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Anticonvulsant and toxicity evaluation of some 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-ones

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Abstract—To further investigate anticonvulsant activity of quinoline derivatives, a series of 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one derivatives was synthesized starting from 7-hydroxyl-3,4-dihydro-2(1*H*)-quinoline. In initial (phase I) screening and quantitative (phase II) evaluation, compound 7-benzyloxyl-4,5-dihydro-[1,2,4]thiazolo[4,3-*a*]quinoline-1(2*H*)-one (**3f**) was among the most active but also has the lowest toxicity. In the anti-MES potency test, it showed median effective dose (ED_{50}) of 12.3 mg/kg, median toxicity dose (TD_{50}) of 547.5 mg/kg, and the protective index (PI) of 44.5, which is much greater than PI of the prototype drugs phenytoin, phenobarbital, carbamazepin, and valproate. Compound **3f** was chosen for further evaluation. In phase III pharmacological test, the compound had median hypnotic dose (HD_{50}) and median lethal dose (LD_{50}) of 1204 mg/kg and >3000 mg/kg, respectively, thus demonstrating much greater margin of safety compared to prototype drugs. The compound **3f** also showed significant oral activity against MES-induced seizures and low oral neurotoxicity in mice in phase IV pharmacological test. Possible structure–activity relationship was discussed.

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 is, 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 was first found to have anticonvulsant activities, among which 6-benzyloxy-3,4-dihydro-2(1*H*)-quinoline (compound **I**) 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 of greater than 300 mg/kg.[6] Introduction of triazole ring to the first and second position of this compound **I** 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 **II**), 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-fluoro-benzyloxyl)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline, showed ED_{50} values of 11.1 or 6.7 mg/kg, protective index ($PI = TD_{50}/ED_{50}$) values of 4.6 or 8.1 in the MES or the sc-PTZ tests, respectively, and thus demonstrated comparable anticonvulsant potency to that of phenobarbital in the corresponding tests.[8]

Aimed at exploring effective compounds with lower neurotoxicity, compound **III** was designed and synthesized which substituted by triazolone the triazole in compound **II**. The hypothesis was that triazolone compound

Keywords: 4,5-dihydro-[1,2,4]thiazolo[4,3-*a*]quinoline-1(2*H*)-one; Synthesis; Anticonvulsant activity; MES; sc-PTZ.
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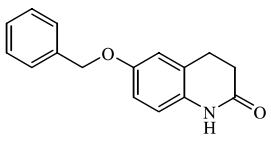
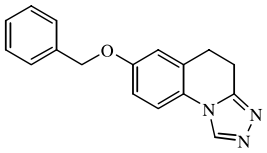
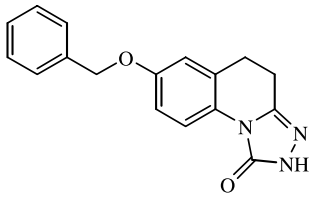
may have higher affinity to the receptor due to the carbonyl group, and thus may increase the anticonvulsant activity. There were some similar design reports.[9–11] For instance, when the triazole in the precursor of compound **IV** (8,9-dimethoxy-6-phenyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepine) was substituted by triazolone, the anticonvulsant activity was remarkably increased. So a series of 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one derivatives was prepared. The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models, that is, maximal electroshock test (MES) and pentylenetetrazol (sc-PTZ)-induced seizure in mice. The rotarod assay was performed in mice to evaluate the neurotoxicity of the compounds. From these tests compound **3f** was selected to undergo further (phases III and IV) pharmacological tests to evaluate its primary toxicology profile (median hypnotic dose (HD_{50}), median lethal dose (LD_{50})), oral activity against MES-induced seizures, and oral neurotoxicity in mice.



compound I



compound Ⅱ



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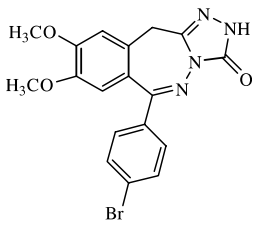
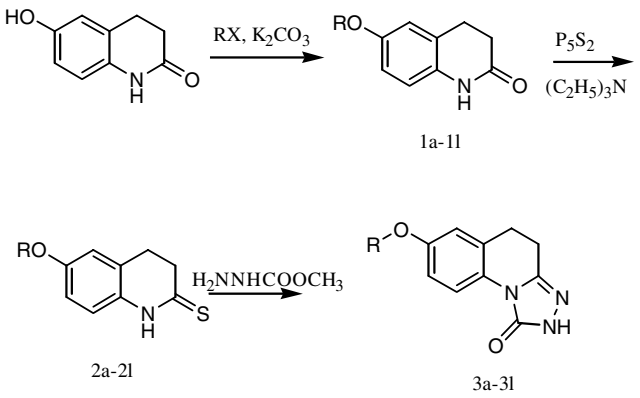


Ⅳ

2. Results and discussion

2.1. Synthesis

Target compounds were prepared along the reaction sequence in Scheme 1. The compound **1a**–**l** was synthesized using the method described in a former paper of our group.[6] 6-Hydroxy-3,4-dihydro-2(1*H*)-quinoline and appropriate alkyl halide reacted in the solution of sodium hydroxide methanol and produced the compound **1a**–**l**. Compound **1a**–**l** then reacted with phosphorus pentasulfide in acetonitrile in the presence of triethylamine under protection of nitrogen,[7] and the resulting compound (**2a**–**1**) reacted further with methyl hydrazine carboxylate in cyclohexanol to produce the target compounds 7-alkoxy-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-ones (**3a**–**l**) according to the Refs. 12 and 13.



R:

3a = n-C_4H_9	3e = n-C_8H_{17}	3i = $CH_2C_6H_4$(*p*-F)
3b = n-C_5H_{11}	3f = $CH_2C_6H_5$	3j = $CH_2C_6H_4$(*p*-CH_3)
3c = n-C_6H_{13}	3g = $CH_2C_6H_4$(*o*-F)	3k = $CH_2C_6H_4$(*p*-Cl)
3d= n-C_7H_{15}	3h = $CH_2C_6H_4$(*m*-F)	3l = $CH_2C_6H_4$(*p*-OCH_3)

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

2.2. Pharmacological evaluations

Pharmacological tests of the 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-ones (**3a**–**l**) were conducted at the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program.[14,15]

The results of preliminary (phase I) screening of **3a**–**l** are summarized in Table 1. All synthesized compounds exhibited strong anticonvulsant activity, among which seven compounds, including all of five alkyl-substituted derivatives **3a**–**e**, benzyl-substituted derivative **3f**, and

Table 1. Phase I anticonvulsant and toxicity data in mice (ip)[a]

Compound	MES[b]		scPTZ[c]		Rotarod toxicity	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
3a	30[d]	—[e]	—	—	—	—
3b	30	—	—	—	100	—
3c	30	—	—	—	100	—
3d	30	100	—	—	100	—
3e	30	—	—	—	—	—
3f	30	30	—	—	—	—
3g	100	—	—	—	100	—
3h	100	—	—	—	100	—
3i	30	—	—	—	—	—
3j	100	—	—	—	300	—
3k	100	—	—	—	—	—
3l	100	—	—	—	100	—

[a] All of tested compounds were dissolved in Polyethylene glycol-400.
[b] The maximal electroshock test was carried out 30 min after administration of the test compounds.
[c] Subcutaneous pentylenetetrazol (85 mg/kg) was administered 30 min after administration of the test compounds.
[d] Doses are denoted in milligrams per kilogram.
[e] —, no activity at 300 mg/kg.

(*p*-F) benzyl-substituted derivative **3i**, possessed anticonvulsant activity against MES-induced seizure at the dose of 30 mg/kg, and the remaining five benzyl-substituted compounds, **3g–h** and **3j–l**, were active at the dose of 100 mg/kg. However, none of the 12 compounds exhibited any potency to the convulsion induced by sc-PTZ at the dose of 300 mg/kg, for which we do not yet formulated a plausible activity–structure relation-based explanation. Rotarod toxicity test result indicated that five compounds (**3a**, **3e**, **3f**, **3i**, and **3k**) showed no toxicity at the dose of 300 mg/kg, meaning that they did not cause ataxia or other neurotoxic reactions and thus possessed high safety profile.

In the phase II pharmacology test, 12 compounds were quantitatively evaluated for their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}). Among these compounds, four compounds, **3c**, **3d**, **3e**, and **3f**, were more active than compound **II** in the MES test, and all four had lower neurotoxicity than compound **II**. In fact, all 12 compounds except one (**3b**) had markedly lower neurotoxicity than compound **II**. But all 12 compounds had no activity in the sc-PTZ test at the dose of 300 mg/kg. For the higher anticonvulsant activity of some compounds, we believe it may be due to the carbonyl group in the triazolone ring which enhanced affinity of the triazole ring to the receptor. For the general lower neurotoxicity, it is possible that compound hydrophobicity decreased when triazole was substituted by triazolone, which led to decreased drug concentration in brain and thus lower neurotoxicity.

Length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 7-alkyloxyl derivatives. From compound **3a** to **3d**, as alkyl chain length increased, ED_{50} gradually increased with the compound **3d** (with the *n*-heptyl-substituted group) being the most active. The trend reversed, however, when the alkyl chain had more than seven carbon numbers as seen with compound **3e**. Compound **3d**, with ED_{50} of 9.8 mg/kg, was better than phenobarbital and close to carbamazepin and phenytoin in anti-MES activity. With PI value of 20.8, compound **3d** could be considered superior to all the other drugs compared in terms of combined efficacy and safety profile. In addition, compound **3c**, with ED_{50} of 11.8 mg/kg and lower neurotoxicity and thus a high PI value of 44.7, could also be considered a potentially useful and safe therapeutic.

Among the 7-benzyloxy derivatives, the anticonvulsant potency of compounds containing substituted benzyloxy was lower than that of the compound with non-substituted benzyloxy (i.e., **3f**). The potency order of three F-substituted derivatives was *p*-F > *o*-F > *m*-F. The *p*-F-substituted derivative **3i** exhibited the strongest anticonvulsant activity, and has a higher PI value of 9.3 compared to the other two (**3g** and **3h**), thus its superiority in terms of safety as well. In another three substituted-benzyl derivatives **3j–l**, *p*-OCH_3 derivative **3l** exhibited the weakest anticonvulsant activity with anti-MES ED_{50} of 50.9 mg/kg. Nevertheless, it possessed the lowest neurotoxicity with PI value of 12.9 and thus higher safety profile.

The compound **3f** possessed strong anti-MES activity, with ED_{50} of 12.3 mg/kg, which was close to current epilepsy drugs phenytoin and carbamazepin. Its neurotoxicity was one of the lowest in all the synthesized compounds and was markedly lower than all current drugs compared (Table 2). It had a protective index as high as 44.5, which was many folds higher than current drugs whose PI values were in the range of 1.6–8.1. Compared with compound **3d**, compound **3f** possessed slightly lower anticonvulsant but nearly threefold lower neurotoxicity and thus a much higher PI value (44.5 vs. 20.8). Therefore, the compound **3f** was chosen to be

Table 2. Phase II quantitative anticonvulsant data in mice (test drug administered ip)

Compound	MES, ED_{50}[a]	sc-PTZ, ED_{50}[a]	Tox, TD_{50}[c]	PI[b]	
				MES	sc-PTZ
II	17.3	24.0	61.4	3.5	2.6
3a	19.7 (17.2–22.6)	>300	132.0 (112.5–154.9)	6.7	—
3b	18.2 (15.2–21.8)	>300	52.8 (44.9–61.9)	2.9	—
3c	11.8 (10.2–13.7)	>300	527.9 (440.0–633.4)	44.7	—
3d	9.8 (8.5–11.4)	>300	204.6 (170.5–245.4)	20.8	—
3e	14.2 (12.3–16.4)	>300	203.6 (170.9–242.5)	14.3	—
3f	12.3 (10.8–13.9)	>300	547.5 (459.5–652.2)	44.5	—
3g	49.1 (43.2–55.8)	>300	204.6 (178.9–223.8)	4.2	—
3h	65.4 (57.8–73.8)	>300	306.0 (261.3–358.4)	4.7	—
3i	24.5 (21.2–28.4)	>300	229.1 (195.3–268.8)	9.3	—
3j	33.9 (29.2–39.6)	>300	184.1 (155.7–217.7)	5.4	—
3k	35.3 (29.4–42.4)	>300	183.3 (160.4–209.5)	5.2	—
3l	50.9 (46.4–55.8)	>300	659.9 (550.0–786.4)	12.9	—
Phenytoin	9.5 (8.1–10.4)	>300	65.5 (52.5–72.9)	6.9	<0.22
Carbamazepin	8.8 (5.5–14.1)	>100	71.6 (45.9–135)	8.1	<0.22
Phenobarbital	21.8 (21.8–25.5)	13.2 (5.8–15.9)	69 (62.8–72.9)	3.2	5.2
Valproate	272 (247–338)	149 (123–177)	426 (369–450)	1.6	2.9

[a] The dose is in milligrams per kilogram.
[b] PI = TD_{50}/ED_{50}.
[c] Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administered.

Table 3. Phase III quantitative toxicity profile of **3f** and prototype anticonvulsant drugs

Compound	HD_{50}[a,b]	LD_{50}[a,c]	HD_{50}/ED_{50}
3f	1204 (1085–1373)	>2000	97.8
Phenytoin	178 (153–196)	230 (216–259)	18.8
Carbamazepin	172 (134–198)	629 (556–708)	19.5

[a] Median hypnoitic dose (HD_{50}) in milligrams per kilogram; determined by loss of righting reflex.
[b] 95% confidence intervals in parentheses.
[c] Median lethal dose (LD_{50}) in milligrams per kilogram; mortality was determined 24 h after ip injection.

further evaluated in phase III and phase IV pharmacological tests.

The toxicity profile of **3f** (Table 3) was determined in phase III testing by administering the drug intraperitoneally (ip) to mice at different doses ($1TD_{50}$, $2TD_{50}$, and $4TD_{50}$). The toxicity induced by **3f** was characterized by decreased motor activity, ataxia, sedation, ptosis, muscular relaxation, loss of righting reflex, decreased respiration, and cyanosis. Animals given doses of $2TD_{50}$ and $4TD_{50}$ also experienced hypnosis, analgesia, and anesthesia.

The median hypnotic dose (HD_{50}) for **3f** was established as 1204 mg/kg, which is twice the TD_{50} (547.5 mg/kg). Compound **3f** exhibited a greater margin of safety ($HD_{50}/ED_{50} = 97.8$) against MES-induced seizures than any of the prototype drugs whose HD_{50}/ED_{50} value was below 20. The 24 h median lethal dose (LD_{50}) could not be accurately determined, but it was in excess of 2000 mg/kg. The toxicity of compound **3f** was obviously much lower than any of the prototype drugs.

As in phase II, phase IV tests involved the evaluation of ED_{50} and TD_{50} for **3f**, except that the candidate drug was administered orally (po) rather than ip in mice. With consideration for enhancing drug bioavailability, the compound **3f** was evaluated as hydrochloride salt. As shown in Table 4, the orally anticonvulsant time to peak effect (TPE) was 2 h, and this was comparable with phenytoin and carbamazepin. The data in Table 4 clearly indicated a decrease in anticonvulsant potency and neurotoxicity of **3f** administered po compared to that in ip administration. Nevertheless, the PI values were comparable in the two modes of drug delivery. The po ED_{50}/ip ED_{50} ratios for **3f** were >5.48 and 5.54 calculated by rotarod and MES test, respectively. These ratios suggested that the compound **3f** was adequately absorbed in mice after oral administration. No neurotoxicity was found after oral administration at the very high dose of 3000 mg/kg, so protective index in po administration was 44.0, which was very close to that in ip administration and was better than any of the prototype drugs.

Table 4. Phase IV pharmacological evaluation of **3f** and prototype drugs administered in mice op

Compound	TPE (h)	MES, ED_{50}[a]	TD_{50}[a]	PI
3f[b]	2	68.2 (51.3–81.6)	>3000	>44.0
Phenytoin	2	9.04 (7.39–10.6)	86.7 (80.4–96.1)	9.59
Carbamazepin	2	15.4 (12.4–17.3)	217 (131.5–270.1)	14.1

[a] ED_{50} and TD_{50} values are in units of milligrams per kilogram and determined at the indicated time.
[b] The compound was evaluated as hydrochloride salt.

3. Conclusions

A new anticonvulsant compound, 7-benzyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one, was synthesized and its pharmacological properties were evaluated. The compound has stronger anticonvulsant activity compared with reference compound **II** which is likely due to the addition of a carbonyl group. The compound not only has higher anticonvulsant activity, but also has marked lower neurotoxicity. So a larger protective index was observed for this compound in mice compared with the prototype drugs phenytoin, phenobarbital, carbamazepin, and valproate.

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 and ^{13}C NMR spectra were measured on a BRUKER-300, and all chemical shifts were given in parts per million relative to tetramethylsilane. Mass spectra were measured on an AP12000 (EIS, 70 eV).

4.1.1. General procedure for preparation of 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-ones. Compounds **2a**–**l** (0.010 mol) reacted with methyl hydrazinocarboxylate (0.015 mol) in cyclohexanol 60 mL under nitrogen atmosphere for 30 h, the solvent was removed under reduced pressure, and the residue dissolved with dichloromethane and washed with water three times. The dichloromethane layer was dried with anhydrous $MgSO_4$, filtered, and concentrated the solvent, and the residue was chromatographed on silica gel using ether acetate and hexane (5:3) to afford target compounds **3a**–**l**.

4.1.2. 7-Butyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3a). Yield 58%, mp 181–183 °C. ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, $J = 7.1$ Hz, CH_3), 1.27–1.78 (m, 4H, CH_2), 2.89 (t, 2H, $J = 7.7$ Hz, 4-CH_2), 2.93 (t, 2H, $J = 7.8$ Hz, 5-CH_2), 3.98 (t, 2H, $J = 7.0$ Hz, 7-OCH_2), 6.80 (d, 1H, $J = 2.75$ Hz, 6-H), 6.88 (dd, 1H, $J = 9.15$, 9.15 Hz, 8-H), 8.23 (d, 1H, $J = 8.7$ Hz, 9-H), 9.98 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$) δ 14.1 21.9, 22.4, 26.5, 29.3, 68.4, 113.2, 115.0, 118.4, 126.2, 127.8, 144.7, 153.3, 156.9. MS *m*/*z* 260 (M+1). IR (KBr) cm^{-1}: 3186, 1699.

4.1.3. 7-Pentyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3b). Yield 56%, mp 197–199 °C. ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.3 Hz, CH_2), 1.37–1.76 (m, 6H, CH_2), 2.87 (t, 2H, J = 7.7 Hz, 4-CH_2), 2.93 (t, 2H, J = 8.7 Hz, 5-CH_2), 3.92 (t, 2H, J = 6.5 Hz, 7-OCH_2), 6.76 (d, 1H, J = 2.75 Hz, 6-H), 6.82 (dd, 1H, J = 9.15, 9.15 Hz, 8-H), 8.21 (d, 1H, J = 8.7 Hz, 9-H), 9.99 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$) δ 14.1 21.9, 22.5, 26.5, 28.3, 29.0, 68.4, 113.2, 115.0, 118.4, 126.2, 127.8, 144.7, 153.3, 156.9. MS *m*/*z* 274 (M+1). IR (KBr) cm^{-1}: 3189, 1698.

4.1.4. 7-Hexyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3c). Yield 62%, mp 148–151 °C. ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.3 Hz, CH_3), 1.34–1.76 (m, 8H, CH_2), 2.89 (t, 2H, J = 7.7 Hz, 4-CH_2), 2.92 (t, 2H, J = 8.6 Hz, 5-CH_2), 3.93 (t, 2H, J = 6.5 Hz, 7-OCH_2), 6.77 (d, 1H, J = 2.75 Hz, 6-H), 6.83 (dd, 1H, J = 9.15, 9.15 Hz, 8-H), 8.22 (d, 1H, J = 8.7 Hz, 9-H), 9.95 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$) δ14.1, 21.9, 22.7, 25.8, 26.5, 29.3, 31.7, 68.4, 113.2, 115.0, 118.4, 126.2, 127.8, 144.7, 153.3, 156.9. MS *m*/*z* 288 (M+1). IR (KBr) cm^{-1}: 3191, 1698.

4.1.5. 7-Heptyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3d). Yield 59%, mp 120–121 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H,J = 7.1 Hz, CH_3), 1.29–1.78 (m, 10H, CH_2), 2.89 (t, 2H, J = 6.8 Hz, 4-CH_2), 2.93 (t, 2H, J = 7.3 Hz, 5-CH_2), 3.93 (t, 2H, J = 6.3 Hz, 7-OCH_2), 6.77 (d, 1H, J = 2.75 Hz, 6-H), 6.84 (dd, 1H, J = 8.7, 8.7 Hz, 8-H), 8.23 (d, 1H, J = 8.7 Hz, 9-H), 10.07 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$) δ 14.2, 21.9, 22.7, 26.1, 26.5, 29.1, 29.3, 31.8, 68.4, 113.2, 115.0, 118.4, 126.2, 127.8, 144.7, 153.3, 156.9. MS *m*/*z* 302 (M+1). IR (KBr) cm^{-1}: 3192, 1695.

4.1.6. 7-Octyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3e). Yield 65%, mp 114–116 °C. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 7.1 Hz, CH_3), 1.25 ~ 1.79 (m, 12H, CH_2), 2.89 (t, 2H, J = 7.3 Hz, 4-CH_2), 2.93 (t, 2H, J = 6.65 Hz, 5-CH_2), 3.94 (t, 2H, J = 6.6 Hz, 7-OCH_2), 6.77 (d, 1H, J = 2.75 Hz, 6-H), 6.84 (dd, 1H, J = 8.7, 8.7 Hz, 8-H), 8.23 (d, 1H, J = 8.7 Hz, 9-H), 10.16 (s, 1H, N-H). ^{13}C NMR δ ($CDCl_3$) 14.2, 21.9, 22.7, 26.1, 26.5, 29.4, 29.3, 29.4, 31.9, 68.4, 113.2, 115.0, 118.4, 126.2, 127.8, 144.7, 153.3, 156.9. MS *m*/*z* 316 (M+1). IR (KBr) cm^{-1}: 3185, 1698.

4.1.7. 7-Benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3f). Yield 52%, mp 187–189 °C. ^{1}H NMR ($CDCl_3$) δ 2.89 (t, 2H, J = 6.9 Hz, 4-CH_2), 2.94 (t, 2H, J = 7.8 Hz, 5-CH_2), 5.06 (s, 2H, 7-OCH_2), 6.86–8.26 (m, 8H, Ar-H), 10.03 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 21.8, 26.5, 70.4, 113.6, 115.5, 118.5, 127.6, 128.1, 128.6, 128.7, 136.8, 144.6, 153.3, 154.9, 156.5. MS *m*/*z* 294 (M+1). IR (KBr) cm^{-1}: 3202, 1695.

4.1.8. 7-(2-Fluoro-benzyloxy)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-one (3g). Yield 48%, mp 173–176 °C. ^{1}H NMR ($CDCl_3$) δ 2.88 (t, 2H, J = 7.8 Hz, 4-CH_2), 2.93 (t, 2H, J = 8.25 Hz, 5-CH_2), 5.07 (s, 2H, 7-OCH_2), 6.74–8.26 (m, 7H, Ar-H), 10.16 (s,1H). ^{13}C NMR ($CDCl_3$) δ 25.8, 30.7, 64.3, 113.6, 114.9, 114.2 (d, J_{C-F} = 20.3 Hz), 115.1, 115.2, 115.3, 116.3, 124.3, 124.7, 124.4, 128.0, 129.8 (d, J_{C-F} = 7.5 Hz), 154.5, 160.5 (d, J_{C-F} = 254 Hz). MS *m*/*z* 312 (M+1). IR (KBr) cm^{-1}: 3201, 1688.

4.1.9. 7-(3-Fluoro-benzyloxy)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-one (3h). Yield 46%, mp 144–148 °C. ^{1}H NMR ($CDCl_3$) δ 2.61 (t, 2H, J = 7.3 Hz, 4-CH_2), 2.92 (t, 2H, J = 7.8 Hz, 5-CH_2), 5.01 (s, 2H, 7-OCH_2), 6.72–8.27 (m, 7H, Ar-H), 10.01 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 25.8, 30.7, 69.7, 113.6, 114.2, 114.4, 114.8 (d, J_{C-F} = 20.9 Hz), 115.0, 115.1, 116.3, 122.7, 125.3, 130.3 (d, J_{C-F} = 7.5 Hz), 132.4, 139.7, 154.5, 163.1 (d, J_{C-F} = 254 Hz). MS *m*/*z* 312 (M+1). IR (KBr) cm^{-1}: 3201, 1689.

4.1.10. 7-(4-Fluoro-benzyloxy)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-one (3i). Yield 53%, mp 171–174 °C. ^{1}H NMR ($CDCl_3$) δ 2.89 (t, 2H, J = 7.7 Hz, 4-CH_2), 2.95 (t, 2H, J = 7.35 Hz, 5-CH_2), 5.02 (s, 2H, 7-OCH_2), 6.84–8.26 (m, 7H, Ar-H), 9.83 (s,1H). ^{13}C NMR ($CDCl_3$) δ 21.8, 26.5, 69.7, 113.5, 115.4, 115.6 (d, J_{C-F} = 20.9 Hz), 118.4, 126.7, 127.9, 129.3 (d, J_{C-F} = 7.5 Hz), 132.4, 144.5, 153.2, 156.2, 162.2 (d, J_{C-F} = 254 Hz). MS *m*/*z* 312 (M+1). IR (KBr) cm^{-1}: 320, 1689.

4.1.11. 7-(4-Methyl-benzyloxy)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-one (3j). Yield 43%, mp 159–162 °C. ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, Ar-CH_3), 2.62 (t, 2H, J = 7.5 Hz, 5-CH_2), 2.94 (t, 2H, J = 7.8 Hz, 5-CH_2), 5.04 (s, 2H, 7-OCH_2), 6.70–8.28 (m, 7H, Ar-H), 9.32 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.3, 21.8, 26.5, 70.4, 113.6, 115.5, 118.5, 127.7, 129.6, 128.7, 133.9, 137.8, 144.6, 153.3, 154.9, 156.6. MS *m*/*z* 308 (M+1). IR (KBr) cm^{-1}: 3202, 1695.

4.1.12. 7-(4-Chloro-benzyloxy)-4,5-dihydro-[1,2,4]triazolo-[4,3-*a*]quinoline-1(2*H*)-one (3k). Yield 52%, mp 189–192 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (t, 2H, J = 7.3 Hz, 4-CH_2), 2.93 (t, 2H, J = 7.8 Hz, 5-CH_2), 5.03 (s, 2H, 7-OCH_2), 6.70–8.29 (m, 7H, Ar-H), 9.49 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 21.8, 26.5, 69.7, 113.6, 115.0, 115.5, 118.5, 126.8, 128.0, 128.9, 131.3, 144.6, 153.1, 156.2, 171.3. MS *m*/*z* 329 (M+1). IR (KBr) cm^{-1}: 3200, 1691.

4.1.13. 7-(4-Methoxy-benzyloxy)-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline-1(2*H*)-one (3l). Yield 51%, mp 177–179 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (t, 2H, J = 7.2 Hz, 4-CH_2), 2.95 (t, 2H, J = 7.8 Hz, 5-CH_2), 3.84 (s, 3H, Ar-OCH_3), 5.01 (s, 2H, 7-OCH_2), 6.67–8.28 (m, 7H, Ar-H), 9.56 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 21.8, 26.5, 30.7, 70.4, 113.6, 115.0, 118.5, 127.5, 127.6, 128.7, 128.8, 136.7, 144.6, 153.3, 154.9, 156.5. MS *m*/*z* 324 (M+1). IR (KBr) cm^{-1}: 3203, 1689.

4.2. Pharmacology

The MES test, sc-PTZ test, and rotarod test were carried out by the Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institutes of Health,

Bethesda, MD, USA.[11,12] All compounds were tested for anticonvulsant activity with C57B/6 mice in the 18–25 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were dissolved in polyethylene glycol-400.

In phase I screening (Table 1) each compound was administered at three dose levels (30, 100, and 300 mg/kg ip, to a total of six mice, using two for each dose) with anticonvulsant activity and neurotoxicity assessed at 30 min and 4 h intervals after administration. Anticonvulsant efficacy was measured in the MES test and the sc-PTZ test. In the MES test, 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. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures. The sc-PTZ test involved subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice). Elevation of the pentylenetetrazol-induced seizure threshold was indicated by the absence of clonic spasms for at least 5 s duration over a 30 min period following administration of the test compound. Anticonvulsant drug-induced neurologic deficit was detected in mice using the rotarod ataxia test.

The pharmacologic parameters estimated in phase I screening was quantified for compounds **3a**–**l** in phase II screening (Table 2). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50} values, 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 plot of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA.

In phase III testing, the general behavior of mice was assessed at regular time intervals up to 24 h following ip administration of TD_{50}, $2TD_{50}$, and $4TD_{50}$ doses of the test compound. The median hypnotic dose (HD_{50}), assessed by loss of righting reflex, and the 24 h median lethal dose (LD_{50}) were determined (Table 3) using the procedure described previously for evaluation of the ED_{50} and TD_{50} values.

Phase IV testing (Table 4) involved the same procedures for determining ED_{50} and TD_{50} as used in phase II screening, except that the test drug was administered po to mice.

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