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# Synthesis and Anticonvulsant Activity Evaluation of 7-Alkoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-ones

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A new series of 7-alkoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-ones were synthesized and evaluated for their anticonvulsant activities. Among these compounds, 7-propoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-one (**4c**) and 7-butoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-one (**4d**) showed the highest activity against maximal electroshock (MES)-induced tonic extension [effective dose $(ED)_{50}$: 11.4 and 13.6 mg/kg, respectively]. It is worth mentioning that compound **4d** showed especially low neurotoxicity, which led to a high protective index (PI >51). The orally anticonvulsant activity data of compound **4d** further confirmed its efficacy, in an MES test, and its high safety with a PI value of 50.2. In addition, the potency of compound **4h** against seizures induced by pentylenetetrazole, 3-mercaptopropionic acid, and bicuculline in the chemical-induced seizure tests suggested that compound **4d** may exert its anticonvulsant activity through affecting the GABAergic system.

**Keywords:** Anticonvulsant activity / Benzothiazole / Maximal electroshock / Pentylenetetrazole / Triazolone

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## Introduction

Epilepsy is a syndrome of different cerebral disorders of the central nervous system (CNS), characterized by paroxysmal, excessive, and hypersynchronous discharges of large numbers of neurons [1]. In recent years, extensive efforts have been devoted to the development of novel therapeutics, and these efforts have resulted in the development of several new promising anticonvulsant drug candidates [2, 3]. Currently however, available anticonvulsants are only effective in reducing the severity and number of seizures in <70% of patients. Furthermore, their usage is often associated with numerous undesirable side effects [4–6] and even life-threatening conditions [7]. Research to find more effective and safer antiepileptic drugs is therefore imperative, yet challenging in medicinal chemistry.

In our previous work, we reported the synthesis and anticonvulsant activity of 7-alkoxy-triazolo[3,4-*b*]benzo[*d*]thiazoles (Fig. 1, **I**) [8]. Among these compounds, 7-octyloxy-triazolo-[3,4-*b*]benzo[*d*]thiazole was the most active compound with an $ED_{50}$ of 8.0 mg/kg and a PI [toxic dose $(TD)_{50}/ED_{50}$] of 15.0 in the maximal electroshock test (MES). In the current study, a series of 7-alkoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-ones (**II**) were designed and synthesized, which replaced the triazole in compounds **I** by triazolone. The design is based on a hypothesis that the carbonyl group in the triazolone may increase anticonvulsant activity of the aim compounds. This hypothesis has already been confirmed in our previous work [9, 10]. The structures of target compounds were characterized using IR, $^1H$ NMR, MS, and elemental analysis techniques. The anticonvulsant activities of the newly synthesized compounds were evaluated using MES-induced seizures in mice as an experimental epilepsy model. The rotarod assay was performed in mice to evaluate the neurotoxicity of these compounds. To elucidate the possible mechanism of action, the most active compound, **4d**, was tested in pentylenetetrazole (PTZ), 3-mercaptopropionic acid (3-MP), and bicuculline (BIC)-induced seizure tests. Orally anticonvulsant activity against MES-induced seizures and orally neurotoxicity in mice were also evaluated for compound **4d**.

## Chemistry

Based on previous studies in our laboratory, we designed and prepared 7-alkoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-one

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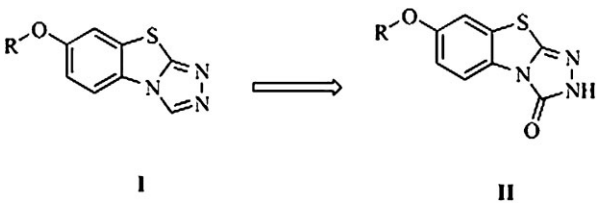


I II

**Figure 1.** Structure of compounds **I** and **II**.

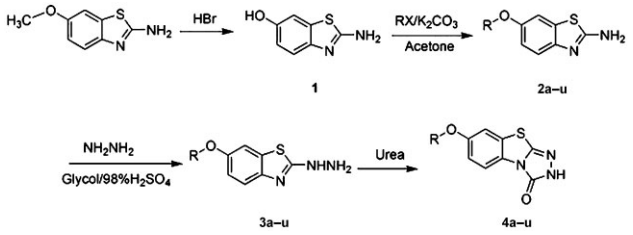
derivatives (**4a–u**). Target compounds **4a–u** were synthesized according to Scheme 1. The starting material, 6-methoxy-1,3-benzothiazol-2-amine, was reacted with hydrobromic acid (48% water solutions) to obtain compound **1** [11], which was reacted further with appropriate alkanol and substituted phenol in acetone to obtain derivatives **2a–u** [12]. Compounds **2a–u** were treated with hydrazine hydrate in the presence of sulfuric acid (98% water solutions) to produce derivatives **3a–u** [8]. Finally, compounds **3a–u** were converted to the target compounds **4a–u** by mixing with urea in the molten state [13]. Their chemical structures were characterized using IR, $^1$H NMR, MS, and elemental analysis techniques. A detailed overview of their physical and analytical data has been provided in the Experimental part.

## Results and discussion

### Preliminary screening of the anticonvulsant activity

During the preliminary screening process, compounds were administered by intraperitoneal (i.p.) injection at dosages of 200, 100, and 30 mg/kg. Their anticonvulsant effects and neurovirulence were assessed at 0.5-h intervals following administration in mice. The following observations were obtained and are listed in Table 1.

From Table 1, we can see that **4b–g** and **4k–p** displayed noticeable anticonvulsant activity at a dosage of 100 mg/kg. Of these compounds, compounds **4c** and **4d** exhibited absolute protection at a dosage of 30 mg/kg, whereas compounds **4e** and **4l** showed partial protection (1/3 or 2/3) at this dosage. Some of these compounds displayed a little neurovirulence at a dosage of 100 mg/kg, but not so strong.




**Scheme 1.** Synthetic route of the target compounds (**4a–u**).

**Table 1.** Preliminary screening of anticonvulsant activity in mice (i.p.).

| | | MES[a] (mg/kg) | | | TOX[b] (mg/kg) | |
|---|---|---|---|---|---|---|
| Compound | R | 30 | 100 | 200 | 100 | *C* log *p*[c] |
| **4a** | $-CH_3$ | – | 0/3 | 0/3 | 0/3 | 1.4 |
| **4b** | $-C_2H_5$ | 0/3 | 1/3 | 2/3 | 1/3 | 1.9 |
| **4c** | $-C_3H_7$ | 3/3 | 3/3 | – | 2/3 | 2.4 |
| **4d** | $-C_4H_9$ | 3/3 | 3/3 | – | 0/3 | 3.0 |
| **4e** | $-C_5H_{11}$ | 1/3 | 2/3 | 3/3 | 0/3 | 3.5 |
| **4f** | $-C_6H_{13}$ | 0/3 | 1/3 | 2/3 | 0/3 | 4.01 |
| **4g** | $-C_7H_{15}$ | 0/3 | 1/3 | 3/3 | 1/3 | 4.54 |
| **4h** | $-C_8H_{17}$ | – | 0/3 | 1/3 | 0/3 | 5.07 |
| **4i** | $-C_{12}H_{25}$ | – | 0/3 | 0/3 | 0/3 | 7.19 |
| **4j** | $-C_{14}H_{29}$ | – | 0/3 | 0/3 | 0/3 | 8.24 |
| **4k** | $-C_6H_5$ | 0/3 | 1/3 | 2/3 | 1/3 | 3.13 |
| **4l** | $-C_6H_5$(*o*-F) | 2/3 | 3/3 | – | 1/3 | 3.28 |
| **4m** | $-C_6H_5$(*m*-F) | 0/3 | 1/3 | 2/3 | 1/3 | 3.28 |
| **4n** | $-C_6H_5$(*p*-F) | 0/3 | 1/3 | 1/3 | 0/3 | 3.28 |
| **4o** | $-C_6H_5$(*o*-Cl) | 0/3 | 1/3 | 2/3 | 2/3 | 3.85 |
| **4p** | $-C_6H_5$(*m*-Cl) | 0/3 | 2/3 | 3/3 | 1/3 | 3.85 |
| **4q** | $-C_6H_5$(*p*-Cl) | – | 0/3 | 0/3 | 0/3 | 3.85 |
| **4r** | $-C_6H_5$(*p*-$CH_3$) | – | 0/3 | 0/3 | 0/3 | 3.63 |
| **4s** | $-C_6H_5$(*m*-$CF_3$) | – | 0/3 | 0/3 | 0/3 | 4.02 |
| **4t** | $-C_6H_5$(*o*-CN) | – | 0/3 | 0/3 | 0/3 | 2.71 |
| **4u** | $-C_6H_5$(2,6-2Cl) | – | 0/3 | 0/3 | 0/3 | 4.56 |

[a] Maximal electroshock seizure test (number of animals protected/number of animals tested).
[b] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested).
[c] Lipid–water partition coefficient, calculated by ChemDraw Ultra 8.0.

### Quantitative evaluation of the anticonvulsant activity

Based on the considerable anticonvulsant activity demonstrated during the preliminary screening test, compounds **4c–e** and **4l** were subjected to quantitative evaluation trials for quantification of their anticonvulsant activity (indicated by $ED_{50}$) and neurotoxicity (indicated by $TD_{50}$) in mice. Results of the quantitative testing of selected compounds, together with data of the antiepileptic drug carbamazepine (measured in the same condition), are shown in Table 2.

The four compounds tested gave $ED_{50}$ values in the range of 11.4–73.7 mg/kg. Compound **4c** is 11.4 mg/kg (45.78 mmol/kg); **4d** is 13.6 mg/kg (51.71 mmol/kg); **4e** is 73.3 mg/kg (264.62 mmol/kg); and **4l** is 26.4 mg/kg (83.8 mmol/kg), and $TD_{50}$ values in the range of 96.0 to more than 700 mg/kg, leading to PI values in the range of 4.2 to more than 51. Among the compounds tested, compound **4c** showed equal MES activity ($ED_{50}$ = 11.4 mg/kg, 45.78 mmol/kg) and PI (8.5) to carbamazepine ($ED_{50}$ = 11.8 mg/kg, 49.94 mmol/kg, PI = 6.4). Compound **4d** had a comparable MES activity ($ED_{50}$ = 13.6 mg/kg, 51.71 mmol/kg) and a much lower neurotoxicity

**Table 2.** Quantitative anticonvulsant data in mice (i.p.).

| Compound | R | MES, $ED_{50}$[a)] (mg/kg) | TOX, $TD_{50}$[b)] (mg/kg) | PI[c)] |
|---|---|---|---|---|
| **4c** | $-C_3H_7$ | 11.4 (9.85–13.11)[d)] | 96.0 (71.93–129.65) | 8.5 |
| **4d** | $-C_4H_9$ | 13.6 (11.81–15.72) | >700 | >51 |
| **4e** | $-C_5H_{11}$ | 73.3 (64.14–83.81) | 311.1 (238.13–406.53) | 4.2 |
| **4l** | $-C_6H_5$(*o*-F) | 26.4 (22.89–30.47) | 126.8 (109.87–146.23) | 4.8 |
| Carbamazepine | – | 11.8 (8.5–16.4) | 76.1 (55.8–103.7) | 6.4 |

a) $ED_{50}$: median effective dose affording anticonvulsant protection in 50% of animals; the dose is measured in mg/kg.
b) $TD_{50}$: median toxic dose eliciting minimal neurological toxicity in 50% of animals; the dose is measured in mg/kg.
c) PI: protective index ($TD_{50}/ED_{50}$).
d) 95% confidence intervals given in parentheses.

($TD_{50} > 700$ mg/kg) compared to carbamazepine, resulting in a PI of more than 51.

## Structure–activity relationships

Following activity analysis of the synthesized compounds, the following structure–activity relationships (SARs) were obtained. For the 12 alkyl chain-substituted derivatives **4a–j**, the length of the alkyl chain appeared to have a direct impact on the anticonvulsant activity of the derivatives. For example, as the alkyl chain length increased from **4a** to **4c**, the anticonvulsant activity gradually increased, with compound **4c** (with the *n*-propane chain) being the most active compound. From **4c** to **4k**, although the alkyl chain length increased further, the anticonvulsant activity decreased and ultimately disappeared. This SARs might be associated with the lipid–water partition coefficients of the compounds, which affects drug absorption, hydrophobic drug–receptor interactions, metabolism of molecules, and especially the ability to cross the blood–brain barrier (BBB). All these factors will have an effect on drug efficiency in the CNS. The optimum log *p*-value of drugs acting on the CNS is around 2.0 [14], since at this point their movement through the aqueous and lipophilic phases of living tissues will be inhibited to a lesser extent. From the calculated log *p* values (*C* log *p*), it can be observed that compounds **4a–j** exhibited *C* log *p* values ranging from 1.4 to 7.2 (Table 1), and the *C* log *p* of the most active compound, **4c**, was 2.4, which is quite close to the optimum log *p*-value of 2.0. To some compounds, which have large substituent group (like **4i**, **4j**, **4s**, etc.), their low activity may be related to their large substituent.

Compound **4k** was substituted with a benzyloxy group at the 6-position of the triazolobenzothiazole core and F, Cl, $CH_3$, $CF_3$, and CN groups were subsequently added onto the benzyloxy group of **4k** in different positions, providing compounds **4l–u**. The anticonvulsant activities of these compounds were weaker than for the compounds mentioned above. Only one compound (**4l**) showed an MES activity below 30 mg/kg, whereas the others showed weak activity at 100 or 200 mg/kg. Among **4k–u**, compound **4l** was the most promising compound with an $ED_{50}$ of 26.4 mg/kg, $TD_{50}$ of 126.8 mg/kg, and PI of 4.8. Comparing the derivatives with different F-substitution positions on the benzyl ring, their activity order was 2-F > 3-F > 4-F. The activity order of the Cl-substituted derivatives was 3-Cl > 2-Cl > 4-Cl > 2.4-2Cl. Some derivatives **4r–t**, which have other substituent groups containing 4-$CH_3$, 3-$CF_3$, and 2-CN, were also designed and prepared. However none of these exhibited any activity, independent of the dosage administered.

As a result of the quantitative evaluation, compound **4d** showed strong anticonvulsant activity and the best PI value in MES test, so it was then selected for further investigations against seizures induced by PTZ, 3-MP, and BIC to prove its anticonvulsant activity and speculate about the possible mechanism of anticonvulsant action. We used 10 mice per group in our experiment. Compound **4d** was administered to mice at 30 mg/kg i.p., which was higher than its $ED_{50}$ value and far below its $TD_{50}$ value. The reference drug carbamazepine was also administered at 30 mg/kg i.p.

In the sc-PTZ model, carbamazepine inhibited the clonic seizures, tonic seizures, and death at the rates of 0%, 100%, and 90%, respectively. Compound **4d** inhibited the clonic seizures, tonic seizures, and lethality at the rates of 10%, 70%, and 70% induced by sc-PTZ, respectively (Table 3). From these data, we can see that compound **4d** can inhibit the tonic seizures and lethality induced by PTZ. PTZ has been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission [15, 16]. GABA is the main inhibitory

**Table 3.** Effects of compound **4d** on sc pentylenetetrazol-induced seizures in mice[a)] (i.h.).

| Compound | Doses (mg/kg) | Test time (h) | Clonic seizures (%) | Tonic seizures (%) | Lethality (%) |
|---|---|---|---|---|---|
| DMSO | | 0.5 | 100 | 100 | 100 |
| Carbamazepine | 30 | 0.5 | 100 | 0 | 10 |
| **4d** | 30 | 0.5 | 90 | 30 | 30 |

a) The number of animals tested is 10.

neurotransmitter in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures [17]. While GABAergic enhancement is known to inhibit or attenuate seizures. The inhibition of compound **4d** upon the PTZ-induced tonic seizures might be related to the enhancing of GABAergic neurotransmission or activity.

In the 3-MP-induced seizure model, carbamazepine inhibited the clonic seizures, tonic seizures, and death at rates of 0%, 100%, and 100%, respectively. By comparison, compound **4d** showed an anticonvulsant effect similar to that of carbamazepine in inhibiting the clonic and tonic seizures. But its protection for the death induced by 3-MP cannot compare with carbamazepine, with inhibition of death induced by 3-MP at rate of 30% (Table 4). 3-MP is competitive inhibitor of the GABA synthesis enzyme glutamate decarboxylase (GAD), and it inhibits the synthesis of GABA resulting in decrease of GABA levels in the brain [18]. Compound **4d** exhibited the efficacy to inhibit the tonic seizures induced by 3-MP, though the protection for death is less satisfactory than carbamazepine, suggesting that it might activate GAD or inhibit GABA transaminase (GABA-T) in the brain.

In the BIC-induced seizure model, both carbamazepine and **4d** inhibited tonic seizures and death, but did not inhibit clonic seizures. Carbamazepine showed inhibition of clonic and tonic seizures and death at rates of 0%, 100%, and 40%, respectively. Compound **4d** showed inhibition of tonic seizures at rate of 80%, but no protection for the clonic and seizures and death (Table 5). BIC is a competitive antagonist of the $GABA_A$ receptor [19]. As compound **4d** inhibited the tonic seizures induced by BIC, it likely exerts anticonvulsant activity, at least partially, through $GABA_A$-mediated mechanisms.

**Table 4.** Effects of compound **4d** on 3-mercaptopropionic acid-induced seizures in mice[a] (i.h.).

| Compound | Doses (mg/kg) | Test time (h) | Clonic seizures (%) | Tonic seizures (%) | Lethality (%) |
|---|---|---|---|---|---|
| DMSO | | 0.5 | 100 | 100 | 100 |
| Carbamazepine | 30 | 0.5 | 100 | 0 | 0 |
| **4d** | 30 | 0.5 | 100 | 20 | 70 |

[a] The number of animals tested is 10.

**Table 5.** Effects of compound **4d** on bicuculline-induced seizures in mice[a] (i.h.).

| Compound | Doses (mg/kg) | Test time (h) | Clonic seizures (%) | Tonic seizures (%) | Lethality (%) |
|---|---|---|---|---|---|
| DMSO | | 0.5 | 100 | 100 | 100 |
| Carbamazepine | 30 | 0.5 | 80 | 0 | 60 |
| **4d** | 30 | 0.5 | 100 | 20 | 100 |

[a] The number of animals tested is 10.

### Pharmacological evaluation of compound 4d administered orally to mice

To obtain the oral time to peak effect (TPE) of compound **4d**, we conducted a time-course test, in which compound **4d** reached the TPE at 1.5 h after administering oral medications (p.o.; Fig. 2). We next evaluated the orally anticonvulsant activity of compound **4d** against MES-induced seizures and oral neurotoxicity in mice (Table 6) with carbamazepin as the reference. We also got the oral $LD_{50}$ data of **4d** (Table 6). The data in Table 6 clearly indicate a decrease in the anticonvulsant potency and neurotoxicity of **4d** in p.o. compared to intraperitoneal (i.p.) administration. Additionally, the oral MES activity was lower than that of carbamazepin. Nevertheless, the PI value was still relatively high (PI = 50.2), which gives compound **4d** a broader safety margin than that of carbamazepin (PI = 50.2 vs. 11.1).

## Conclusion

Among the series of 7-alkoxy[1,2,4]triazolo[3,4-*b*]benzothiazol-3(2*H*)-ones, compounds **4c** and **4d** were the most active in this study with high activity in the MES assay ($ED_{50}$ = 11.4, 13.6 mg/kg, respectively). Compound **4d** showed a special low neurotoxicity, much lower than compound **4c**. It also led to the conclusion that **4d** has a high PI (>51). The PI has also higher safety than marketed drug carbamazepine (PI = 6.4). The orally anticonvulsant activity data of compound **4d** also showed that **4d** has a high safety with a PI value of 50.2. The potency of compound **4d** against seizures induced by pentylenetetrazole, 3-MP, and BIC in the chemical-induced seizure test suggested that compound **4d** displayed wide

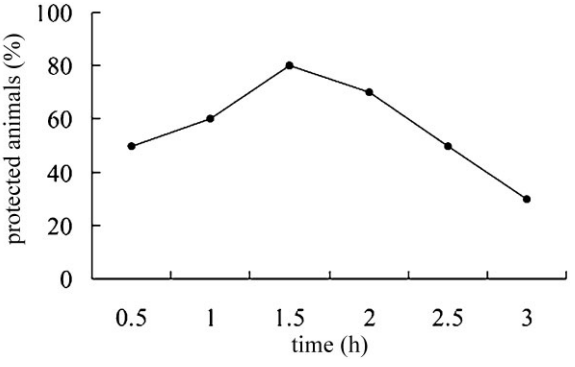



**Figure 2.** Time-course of compound **4d** (50 mg/kg) in the maximal electroshock seizure test (the number of animals at each point was 8; o.p.).

**Table 6.** Pharmacological evaluation of compound **4d** and carbamazepin administered orally to mice.

| Compound | R | MES, $ED_{50}$ (mg/kg) | TOX, $TD_{50}$ (mg/kg) | PI |
|---|---|---|---|---|
| **4d** | $-C_4H_9$ | 34.1 (29.54–39.32) | 1710.4 (1587.24–1843.09) | 50.2 |
| Carbamazepine | – | 18.3 (16.04–20.95) | 203.7 (178.19–232.82) | 11.1 |

spectrum activity in several models. Though the specific mechanism is still unclear, the results suggest that GABA-mediated mechanisms might be involved in its anticonvulsant activity, such as enhancing of GABAergic neurotransmission or activity, activating GAD or inhibiting GABA-T, and mediating $GABA_A$-receptors.

## Experimental

### Chemistry

#### *General methods*

Melting points were determined in open capillary tubes and are 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 ppm relative to tetramethylsilane. 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). The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade.

#### *Synthesis of 6-hydroxy-2-aminobenzothiazole (**1**)*

A mixture of 6-methoxy-2,3-dihydrobenzo[*d*]thiazol-2-amine (10 g, 55.56 mmol) and 40 mL of hydrobromic acid (48% water solutions) was refluxed for 20 h. The mixture was allowed to cool to room temperature and neutralized with NaOH solution to pH 7–8. The precipitate formed was filtered and washed with water. The filtrate was stirred with 100 mL of hot water for 0.5 h, and the remaining precipitate was filtered to yield a brown solid.

#### *General procedure for the synthesis of 6-alkoxy-2-aminobenzothiazoles (**2a–u**) [8]*

A mixture of compound **1** (2 g, 12 mmol), potassium carbonate (2 g, 14.4 mmol), appropriate alkyl bromide or benzyl chloride derivatives (1.32 mmol), and a catalytic amount of benzyltriethylamine chloride (TEBA) in 50 mL of acetonitrile was heated under reflux for 24–48 h. After removing the solvent under reduced pressure, 80 mL of hot water was poured into the flask, and the mixture was stirred for 0.5 h to eliminate excess potassium carbonate. The remaining precipitate was filtered to yield a Russet solid, which was used without further purification.

#### *General procedure for the synthesis of 6-alkoxy-2-hydrazinobenzothiazoles (**3a–u**) [8]*

A mixture of compounds **2a–u** (20 mmol) and 0.6 mL of 98% $H_2SO_4$ solution (water solutions) in 20 mL of glycol was refluxed for 0.5 h at 80°C. Then 10 mL of hydrazine hydrate was added into the mixture and heated at 140°C for 5 h. After cooling to the room temperature, the mixture was added into 50 mL of ice water. The precipitate formed was filtered and washed with water to obtain a light green needle-like solid.

#### *General procedure for the synthesis of 7-alkoxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-ones (**4a–u**)*

A mixture of compounds **3a–u** (10 mmol) and diaminomethanal (80 mmol) was heated to be fused for 4 h. Then the mixture was poured into hot water with stirring for 0.5 h. The remaining precipitate was filtered to obtain a crude product, which was purified by silica gel column chromatography with $CH_2Cl_2$–$CH_3OH$ (50:1) to a white solid.

The yield, melting point, analytical data, and spectral data of each compound are given below.

#### *7-Methoxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4a**)*

M.p. 266–268°C; yield: 68.6%; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ 3.79 (t, $J = 7.1$ Hz, 3H, –O–$CH_3$), 6.95 (dd, $J = 8.8$, 2.6 Hz, 1H, Ar–H), 7.58 (d, $J = 2.6$ Hz, 1H, Ar–H), 7.73 (d, $J = 8.8$ Hz, 1H, Ar–H), 12.15 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 63.8, 109.4, 113.5, 114.5, 124.5, 129.9, 156.8, 157.3, 158.2. IR (KBr, $cm^{-1}$): 3156 (NH), 1699 (C=O). MS *m*/*z* 222 (M+1). Anal. calcd. for $C_9H_7N_3O_2S$: C, 48.86; H, 3.19; N, 18.99. Found: C, 49.65; H, 3.30; N, 18.86.

#### *7-Ethoxyl[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4b**)*

M.p. 249–251°C; yield: 55.8%; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ 1.44 (t, $J = 6.7$ Hz, 3H, $-CH_3$), 4.06 (d, $J = 6.7$ Hz, 2H, $-OCH_2-$), 6.95 (d, $J = 8.8$, 2.6 Hz, 1H, Ar–H), 7.06 (d, $J = 2.6$ Hz, 1H, Ar–H), 7.89 (d, $J = 8.8$ Hz, 1H, Ar–H), 9.65 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 14.2, 66.5, 109.4, 113.5, 114.5, 124.6, 129.8, 156.9, 157.1, 157.6. IR (KBr, $cm^{-1}$): 3155 (NH), 1698 (C=O). MS *m*/*z* 236 (M+1). Anal. calcd. for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86. Found: C, 51.18; H, 3.77; N, 17.96.

#### *7-Propoxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4c**)*

M.p. 178–180°C; yield: 46.3%; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ 1.06 (t, $J = 7.4$ Hz, 3H, $-CH_3$), 1.66–1.89 (m, 2H, $-CH_2-$), 3.95 (t, $J = 6.5$ Hz, 2H, $-OCH_2-$), 6.96 (dd, $J = 8.8$, 2.4 Hz, 1H, Ar–H), 7.07 (d, $J = 2.4$ Hz, 1H, Ar–H), 7.90 (d, $J = 8.8$ Hz, 1H, Ar–H), 9.88 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 10.5, 22.5, 70.3, 109.5, 113.5, 114.5, 124.6, 130.0, 148.3, 151.2, 157.3. IR (KBr, $cm^{-1}$): 3154 (NH), 1696 (C=O). MS *m*/*z* 250 (M+1). Anal. calcd. for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86. Found: C, 51.99; H, 4.67; N, 17.02.

#### *7-Butoxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4d**)*

M.p. 162–164°C; yield: 36.5%; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ 0.99 (t, $J = 7.2$ Hz, 3H, $-CH_3$), 1.39–1.62 (m, 2H, $-CH_2-$), 1.67–1.90 (m, 2H, $-CH_2-$), 3.99 (t, $J = 6.3$ Hz, 2H, $-OCH_2-$), 6.95 (dd, $J = 8.8$, 2.6 Hz, 1H, Ar–H), 7.06 (d, $J = 2.6$ Hz, 1H, Ar–H), 7.89 (d, $J = 8.8$ Hz,

1H, Ar–H), 10.15 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 13.8, 19.2, 31.2, 68.5, 109.5, 113.2, 114.5, 124.6, 129.9, 148.3, 151.2, 157.3. IR (KBr, $cm^{-1}$): 3152 (NH), 1697 (C=O). MS *m/z* 264 (M+1). Anal. calcd. for $C_{12}H_{13}N_3O_2S$: C, 54.74; H, 4.98; N, 15.96. Found: C, 54.83; H, 4.79; N, 16.01.

### *7-Pentyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4e**)*

M.p. 150–152°C; yield: 46.3%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.96 (t, *J* = 6.6 Hz, 3H, $-CH_3$), 1.43–1.82 (m, 6H, $-(CH_2)_3-$), 3.98 (d, *J* = 6.2 Hz, 2H, $-OCH_2-$), 6.95 (dd, *J* = 8.8, 2.6 Hz, 1H, Ar–H), 7.07 (d, *J* = 2.6 Hz, 1H, Ar–H), 7.90 (d, *J* = 8.8 Hz, 1H, Ar–H), 9.53 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.0, 22.4, 28.1, 28.9, 68.8, 109.4, 113.5, 114.5, 124.6, 130.0, 148.3, 151.2, 157.3. IR (KBr, $cm^{-1}$): 3151 (NH), 1696 (C=O). MS *m/z* 278 (M+1). Anal. calcd. for $C_{13}H_{15}N_3O_2S$: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.39; H, 5.38; N, 15.29.

### *7-Hexyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4f**)*

M.p. 139–141°C; yield: 45.2%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.91 (t, *J* = 7.3 Hz, 3H, $-CH_3$), 1.33–1.82 (m, 8H, $-(CH_2)_4-$), 3.97 (t, *J* = 6.1 Hz, 2H, $-OCH_2-$), 6.94 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar–H), 7.04 (d, *J* = 8.8, 2.4 Hz, 1H, Ar–H), 7.89 (d, *J* = 8.8 Hz, 1H, Ar–H), 10.46 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.0, 19.9, 26.9, 28.9, 31.3, 68.5, 109.4, 113.5, 114.5, 124.6, 129.9, 156.9, 157.4, 157.6. IR (KBr, $cm^{-1}$): 3149 (NH), 1697 (C=O). MS *m/z* 292 (M+1). Anal. calcd. for $C_{14}H_{17}N_3O_2S$: C, 57.71; H, 5.88; N, 14.42. Found: C, 57.97; H, 5.66; N, 14.62.

### *7-Heptyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4g**)*

M.p. 158–160°C; yield: 43.3%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.90 (t, *J* = 6.6 Hz, 3H, $-CH_3$), 1.30–1.86 (m, 10H, $-(CH_2)_5-$), 3.98 (t, *J* = 6.5 Hz, 2H, $-OCH_2-$), 6.95 (dd, *J* = 8.8, 2.2 Hz, 1H, Ar–H), 7.07 (d, *J* = 2.2 Hz, 1H, Ar–H), 7.90 (d, *J* = 8.8 Hz, 1H, Ar–H), 9.60 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.1, 22.6, 26.0, 28.7, 29.0, 31.3, 68.5, 109.4, 113.6, 114.1, 124.5, 129.9, 156.9, 157.3, 157.7. IR (KBr, $cm^{-1}$): 3148 (NH), 1694 (C=O). MS *m/z* 306 (M+1). Anal. calcd. for $C_{15}H_{19}N_3O_2S$: C, 58.99; H, 6.27; N, 13.76. Found: C, 59.10; H, 6.39; N, 13.92.

### *7-Octyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4h**)*

M.p. 152–154°C; yield: 52.2%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.89 (s, *J* = 6.8 Hz, 3H, $-CH_3$), 1.37–1.68 (m, 10H, $-(CH_2)_5-$), 1.75–1.84 (m, 2H, $-CH_2-$), 3.96 (t, *J* = 6.6 Hz, 2H, $-OCH_2-$), 7.00 (dd, *J* = 8.7, 3.1 Hz, 1H, Ar–H), 7.09 (d, *J* = 3.1 Hz, 1H, Ar–H), 7.89 (d, *J* = 8.7 Hz, 1H, Ar–H), 10.29 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.1, 22.5, 27.1, 29.0, 29.1, 29.5, 31.8, 68.5, 109.4, 113.5, 114.5, 124.5, 129.8, 156.9, 157.4, 157.5. IR (KBr, $cm^{-1}$): 3146 (NH), 1693 (C=O). MS *m/z* 320 (M+1). Anal. calcd. for $C_{16}H_{21}N_3O_2S$: C, 60.16; H, 6.63; N, 13.16. Found: C, 60.39; H, 6.87; N, 13.02.

### *7-Dodecyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4i**)*

M.p. 134–136°C; yield: 45.2%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.88 (t, *J* = 6.4 Hz, 3H, $-CH_3$), 1.27–1.82 (m, 20H, $-(CH_2)_{10}-$), 3.98 (t, *J* = 6.4 Hz, 2H, $-OCH_2-$), 6.95 (dd, *J* = 8.8, 2.9 Hz, 1H, Ar–H), 7.07 (d, *J* = 2.9 Hz, 1H, Ar–H), 7.89 (d, *J* = 8.8 Hz, 1H, Ar–H), 9.70 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.1, 22.5, 27.1, 29.1, 29.1, 29.3, 29.3, 29.4, 29.4, 29.8, 31.8, 68.6, 109.4, 113.5, 114.5, 124.6, 129.9, 156.9, 157.3, 157.6. IR (KBr, $cm^{-1}$): 3142 (NH), 1695 (C=O). MS *m/z* 376 (M+1). Anal. calcd. for $C_{20}H_{29}N_3O_2S$: C, 63.97; H, 7.78; N, 11.19. Found: C, 64.18; H, 7.59; N, 11.31.

### *7-Tetradecyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4j**)*

M.p. 211–213°C; yield: 70.2%; $^1$H NMR (300 MHz, $CDCl_3$), δ 0.88 (t, *J* = 6.1 Hz, 3H, $-CH_3$), 1.26 (s, 22H, $-(CH_2)_{11}-$), 1.76–1.87 (m, 2H, $-CH_2-$), 3.98 (t, *J* = 6.1 Hz, 2H, $-OCH_2-$), 6.95 (dd, *J* = 8.6, 2.3 Hz, 1H, Ar–H), 7.06 (d, *J* = 2.3 Hz, 1H, Ar–H), 7.90 (d, *J* = 8.6 Hz, 1H, Ar–H), 10.05 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 14.1, 22.5, 27.1, 29.1, 29.1, 29.2, 29.3, 29.3, 29.3, 29.4, 29.4, 29.8, 31.8, 68.5, 109.2, 113.8, 114.5, 124.8, 129.7, 157.0, 157.4, 157.9. IR (KBr, $cm^{-1}$): 3140 (NH), 1690 (C=O). MS *m/z* 404 (M+1). Anal. calcd. for $C_{22}H_{33}N_3O_2S$: C, 65.47; H, 8.24; N, 10.41. Found: C, 65.32; H, 8.09; N, 10.56.

### *7-Benzyloxy[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4k**)*

M.p. 231–233°C; yield: 47.2%; $^1$H NMR (300 MHz, DMSO), δ 5.02 (s, 2H, $-OCH_2-$), 6.93–7.75 (m, 8H, Ar–H), 11.33 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 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. IR (KBr, $cm^{-1}$): 3165 (NH), 1720 (C=O). MS *m/z* 298 (M+1). Anal. calcd. for $C_{15}H_{11}N_3O_2S$: C, 60.59; H, 3.73; N, 14.13. Found: C, 60.82; H, 3.60; N, 14.37.

### *7-(2-Fluorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4l**)*

M.p. 164–166°C; yield: 45.8%; $^1$H NMR (300 MHz, $CDCl_3$), δ 5.17 (s, 2H, $-OCH_2-$), 7.03–7.93 (m, 7H, Ar–H), 10.06 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 61.3, 114.6, 114.9, 114.2, 115.1, 115.2, 115.3, 116.3, 124.3, 124.6, 124.4, 128.0, 129.8, 154.5, 160.5. IR (KBr, $cm^{-1}$): 3167 (NH), 1721 (C=O). MS *m/z* 316 (M+1). Anal. calcd. for $C_{15}H_{10}FN_3O_2S$: C, 57.14; H, 3.20; N, 13.33. Found: C, 57.32; H, 3.39; N, 13.51.

### *7-(3-Fluorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4m**)*

M.p. 238–240°C; yield: 49.9%; $^1$H NMR (300 MHz, DMSO), δ 5.02 (s, 2H, $-OCH_2-$), 6.92–7.77 (m, 7H, Ar–H), 11.69 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 61.7, 114.8, 115.1, 115.4, 115.8, 115.0, 115.1, 116.3, 122.7, 125.3, 130.3, 132.4, 139.7, 154.5, 163.1. IR (KBr, $cm^{-1}$): 3168 (NH), 1722 (C=O). MS *m/z* 316 (M+1). Anal. calcd. for $C_{15}H_{10}FN_3O_2S$: C, 57.14; H, 3.20; N, 13.33. Found: C, 57.29; H, 3.36; N, 13.45.

### *7-(4-Fluorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4n**)*

M.p. 230–232°C; yield: 42.6%; $^1$H NMR (300 MHz, $CDCl_3$), δ 5.03 (s, 2H, $-OCH_2-$), 6.90–7.40 (m, 7H, Ar–H), 9.31 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), δ 61.7, 113.8, 115.4, 115.6, 118.4, 116.5, 122.6, 126.8, 127.9, 129.3, 132.5, 144.5, 153.2, 156.2, 162.2. IR (KBr, $cm^{-1}$): 3166 (NH), 1721 (C=O). MS *m/z* 316 (M+1). Anal. calcd. for $C_{15}H_{10}FN_3O_2S$: C, 57.14; H, 3.20; N, 13.33. Found: C, 56.93; H, 3.03; N, 13.21.

### *7-(2-Chlorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4o**)*

M.p. 207–209°C; yield: 52.3%; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ 5.20 (s, 2H, $-OCH_2-$), 7.04–7.94 (m, 7H, Ar–H), 9.83 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 64.3, 113.6, 114.9, 114.2, 115.1, 115.2, 115.3, 116.3, 124.0, 124.1, 124.4, 128.2, 129.9, 154.5, 170.3. IR (KBr, $cm^{-1}$): 3172 (NH), 1718 (C=O). MS *m/z* 333 (M+1). Anal. calcd. for $C_{15}H_{10}ClN_3O_2S$: C, 54.30; H, 3.04; N, 12.67. Found: C, 54.52; H, 2.89; N, 12.85.

### *7-(3-Chlorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4p**)*

M.p. 180–182°C; yield: 60.5%; $^1$H NMR (300 MHz, DMSO), $\delta$ 5.01 (s, 2H, $-OCH_2-$), 6.93–7.76 (m, 7H, Ar–H), 11.68 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 69.7, 113.6, 114.2, 114.4, 114.8, 115.0, 115.1, 116.5, 122.6, 125.3, 130.5, 132.2, 139.8, 154.5, 171.2. IR (KBr, $cm^{-1}$): 3174 (NH), 1717 (C=O). MS *m/z* 333 (M+1). Anal. calcd. for $C_{15}H_{10}ClN_3O_2S$: C, 54.30; H, 3.04; N, 12.67. Found: C, 54.44; H, 2.97; N, 12.91.

### *7-(4-Chlorobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4q**)*

M.p. 227–229°C; yield: 42.8%; $^1$H NMR (300 MHz, DMSO), $\delta$ 4.97 (s, 2H, $-OCH_2-$), 6.94–7.76 (m, 7H, Ar–H), 11.79 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 69.7, 113.5, 115.4, 115.6, 118.4, 116.5, 122.4, 126.7, 127.9, 129.3, 131.4, 144.4, 153.1, 156.3, 171.8. IR (KBr, $cm^{-1}$): 3175 (NH), 1716 (C=O). MS *m/z* 333 (M+1). Anal. calcd. for $C_{15}H_{10}ClN_3O_2S$: C, 54.30; H, 3.04; N, 12.67. Found: C, 54.47; H, 2.79; N, 12.82.

### *7-(4-Methylbenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4r**)*

M.p. 188–190°C; yield: 43.3%; $^1$H NMR (300 MHz, DMSO), $\delta$ 2.27 (s, 3H, $-CH_3$), 4.97 (s, 2H, $-OCH_2-$), 6.91–7.76 (m, 7H, Ar–H), 11.40 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 22.5, 70.5, 113.6, 115.4, 115.5, 116.0, 118.5, 127.7, 129.6, 128.7, 133.8, 137.8, 144.6, 153.4, 155.0, 156.6. IR (KBr, $cm^{-1}$): 3169 (NH), 1724 (C=O). MS *m/z* 312 (M+1). Anal. calcd. for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50. Found: C, 61.55; H, 4.03; N, 13.32.

### *7-(3-Trifluoromethylbenzyloxy)-[1,2,4]triazolo[3,4-b]-benzothiazol-3(2H)-one (**4s**)*

M.p. 168–170°C; yield: 45.2%; $^1$H NMR (300 MHz, DMSO), $\delta$ 5.08 (s, 2H, $-OCH_2-$), 6.93–7.79 (m, 7H, Ar–H), 11.67 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 70.5, 113.6, 115.4, 124.9, 125.6, 126.0, 128.3, 128.7, 131.3, 133.7, 133.3, 136.2, 141.6, 153.5, 154.7, 156.7. IR (KBr, $cm^{-1}$): 3167 (NH), 1719 (C=O). MS *m/z* 366 (M+1). Anal. calcd. for $C_{16}H_{10}F_3N_3O_2S$: C, 52.60; H, 2.76; N, 11.50. Found: C, 52.73; H, 2.89; N, 11.62.

### *7-(2-Cyanobenzyloxy)-[1,2,4]triazolo[3,4-b]benzothiazol-3(2H)-one (**4t**)*

M.p. 239–241°C; yield: 52.3%; $^1$H NMR (300 MHz, DMSO), $\delta$ 5.27 (s, 2H, $-OCH_2-$), 7.16–7.96 (m, 7H, Ar–H), 11.79 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 70.5, 113.6, 115.4, 115.5, 116.0, 128.5, 128.7, 129.6, 128.7, 133.3, 133.7, 136.2, 144.5, 153.4, 155.2, 156.3. IR (KBr, $cm^{-1}$): 3170 (NH), 1720 (C=O). MS *m/z* 323 (M+1). Anal. calcd. for $C_{16}H_{10}N_4O_2S$: C, 59.62; H, 3.13; N, 17.38. Found: C, 59.85; H, 3.40; N, 17.21.

### *7-(2,6-Dichlorobenzyloxy)-[1,2,4]triazolo[3,4-b]-benzothiazol-3(2H)-one (**4u**)*

M.p. 230–232°C; yield: 56.3%; $^1$H NMR (300 MHz, DMSO), $\delta$ 5.18 (s, 2H, $-OCH_2-$), 6.90–7.82 (m, 6H, Ar–H), 11.67 (s, 1H, –NH). $^{13}$C NMR (300 MHz, $CDCl_3$), $\delta$ 61.5, 111.6, 112.4, 122.5, 126.1, 128.3, 128.9, 130.2, 131.7, 133.8, 136.8, 144.6, 153.4, 155.0, 156.6. IR (KBr, $cm^{-1}$): 3176 (NH), 1722 (C=O). MS *m/z* 367 (M+1). Anal. calcd. for $C_{15}H_9Cl_2N_3O_2S$: C, 49.19; H, 2.48; N, 11.47. Found: C, 48.83; H, 2.33; N, 11.56.

## Pharmacology

The MES test, chemical-induced epileptic test, and rotarod test were carried out according to the procedures described in Anticonvulsant Screening Program with some modification [20, 21]. In the MES test and rotarod test, the anticonvulsant effects and the neurovirulence of the compounds were assessed at 0.5-h intervals following administration in mice. And in preliminary neurotoxicity screening, compounds only were administered by intraperitoneal (i.p.) injection at dosages of 100 mg/kg to avoid wasting animals. Electro-convulsions were produced by an electric stimulation generator (JTC-1, ChengDu, China). All compounds, which were dissolved in dimethylsulfoxide (DMSO), were evaluated for anticonvulsant activities with KunMing mice in the 18–22 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University.

### *The maximal electroshock (MES) test*

Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via ear-clip electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the absence of tonic extension of the hind leg. At 30 min after the administration of the compounds, the activities were evaluated in MES test. In preliminary screening, each compound was administered at the dose levels of 200, 100, and 30 mg/kg for evaluating the preliminary anticonvulsant activity. For determination of the median $ED_{50}$ and the median $TD_{50}$, the quantitative evaluation was prepared. Groups of 10 mice were given a range of intraperitoneal doses of the tested compound until at least three points were established in the range of 10–90% seizure protection or neurotoxicity. From these data, the respective $ED_{50}$, $TD_{50}$ values, and 95% confidence intervals were calculated by probit analysis.

### *Pentylenetetrazol-induced seizure test [22]*

At 30 min after the administration of the test compound, 100 mg/kg of PTZ dissolved in saline was administered sc. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was noted.

### *3-Mercaptopropionic acid-induced seizures [23]*

At 30 min after the administration of the test compound, 40 mg/kg of 3-MP dissolved in saline solution was injected sc. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was noted.

### *Bicuculline-induced seizures test [24]*

At 30 min after the administration of the test compound, 5.4 mg/kg of BIC (within 15–45 min after preparation due to

instability) was injected s.c. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths was noted.

### *Rotarod test [25]*

The neurotoxicity of the compounds was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod of diameter an inch that rotates at 10 rpm. Trained animals were given i.p. injection of the test compounds. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

### *Pharmacological evaluation of compound **4d** administered orally to mice*

The time-course effect of compound **4d** in the MES test was determined. A suspension of compound **4d** was given in mice by oral gavages (50 mg/kg in a dose volume of 0.5% methylcellulose). The mice were divided into seven groups ($n = 8$). Subsequently, the animals were subjected to the MES test at various times: 0.5, 1, 1.5, 2, 2.5, and 3 h. The TPE was 1.5 h after the p.o. injection. Then, compound **4d** was evaluated for its orally anticonvulsant activity against MES-induced seizures and oral neurotoxicity at its TPE. This test involved the same procedures for determining $ED_{50}$ and $TD_{50}$ as used in the MES test and the Tox test screening, except that the test drug was administered orally to mice.

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