European Journal of Medicinal Chemistry 44 (2009) 954–958

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]quinoline derivatives with anticonvulsant activity

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ARTICLE INFO

Article history:
Received 4 April 2008
Received in revised form 10 July 2008
Accepted 11 July 2008
Available online 19 July 2008

Keywords:
1,2,4-Triazolo[4,3-*a*]quinoline
Triazole
Quinoline
Anticonvulsant
Maximal electroshock
Neurotoxicity
Pentylenetetrazole
Isoniazid
Thiosemicarbazide
3-Mercaptopropionic acid
Strychnine

ABSTRACT

A series of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]quinoline derivatives were synthesized using 4-hydroxyquinolin-2(1*H*)-one as the starting material. Their anticonvulsant activities were evaluated by the maximal electroshock test (MES) and their neurotoxicities were measured by the rotarod test. The results of these tests demonstrated that 5-hexyloxy-[1,2,4]triazolo[4,3-*a*]quinoline (**3f**) was the most potent anticonvulsant, with median effective dose (ED_{50}) of 19.0 mg/kg and protective index ($PI = TD_{50}/ED_{50}$) values of 5.8 in the MES test. Compound 5-benzyloxy-[1,2,4]triazolo[4,3-*a*]quinoline (**3j**), exhibited a little weaker activity than compound **3f** in controlling the seizure induced by MES test at the dose of 22.8 mg/kg, but it possessed lower neurotoxicity with PI value of 12.0, which was safer than marketed drug carbamazepine. To explain the possible mechanism of anticonvulsant activity, compound **3j** 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 anemia [2–4], and even life threatening conditions [5]. Research to find more effective and safer antiepileptic drugs are, therefore, imperative and challenging in medicinal chemistry.

In our previous work, a series of derivatives of 6-alkoxy 3,4-dihydro-2(1*H*)-quinoline were first found to have anticonvulsant activities, among which 6-benzyloxy-3,4-dihydro-2(1*H*)-quinoline showed the strongest activity with an ED_{50} value of 29.6 mg/kg in the MES and a TD_{50} value of greater than 300 mg/kg [6]. Introduction of triazole ring to the first and second position of this 6-benzyloxy-3,4-dihydro-2(1*H*)-quinoline caused a remarkable increase in the anticonvulsant activity, as seen in 7-benzyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline (compound **I**), which showed ED_{50} values of 17.3 and 24 mg/kg in the MES and the sc-PTZ tests, respectively. Another derivative in the group of 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline (compounds **II**), 7-(4-fluorobenzyloxyl)-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline, showed an ED_{50} of 11.1 mg/kg, PI value of 4.6 in the MES test [7] (Fig. 1).

In the present study, we report the synthesis and anticonvulsant activities of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]quinoline derivatives (compounds **III**) to investigate the contribution of different alkoxyl groups at position 5 of the 1,2,4-triazolo[4,3-*a*]quinoline to the anticonvulsant activity. On the basic of compounds **II**, compounds **III** in this paper were introduced a double bond into the 4th and 5th positions. Via the conjugation effect, the electron cloud density at triazole ring was increased because of the existence of lone pair electrons of oxygen atom of 5-alkoxy, so as to increase the combination ability of compounds **III** to the receptor. Their structures were characterized using IR, ^{1}H NMR, MS, and elemental analysis techniques. In additional, their anticonvulsant activity was evaluated using the MES test and reported for the first time. Their neurotoxicity was evaluated using the rotarod test in mice. For

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doi:10.1016/j.ejmech.2008.07.010

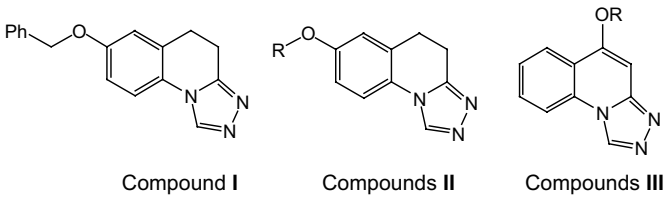


Fig. 1. Structure of compounds **I**, **II** and **III**.

explaining the possible mechanism of action, compound **3j** was tested in pentylenetetrazole (PTZ), isoniazid, thiosemicarbazide, 3-mercaptopropionic acid (3-MP) and strychnine-induced test. In the same condition, the anti-MES activity and the neurotoxicity of the marketed agent carbamazepine were evaluated in our laboratory taken as comparison.

2. Result and discussion

2.1. Chemistry

Based on the previous studies in our laboratory, we designed and synthesized a series of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]quinoline derivatives (**3a**–**u**). Target compounds **3a**–**u** were synthesized according to Scheme 1. Starting material 4-hydroxyquinolin-2(1*H*)-one reacted with appropriate alkyl halide to obtain derivatives **1a**–**i** in acetone with stirring and refluxing [8], derivatives **1j**–**u** were prepared by the reaction of 4-hydroxyquinolin-2(1*H*)-one with appropriate benzyl chloride in DMF with stirring and refluxing. Derivatives **1a**–**u** reacted further with $POCl_3$ at 80 °C to yield 2-chloro-4-alkoxyquinolines (**2a**–**u**) [8]. Derivatives **2a**–**u** reacted with formyl hydrazine in *n*-butanol to obtain target compounds **3a**–**u**.

2.2. Pharmacology

The results of pharmacology test of all synthesized compounds and reference drug were shown in Table 1. As shown in Table 1, most of the compounds showed remarkable anticonvulsant activity. Length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 4-alkyloxyl derivatives. From compound **3a** to **i**, as alkyl chain length increased, ED_{50} gradually increased with the compound **3f** (with the *n*-hexyloxy substituted group) being the most active. The trend reversed, however, when the alkyl chain had more than six carbon numbers. These alkoxy derivatives, except compound **3f**, exhibited smaller PI value ranging form 1.1 to 2.6 than compared to the drug carbamazepine. Compound **3f**, with ED_{50} value of 19.0 mg/kg, was a little weaker than compound **I** in anti-MES test, but it possessed lower neurotoxicity with PI value of 5.8. And the anticonvulsant activity decreased obviously when alkyl chain number lengthened to eight, even showed no activity at a dose of 100 mg/kg.

In those benzyloxy substituted derivatives **3j**–**u**, only 5-benzyloxy-[1,2,4]triazolo[4,3-*a*]quinoline compound (**3j**) and 5-(2-fluoro benzyloxy)-[1,2,4]triazolo[4,3-*a*]quinoline (**3k**) possessed excellent anticonvulsant activity with ED_{50} values of 22.8 and 25.4 mg/kg, respectively. Compound **3j** exhibited stronger activity than compound **3k** against seizure induced by MES, in additional, compound **3j** possessed lower neurotoxicity with PI value of 12, and it was the safest compound in all 21 derivatives of this paper. Compared with the reference compound **I**, the two compounds **3j** and **k** showed possessed lower neurotoxicity.

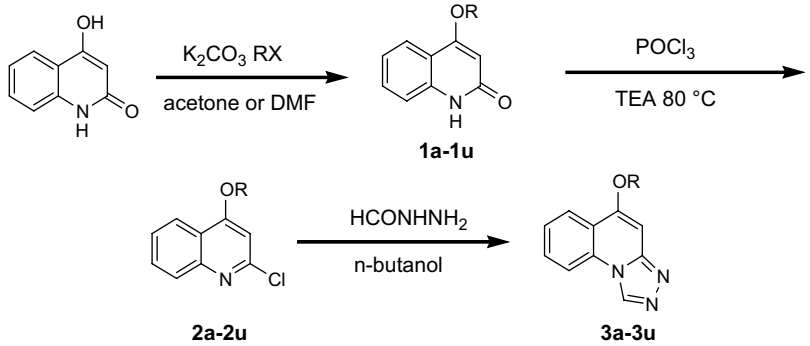
As a result of the first test, taking consideration of the safety, compound **3j** was then selected for further investigations against seizures induced by PTZ, 3-MP, thiosemicarbazide, isoniazid and strychnine to prove its anticonvulsant activity and speculate about the possible mechanism of anticonvulsant action. As shown in Table 2, compound **3j** was effective against the seizures induced by PTZ, isoniazid, 3-MP and thiosemicarbazide, and they exhibited stronger potency than carbamazepine in most of the chemically induced seizures.

PTZ and isoniazid have been reported to produce seizures by inhibiting gamma-aminobutyic acid (GABA) neurotransmission [9]. 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 promote and facilitate seizures [10], 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 PTZ-induced and isoniazid-induced seizures in mice by enhancing GABAergic neurotransmission.

3-MP acid and thiosemicarbazide were seen as the competitive inhibitor of GABA synthesis enzyme glutamate decarboxylase (GAD), inhibited the synthesis of GABA to decrease the GABA level in the brain [11]. The moderate antagonism of 3-MP-induced and thiosemicarbazide-induced seizures suggests that these series of compounds might activate GAD or inhibit (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

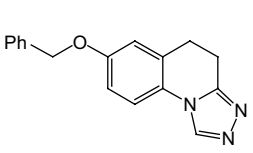
As shown in Table 2, compound **3j** failed to protect animals from seizure induced by strychnine. It is known that strychnine directly antagonize the inhibitory spinal reflexes of glycine [12], so the result suggested that compound**s** could not influence glycine system.

In conclusion, in the present study, we found that 5-benzyloxy-[1,2,4]triazolo[4,3-*a*]quinoline (**3j**) possessed the most potential anticonvulsant activity with ED_{50} value of 22.8 mg/kg and protective index value of 12.0. Especially, compound **3j** produced significant antagonism activity against seizures induced by PTZ, 3-MP,

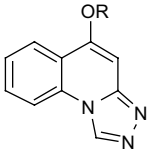


Scheme 1. Synthesis of Compounds **3a**–**3u**.

Table 1
Quantitative anticonvulsant data in mice (test drug administered ip)[a]



Compound **I**



3a-3u

Compound	R	MES, ED_{50}[b]	Toxicity, TD_{50}[c]	PI = TD_{50}/ED_{50}
Compound **I**	–	17.3 (14.8—20.4)[d]	61.4 (51.4—73.3)	3.5
3a	$-CH_3$	93.0 (69.0–125.3)	101.8 (80.8–128.3)	1.1
3b	$-C_2H_5$	91.3 (67.0–124.4)	131.4 (98.2–175.9)	1.4
3c	$-C_3H_7$	65.7 (47.4–91.1)	94.7 (69.9–128.2)	1.4
3d	$-C_4H_9$	36.7 (29.7–45.2)	54.8 (40.9–73.4)	1.5
3e	$-C_5H_{11}$	24.5 (19.8–30.2)	63.4 (46.5–86.4)	2.6
3f	$-C_6H_{13}$	19.0 (13.4–26.8)	109.5 (81.8–146.6)	5.8
3g	$-C_7H_{15}$	68.2 (55.2–84.2)	109.5 (81.8–146.6)	1.6
3h	$-C_8H_{17}$	>100	–[e]	–
3i	$-C_{12}H_{25}$	–	–	–
3j	$-CH_2Ph$	22.8 (16.7–31.0)	273.9 (204.6–366.7)	12.0
3k	$-CH_2Ph$(*o*-F)	25.4 (19.8–32.5)	228.2 (170.4–305.5)	9.0
3l	$-CH_2Ph$(*m*-F)	>100	–	–
3m	$-CH_2Ph$(*p*-F)	>100	–	–
3n	$-CH_2Ph$(*o*-Cl)	>100	–	–
3o	$-CH_2Ph$(*m*-Cl)	–	–	–
3p	$-CH_2Ph$(*p*-Cl)	>100	–	–
3q	$-CH_2Ph(2,4\text{-}Cl_2)$	>100	–	–
3r	$-CH_2Ph(2,6\text{-}Cl_2)$	>100	–	–
3s	$-CH_2Ph$(*o*-Br)	>100	–	–
3t	$-CH_2Ph$(*p*-Br)	>100	–	–
3u	$-CH_2Ph$(*p*-CH_3)	>100	–	–
Carbamazepine	–	11.8 (8.5–16.4)	76.1 (55.8–103.7)	6.4

[a] All of the tested compounds were dissolved in polyethylene glycol-400.
[b] The dose is measured in mg/kg.
[c] Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administrated.
[d] The 95% confidence limits.
[e] Not tested.

thiosemicarbazide and isoniazid, suggested that the compound **3j** might have effects on GABAergic neurotransmission and activate GAD or inhibit GABA-T in the brain.

3. Experimental section

3.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Perkin–Elmer, USA). ^{1}H NMR spectra were measured on a BRUKER-300 (Bruker Bioscience, Billerica, MA, USA), and all chemical shifts were given in parts per million relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Hewlett-Packard, PaloAlto, CA, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer (Heraeus GmbH, Hanau, Germany).

3.2. General procedure for the synthesis of 5-alkoxy-[1,2,4]triazolo[4,3-a]quinolines (**3a**–**u**)

A solution of **2a**–**u** (10 mmol) in appropriate *n*-butanol and formyl hydrazine (12 mmol) was refluxed for 8–12 h (TLC monitoring), the solvent was evaporated to dryness under reduced pressure, and the residue was extracted twice with dichloromethane (60 mL). The dichloromethane layer was washed three times with saturated aqueous NaCl (60 × 3) and dried over anhydrous $MgSO_4$. After removing the solvents, products were purified by silica gel column chromatography with ethyl acetate:methanol (12:1). The yield, melting point and spectral data of each compound were given below.

3.2.1. 5-Methoxy-[1,2,4]triazolo[4,3-a]quinoline (**3a**)

Yield 46%, mp 202–204 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.07 (s, 3H, $-OCH_3$), 6.88 (s, 1H, H-4), 7.53–8.24 (m, 4H, Ar-H), 9.10 (s, 1H, H-1). IR (KBr) cm^{-1}: 1640 (C=N), 1300 (C–N), 1240, 1015 (C–O–C), 1146 (N–N). MS *m*/*z* 200 (M + 1), Anal. Calcd for $C_{11}H_9N_3O$: C 66.32, H 4.55, N 21.09. Found: C 66.24, H 4.36, N 21.13.

3.2.2. 5-Ethoxy-[1,2,4]triazolo[4,3-a]quinoline (**3b**)

Yield 42%, mp 201–203 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.60 (t, 3H, J = 6.9 Hz, $-CH_3$), 4.28 (q, 2H, J = 6.9 Hz, $-OCH_2-$), 6.87 (s, 1H, H-4) 7.54–8.25 (m, 4H, Ar-H), 9.10 (s, 1H, H-1). IR (KBr) cm^{-1}: 1638 (C=N), 1302 (C–N), 1239, 1013 (C–O–C), 1145 (N–N). MS *m*/*z* 214 (M + 1), Anal. Calcd for $C_{12}H_{11}N_3O$: C 67.59, H 5.20, N 19.71. Found: C 67.32, H 5.31, N 19.53.

3.2.3. 5-Propoxy-[1,2,4]triazolo[4,3-a]quinoline (**3c**)

Yield 47%, mp 200–202 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.16 (t, 3H, J = 7.4 Hz, $-CH_3$), 1.94–2.06 (m, 2H, $-CH_2-$), 4.18 (t, 2H, J = 6.4 Hz, $-OCH_2-$), 6.86 (s, 1H, H-4), 7.54–8.25 (m, 4H, Ar-H), 9.09 (s, 1H, H-1). IR (KBr) cm^{-1}: 1636 (C=N), 1308 (C–N), 1236, 1012 (C–O–C), 1146 (N–N). MS *m*/*z* 228 (M + 1), Anal. Calcd for $C_{13}H_{13}N_3O$: C 68.70, H 5.77, N 18.49. Found: C 68.64, H 5.56, N 18.71.

3.2.4. 5-Butoxy-[1,2,4]triazolo[4,3-a]quinoline (**3d**)

Yield 47%, mp 172–173 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (t, 3H, J = 7.4 Hz, $-CH_3$), 1.58–1.66 (m, 2H, $-CH_2-$), 1.91–1.98 (m, 2H, $-CH_2-$), 4.22 (t, 2H, J = 6.3 Hz, $-OCH_2-$), 6.86 (s, 1H, H-4), 7.54–8.24 (m, 4H, Ar-H), 9.09 (s, 1H, H-1). IR (KBr) cm^{-1}: 1636 (C=N), 1300 (C–N), 1242, 1015 (C–O–C), 1142 (N–N). MS *m*/*z* 242 (M + 1), Anal. Calcd for $C_{14}H_{15}N_3O$: C 69.69, H 6.27, N 17.41. Found: C 69.52, H 6.35, N 17.54.

3.2.5. 5-Pentyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3e**)

Yield 51%, mp 140–142 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (t, 3H, J = 7.1 Hz, $-CH_3$), 1.43–1.60 (m, 4H, $-CH_2CH_2-$), 1.96–2.00 (m, 2H, $-CH_2-$), 4.22 (t, 2H, J = 6.4 Hz, $-OCH_2-$), 6.88 (s, 1H, H-4), 7.58–8.25 (m, 4H, Ar-H), 9.10 (s, 1H, H-1). IR (KBr) cm^{-1}: 1635 (C=N), 1305 (C–N), 1240, 1013 (C–O–C), 1145 (N–N). MS *m*/*z* 256 (M + 1),

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

Compound	Pentylenetetrazole	Isoniazid	Thiosemicarbazide	3-Mercaptopropionic acid	Strychnine
3j	27.4 (20.5–36.7)	38.0 (27.9–51.8)	19.0 (13.4–26.8)	9.5 (7.0–12.9)	–[a]
carbamazepine	11.4 (8.4–15.5)	22.8 (16.7–31.1)	19.7 (14.5–26.8)	13.7 (10.0–18.7)	–

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

Anal. Calcd for $C_{15}H_{17}N_3O$: C 70.56, H 6.71, N 16.46. Found: C 70.71, H 6.82, N 16.28.

*3.2.6. 5-Hexyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3f**)*

Yield 50%, mp 134–136 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H, $J = 6.9$ Hz, $-CH_3$), 1.39–1.61 (m, 6H, $(-CH_2-)_3$), 1.71–2.02 (m, 2H, $-CH_2-$), 4.22 (t, 2H, $J = 6.4$ Hz, $-OCH_2-$), 6.87 (s, 1H, H-4), 7.55–8.25 (m, 4H, Ar-H), 9.10 (s, 1H, H-1). IR (KBr) cm^{-1}: 1637 (C=N), 1302 (C–N), 1237, 1011 (C–O–C), 1142 (N–N). MS *m/z* 270 (M + 1), Anal. Calcd for $C_{16}H_{19}N_3O$: C 71.35, H 7.11, N 15.60. Found: C 71.28, H 7.21, N 15.43.

*3.2.7. 5-Heptyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3g**)*

Yield 61%, mp 144–145 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, 3H, $J = 6.6$ Hz, $-CH_3$), 1.34–1.62 (m, 8H, $(-CH_2-)_4$), 1.92–2.01 (m, 2H, $-CH_2-$), 4.21 (t, 2H, $J = 6.4$ Hz, $-OCH_2-$), 6.86 (s, 1H, H-4), 7.54–8.24 (m, 4H, Ar-H), 9.09 (s, 1H, H-1). IR (KBr) cm^{-1}: 1638 (C=N), 1309 (C–N), 1239, 1010 (C–O–C), 1146 (N–N). MS *m/z* 284 (M + 1), Anal. Calcd for $C_{17}H_{21}N_3O$: C 72.06, H 7.47, N 14.83. Found: C 72.20, H 7.58, N 14.79.

*3.2.8. 5-Octyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3h**)*

Yield 50%, mp 130–132 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, 3H, $J = 6.6$ Hz, $-CH_3$), 1.32–2.00 (m, 12H, $(-CH_2-)_6$), 4.20 (t, 2H, $J = 6.4$ Hz, $-OCH_2-$), 6.85 (s, 1H, H-4), 7.53–8.24 (m, 4H, Ar-H), 9.08 (s, 1H, H-1). IR (KBr) cm^{-1}: 1641 (C=N), 1306 (C–N), 1237, 1014 (C–O–C), 1139 (N–N). MS *m/z* 298 (M + 1), Anal. Calcd for $C_{18}H_{23}N_3O$: C 72.70, H 7.80, N 14.13. Found: C 72.54, H 7.61, N 14.01.

*3.2.9. 5-Dodecyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3i**)*

Yield 51%, mp 126–128 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 3H, $J = 6.5$ Hz, $-CH_3$), 1.28–2.01 (m, 20H, $(-CH_2-)_{10}$), 4.21 (t, 2H, $J = 6.4$ Hz, $-OCH_2-$), 6.86 (s, 1H, H-4), 7.54–8.24 (m, 4H, Ar-H), 9.09 (s, 1H, H-1). IR (KBr) cm^{-1}: 1645 (C=N), 1302 (C–N), 1239, 1015 (C–O–C), 1142 (N–N). MS *m/z* 354 (M + 1), Anal. Calcd for $C_{22}H_{31}N_3O$: C 74.75, H 8.84, N 11.89. Found: C 74.93, H 8.65, N 11.63.

*3.2.10. 5-Benzyloxy-[1,2,4]triazolo[4,3-a]quinoline (**3j**)*

Yield 52%, mp 180–182 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.32 (s, 2H, $-OCH_2-$), 6.99 (s, 1H, H-4), 7.41–8.30 (m, 9H, Ar-H), 9.11 (s, 1H, H-1). IR (KBr) cm^{-1}: 1637 (C=N), 1298 (C–N), 1244, 1001 (C–O–C), 1150 (N–N). MS *m/z* 276 (M + 1), Anal. Calcd for $C_{17}H_{13}N_3O$: C 74.17, H 4.76, N 15.26. Found: C 74.31, H 4.97, N 15.21.

*3.2.11. 5-(2-Fluorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3k**)*

Yield 49%, mp 178–180 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.38 (s, 2H, $-OCH_2-$), 7.02 (s, 1H, H-4), 7.15–8.26 (m, 8H, Ar-H), 9.14 (s, 1H, H-1). IR (KBr) cm^{-1}: 1639 (C=N), 1301 (C–N), 1241, 1004 (C–O–C), 1148 (N–N). MS *m/z* 294 (M + 1), Anal. Calcd for $C_{17}H_{12}FN_3O$: C 69.62, H 4.12, N 14.33. Found: C 69.59, H 4.19, N 14.12.

*3.2.12. 5-(3-Fluorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3l**)*

Yield 54%, mp 202–204 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.31 (s, 2H, $-OCH_2-$), 6.96 (s, 1H, H-4), 7.08–8.29 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1640 (C=N), 1296 (C–N), 1240, 1006 (C–O–C), 1156 (N–N). MS *m/z* 294 (M + 1), Anal. Calcd for $C_{17}H_{12}FN_3O$: C 69.62, H 4.12, N 14.33. Found: C 69.48, H 4.29, N 14.25.

*3.2.13. 5-(4-Fluorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3m**)*

Yield 43%, mp 194–196 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.27 (s, 2H, $-OCH_2-$), 6.99 (s, 1H, H-4), 7.12–8.26 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1636 (C=N), 1301 (C–N), 1238, 1010 (C–O–C), 1150 (N–N). MS *m/z* 294 (M + 1), Anal. Calcd for $C_{17}H_{12}FN_3O$: C 69.62, H 4.12, N 14.33. Found: C 69.46, H 4.19, N 14.13.

*3.2.14. 5-(2-Chlorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3n**)*

Yield 60%, mp 190–192 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 2H, $-OCH_2-$), 6.99 (s, 1H, H-4), 7.33–8.30 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1640 (C=N), 1298 (C–N), 1240, 1006 (C–O–C), 1145 (N–N). MS *m/z* 310 (M + 1), Anal. Calcd for $C_{17}H_{12}ClN_3O$: C 65.92, H 3.90, N 13.57. Found: C 66.06, H 4.01, N 13.35.

*3.2.15. 5-(3-Chlorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3o**)*

Yield 46%, mp 186–188 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.28 (s, 2H, $-OCH_2-$), 6.95 (s, 1H, H-4), 7.39–8.27 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1642 (C=N), 1306 (C–N), 1239, 1001 (C–O–C), 1151 (N–N). MS *m/z* 310 (M + 1), Anal. Calcd for $C_{17}H_{12}ClN_3O$: C 65.92, H 3.90, N 13.57. Found: C 65.79, H 3.80, N 13.42.

*3.2.16. 5-(4-Chlorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3p**)*

Yield 51%, mp 218–220 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.29 (s, 2H, $-OCH_2-$), 7.00 (s, 1H, H-4), 7.42–8.27 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1638 (C=N), 1300 (C–N), 1244, 1008 (C–O–C), 1147 (N–N). MS *m/z* 310 (M + 1), Anal. Calcd for $C_{17}H_{12}ClN_3O$: C 65.92, H 3.90, N 13.57. Found: C 65.84, H 3.70, N 13.46.

*3.2.17. 5-(2,4-Dichlorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3q**)*

Yield 42%, mp 202–204 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.36 (s, 2H, $-OCH_2-$), 6.97 (s, 1H, H-4), 7.32–8.26 (m, 7H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1645 (C=N), 1307 (C–N), 1240, 1012 (C–O–C), 1154 (N–N). MS *m/z* 344 (M + 1), Anal. Calcd for $C_{17}H_{11}Cl_2N_3O$: C 59.32, H 3.22, N 12.21. Found: C 59.46, H 3.12, N 12.11.

*3.2.18. 5-(2,6-Dichlorobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3r**)*

Yield 45%, mp 222–224 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.53 (s, 2H, $-OCH_2-$), 7.11 (s, 1H, H-4), 7.34–8.15 (m, 7H, Ar-H), 9.13 (s, 1H, H-1). IR (KBr) cm^{-1}: 1643 (C=N), 1303 (C–N), 1244, 1016 (C–O–C), 1150 (N–N). MS *m/z* 344 (M + 1), Anal. Calcd for $C_{17}H_{11}Cl_2N_3O$: C 59.32, H 3.22, N 12.21. Found: C 59.15, H 3.12, N 12.28.

*3.2.19. 5-(2-Bromobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3s**)*

Yield 39%, mp 196–198 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.38 (s, 2H, $-OCH_2-$), 7.00 (s, 1H, H-4), 7.30–8.32 (m, 8H, Ar-H), 9.13 (s, 1H, H-1). IR (KBr) cm^{-1}: 1639 (C=N), 1298 (C–N), 1236, 1004 (C–O–C), 1147 (N–N). MS *m/z* 354 (M + 1), Anal. Calcd for $C_{17}H_{12}BrN_3O$: C 57.65, H 3.41, N 11.86. Found: C 57.80, H 3.52, N 11.73.

*3.2.20. 5-(4-Bromobenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3t**)*

Yield 41%, mp 208–210 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.25 (s, 2H, $-OCH_2-$), 6.95 (s, 1H, H-4), 7.39–8.25 (m, 8H, Ar-H), 9.12 (s, 1H, H-1). IR (KBr) cm^{-1}: 1637 (C=N), 1304 (C–N), 1241, 1001 (C–O–C), 1150 (N–N). MS *m/z* 354 (M + 1), Anal. Calcd for $C_{17}H_{12}BrN_3O$: C 57.65, H 3.51, N 11.76. Found: C 57.72, H 3.41, N 11.82.

*3.2.21. 5-(4-Methylbenzyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**3u**)*

Yield 53%, mp 208–209 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H, $-CH_3-$), 5.27 (s, 2H, $-OCH_2-$), 6.98 (s, 1H, H-4), 7.25–8.28 (m, 8H, Ar-H), 9.10 (s, 1H, H-1). IR (KBr) cm^{-1}: 1640 (C=N), 1301 (C–N), 1244, 1010 (C–O–C), 1154 (N–N). MS *m/z* 290 (M + 1), Anal. Calcd for $C_{18}H_{15}N_3O$: C 74.72, H 5.23, N 14.52. Found: C 74.65, H 5.14, N 14.39.

3.3. Pharmacology

The MES 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) [13,14]. 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.

3.3.1. 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.

3.3.2. Pentylenetetrazole-induced seizure [13,14]

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 clonic seizure was determined by a dose-percent effect curve. This dose was then administered to animals 30 min after different treatments. The doses were calculated which prevented 50% of the treated animals from clonic convulsions (ED_{50}).

3.3.3. Isoniazid-induced seizure [15]

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}).

3.3.4. Thiosemicarbazide-induced seizure [16]

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. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

3.3.5. 3-Mercaptopropionic acid-induced seizure [17]

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. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

3.3.6. Strychnine-induced seizure [18]

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.

3.3.7. Rotarod test [19]

At 30 min after the administration of the compounds, the animals were tested on a 1-inch 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.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 30460151 and No. 30760290) and Important Item Foundation of Ministry of Education PR China (No. 20070422029).

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