.4 Hz, 2H, –CH–O–), 6.89 (d, 2H, J = 8.5 Hz, Ar-H), 7.08 (d, 2H, J = 8.5 Hz, Ar-H), 8.02 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1611 (C═N), 1292 (C—N), 1246, 1038 (C—O—C), 1176 (N—N). MS: *m/z* 246 (M+1); Anal. Calcd for $C_{14}H_{19}N_3O$: C, 68.54; H, 7.81; N, 17.13. Found: C, 68.32; H, 7.65; N, 17.01.

4.1.2.16. 3-Ethyl-4-(4-hexyloxyphenyl)-4*H*-1,2,4-triazole (3p). Yield = 87.5%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.82 (t, J = 6.8 Hz, 3H, –CH_3), 1.27 (t, J = 7.5 Hz, 3H, –CH_3), 1.34 (m, 4H, –$(CH_2)_2$–), 1.77 (m, 2H, –CH_2–), 2.77 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 4.01 (t, J = 6.4 Hz, 2H, –CH–O–), 7.01 (d, 2H, J = 7.45 Hz, Ar-H), 7.22 (d, 2H, J = 7.45 Hz, Ar-H), 8.29 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1613 (C═N), 1295 (C—N), 1249, 1036 (C—O—C), 1178 (N—N). MS: *m/z* 274 (M+1); Anal. Calcd for $C_{16}H_{23}N_3O$: C, 70.30; H, 8.48; N, 15.37. Found: C, 70.10; H, 8.25; N, 15.15.

4.1.2.17. 3-Ethyl-4-(4-heptyloxyphenyl)-4*H*-1,2,4-triazole (3q). Yield = 86.3%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, J = 6.8 Hz, 3H, –CH_3), 1.23 (t, J = 7.5 Hz, 3H, –CH_3), 1.44 (m, 6H, –$(CH_2)_3$–), 1.82 (m, 2H, –CH_2–), 2.74 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 4.05 (t, J = 6.4 Hz, 2H, –CH–O–), 7.51 (d, 2H, J = 8.7 Hz, Ar-H), 7.36 (d, 2H, J = 8.7 Hz, Ar-H), 8.52 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1615 (C═N), 1298 (C—N), 1251, 1042 (C—O—C), 1182 (N—N). MS; *m/z* 288 (M+1); Anal. Calcd for $C_{17}H_{25}N_3O$: C, 71.04; H, 8.77; N, 14.62. Found: C, 70.86; H, 8.59; N, 14.35.

4.1.2.18. 3-Ethyl-4-(4-octyloxyphenyl)-4*H*-1,2,4-triazole (3r). Yield = 87.5%, oil. ^{1}H NMR ($CDCl_3$) δ: 0.81 (t, J = 6.8 Hz, 3H, –CH_3), 1.19 (t, J = 7.5 Hz, 3H, –CH_3), 1.39 (m, 8H, –$(CH_2)_4$–), 1.76 (m, 2H, –CH_2–), 2.63 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 3.93 (t, J = 6.4 Hz, 2H, –CH–O–), 6.94 (d, 2H, J = 8.5 Hz, Ar-H), 7.12 (d, 2H, J = 8.5 Hz, Ar-H), 8.08 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1617 (C═N), 1296 (C—N), 1249, 1039 (C—O—C), 1178 (N—N). MS: *m/z* 302 (M+1); Anal. Calcd for $C_{18}H_{27}N_3O$: C, 71.72; H, 9.03; N, 13.94. Found: C, 71.56; H, 8.97; N, 13.78.

4.1.2.19. 3-Ethyl-4-(4-dodecyloxyphenyl)-4*H*-1,2,4-triazole (3s). Yield = 92.5%, mp 58–60 °C. ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, J = 6.8 Hz, 3H, –CH_3), 1.24 (t, J = 7.5 Hz, 3H, –CH_3), 1.42 (m, 14H, –$(CH_2)_7$–), 1.82 (m, 2H, –CH_2–), 2.75 (q, J = 7.5 Hz, 2H, –CH_2CH_3), 4.06 (t, J = 6.4 Hz, 2H, –CH–O–), 7.12 (d, 2H, J = 1.86 Hz, Ar-H),7.38 (d, 2H, J = 1.86 Hz, Ar-H), 8.54 (s, 1H, triazol-H). IR (KBr) cm^{-1}: 1619 (C═N), 1298 (C—N), 1247, 1041 (C–O–C), 1181 (N—N). MS: *m/z* 358 (M+1); Anal. Calcd for $C_{22}H_{35}N_3O$: C, 73.91; H, 9.87; N, 11.75. Found: C, 73.75; H, 9.67; N, 11.54.

4.2. Pharmacology

The MES test, scMet test and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health following previously described testing procedures (USA).[15,16] All compounds, which were dissolved in polyethylene glycol-400, were evaluated for anticonvulsant activities with C57B/6 mice in the 18–25 g weight range. Groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by means of a computer program written by the National Institute of Neurological Disorders and Stroke.

4.2.1. Maximal electroshock seizure. Seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. At 30 min after the administration of the compounds, the activities were evaluated in MES test.

4.2.2. Pentylenetetrazole-induced seizures.[15,16] At 30 min after the administration of the compounds, the animals' sc dose of pentylenetetrazole (85 mg/kg) at which 100% of the animals showed convulsive reaction was determined by a dose–percent effect curve. This dose was then administered to animals 30 min after different treatments, latency and duration of convulsions as well as latency and percentage of lethality were recorded during the 1 h following pentylenetetrazole administration. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

4.2.3. Isoniazid-induced seizures.[17] At 30 min after the administration of the compounds, the animals' ip dose of Isoniazid (250 mg/kg) at which 100% of the animals showed convulsive reaction was determined by a dose–percent effect curve. The mice were placed in individual cages and observed for 1 h. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

4.2.4. Thiosemicarbazide-induced seizures.[18] At 30 min after the administration of the compounds, the animals' ip dose of thiosemicarbazide (50 mg/kg) at which 100% of the animals showed convulsive reaction was determined by a dose–percent effect curve. This dose was then administered to animals 1 h after different treatments, latency and duration of convulsions as well as latency and percentage of lethality were recorded during the 2 h 30 min following thiosemicarbazide administration. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

4.2.5. 3-Mercaptopropionic acid-induced seizures.[19] At 30 min after the administration of the compounds,

40 mg/kg 3-mercaptopropionic acid in saline solution was injected sc. This dose was then administered to animals 1 h after different treatments, latency and duration of convulsions as well as latency and percentage of lethality were recorded during the 1h following 3-mercaptopropionic acid administration. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

4.2.6. Strychnine-induced seizures.[20] At 30 min after the administration of the compounds, the animals' sc dose of strychnine chlorhydrate in saline (1.2 mg/mL, 1 mL/kg) at which 100% of the animals showed convulsive reaction was determined by a dose–percent effect curve. The mice were placed in individual cages and observed for 30 min. The time of onset of the seizure, the number of tonic seizures and the lethality were recorded.

4.2.7. Rotarod test.[21] At 30 min after the administration of the compounds, the animals were tested on a 1-in. diameter, knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials.

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