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Original article

Synthesis and evaluation of anticonvulsant and antidepressant activities of 5-alkoxytetrazolo[1,5-*c*]thieno[2,3-*e*]pyrimidine derivatives

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ABSTRACT

A series of 5-alkoxytetrazolo[1,5-*c*]thieno[2,3-*e*]pyrimidine derivatives were synthesized and their anticonvulsant and antidepressant activities were evaluated. Pharmacological tests showed that four of the synthesized compounds had weak anticonvulsant activity, while most of the compounds had excellent antidepressant activity. The most active compound was 5-(2,4-dichlorobenzyloxy)tetrazolo [1,5-*c*] thieno[2,3-*e*]pyrimidine, which decreased the immobility time by 51.62% at a dose of 100 mg/kg. The results of open-field tests of this compound indicated that it had no significant effects on the locomotor activity compared with the control group at the doses assayed in the forced swimming tests test. This means that the antidepressant activity detected in the FST for the compound is not the result of central nervous system stimulant properties, and further confirms its antidepressant-like effect.

1. Introduction

Antidepressants and anticonvulsants are among the most widely used drugs for the treatment of central nervous system (CNS) disorders [1–4]. Research has revealed that major depression is common in patients with epilepsy [5,6]. Co-morbid depression correlates with poor quality of life among epileptic patients [7], and suicide seems to be one of the leading causes of death in this group [8]. Many patients with epilepsy require treatment with antidepressants. The continued search for new, safer, and more effective drugs with both anticonvulsant and antidepressant activities is therefore imperative, and is a challenge in medicinal chemistry. Considerable interest has focused on the tetrazole structure. The tetrazole nucleus has been incorporated into a wide variety of therapeutically important agents with anticonvulsant [9–11], antituberculosis [12], antiemetic [13], antiamoebic [14], antihistamine [15], antihypertensive [16], and antidepressant activities [17–19].

In our previous study [18], we reported that 5-alkoxytetrazolo [1,5-*a*]quinazolines (**I**) showed anticonvulsant activities (Fig. 1). We have also evaluated the antidepressant activities of these compounds. Most of the compounds showed significant antidepressant activities. Two compounds, 5-(hexyloxy)tetrazolo[1,5-*a*] quinazoline and 5-(4-methoxy-phenoxy)tetrazolo[1,5-*a*]quinazoline, showed significant antidepressant activities; they decreased immobility times by 62.2% and 51.7%, respectively, at doses of 100 mg/kg.

In order to find better anticonvulsant and antidepressant agents, we used thiophene rings instead of the benzene rings in the 5-alkoxytetrazolo[1,5-*a*]quinazolines. A series of 5-alkoxytetrazolo [1,5-*c*]thieno[2,3-*e*]pyrimidine derivatives were synthesized in the present study. Their anticonvulsant activities and antidepressant activities were evaluated using the maximal electroshock (MES) and forced swimming tests (FST), respectively. Open-field tests were performed to analyze whether changes in immobility were associated with changes in motor activity.

2. Results and discussion

2.1. Chemistry

The target compounds **5a**–**5t** were synthesized as shown in Scheme 1. The starting materials 3-amino-2-thiophenecarboxylic acid methyl ester and urea were heated at 200 °C for 1.5 h to obtain compound **1**. This compound reacted further by refluxing

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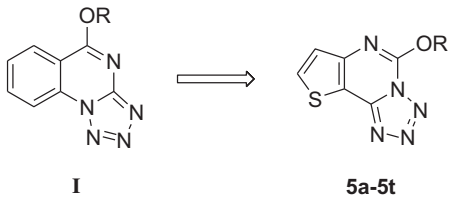


Fig. 1. Structures of compound **I** and target compounds **5a**–**5t**.

with $POCl_3$ to yield compound **2**. Compound **2** reacted with hydrazine hydrate in methanol to afford the compound **3** in high yield. Compound **4** was obtained via the diazotization of compound **3** with $NaNO_2$ at 5 °C in 30% HCl. Finally, the target compounds **5a**–**5t** were obtained by reacting compound **4** with the appropriate substituted phenol in a solution of ethyl acetate in the presence of K_2CO_3. The chemical structures of the compounds synthesized were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis.

2.2. Pharmacology

The anticonvulsant activities of **5a**–**5t** are listed in Table 1. As shown in Table 1, only **5f**, **5g**, **5i**, and **5j** exhibited anticonvulsant activity. None of the compounds exhibited activities at a dose of 30 mg/kg. Compounds **5g** and **5i** displayed weak anticonvulsant activities at a dose of 300 mg/kg in the MES test. Among all the compounds, only compounds **5f** and **5j** exhibited anticonvulsant activities at a dose of 100 mg/kg in the MES test. These two compounds are the most active in this series.

Compounds **5a**–**5t** were screened for antidepressant activity using the FST. This test effectively predicts the activities of a wide variety of antidepressants such as monoamine oxidase (MAO) inhibitors and typical antidepressants. The antidepressant activities of the tested compounds and fluoxetine are shown in Table 2.

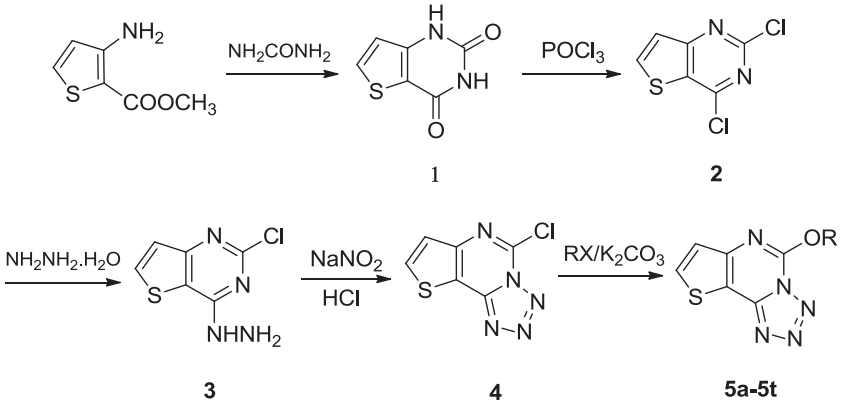
In this study, all of the compounds except **5c**–**5e**, **5j**, and **5o** significantly reduced the immobility times at a dose of 100 mg/kg compared with a control ($p < 0.01$). The most active compound was 5-(2,4-dichlorobenzyloxy)tetrazolo[1,5-*c*]thieno[2,3-*e*]pyrimidine (**5t**) and its antidepressant activity was nearly equal to that of fluoxetine ($p < 0.01$). Compounds **5a**, **5b**, **5h**, **5k**–**5m**, **5q** and **5s** reduced the immobility times to 73.7, 79.3, 94.1, 78.1, 75.1, 78.3, 66.3 and 77.1 s, respectively, compared with an immobility time of 51.6 s for fluoxetine at a dose of 100 mg/kg ($p < 0.01$). The other tested compounds, including **5f**, **5g**, **5i**, **5n**, **5p**, and **5r**, exhibited weak antidepressant activity compared with fluoxetine at a dose of 100 mg/kg.

An analysis of the antidepressant activities of compounds **5a**–**5t** (Table 2) showed the following structure–activity relationships. Among the phenoxy-substituted derivatives, the position of the substituent group on the benzene ring appeared to greatly influence the FST activity. Comparing derivatives with different halogen substituents on the benzene ring, their activity order was *m*-F > *p*-F > *o*-F, 2,4-Cl_2 > *o*-Cl > *p*-Cl > *m*-Cl. Comparing derivatives with different electron-donating substituents (methyl and methoxy) on the benzene ring, their activity order was *m*-CH_3 > *p*-CH_3 > *o*-CH_3, *p*-OCH_3 > *m*-OCH_3 > *o*-OCH_3. The activity order for amino substituents on the benzene ring was *m*-NH_2 > *o*-NH_2. Electron-donating group seemed to be a more profitable structural feature than electron-withdrawing. The *p*-CF_3, *p*-Br, and *o*-F group in place of hydrogen atom being an electron-withdrawing moiety, showed less activity.

Since some compounds that alter motor activity may give false positive/negative effects in the FST, in particular psychomotor stimulants and drugs enhancing motor activity, which decrease immobility time by stimulating locomotor activity [20,21], an additional measurement was carried out with the specific aim of observing motor activity. In this study, the effect of **5t** on spontaneous locomotor activity was evaluated in the open-field test, a classical animal test used to evaluate the autonomic effects of drugs and general activity of animals [22,23]. This study demonstrated that **5t** did not significantly change motor activity (crossing, rearing, or grooming) in mice (Fig. 2). It is unlikely that the effect of **5t** observed in the FST is based on stimulation of general motor activity. This study provides evidence that **5t** has an antidepressant-like effect on mice.

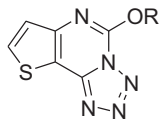
3. Conclusion

In conclusion, a series of 5-alkoxytetrazolo[1,5-*c*]thieno[2,3-*e*]pyrimidine derivatives were synthesized, and their anticonvulsant and antidepressant activities were evaluated. Only four of the synthesized compounds (**5f**, **5g**, **5i**, and **5j**) showed weak anticonvulsant activity; however, most of the synthesized compounds showed significant antidepressant activity in FSTs. 5-(2,4-Dichlorobenzyloxy)tetrazolo[1,5-*c*]thieno[2,3-*e*]pyrimidine (**5t**) showed significant antidepressant activity, and decreased immobility time by 51.62% at an intraperitoneal dose of 100 mg/kg. The results of open-field tests on compound **5t** indicated that it has no significant effects on locomotor activity in comparison with a control group at the doses assayed in the FST test. This means that the antidepressant activity detected in the FST for the compound is not caused by CNS stimulant properties, and further confirmed its antidepressant-like effect.



Scheme 1. The synthesis route of compounds **5a**–**5t**.

Table 1
Anticonvulsant activities of compounds **5a**–**5t** in maximal electroshock tests.



5a-5t

Compound	R	MES[a]		
		30	100	300
5a	$-C_6H_4$(*o*-Cl)	0/3	0/3	0/3
5b	$-C_6H_4$(*p*-Cl)	0/3	0/3	0/3
5c	$-C_6H_4$(*m*-Cl)	0/3	0/3	0/3
5d	α-Naphthalene	0/3	0/3	0/3
5e	$-C_6H_4$(*p*-CF_3)	0/3	0/3	0/3
5f	$-C_6H_4$(*o*-NH_2)	0/3	1/3	3/3
5g	$-C_6H_4$(*m*-NH_2)	0/3	0/3	2/3
5h	8-Quinoline	0/3	0/3	0/3
5i	$-C_6H_5$	0/3	0/3	1/3
5j	$-C_6H_4$(*p*-Br)	0/3	1/3	3/3
5k	$-C_6H_4$(*p*-CH_3)	0/3	0/3	0/3
5l	$-C_6H_4$(*m*-CH_3)	0/3	0/3	0/3
5m	$-C_6H_4$(*o*-CH_3)	0/3	0/3	0/3
5n	$-C_6H_4$(*p*-F)	0/3	0/3	0/3
5o	$-C_6H_4$(*o*-F)	0/3	0/3	0/3
5p	$-C_6H_4$(*m*-F)	0/3	0/3	0/3
5q	$-C_6H_4$(*p*-OCH_3)	0/3	0/3	0/3
5r	$-C_6H_4$(*o*-OCH_3)	0/3	0/3	0/3
5s	$-C_6H_4$(*p*-OCH_3)	0/3	0/3	0/3
5t	$-C_6H_3$ (2,4-Cl)	0/3	0/3	0/3

[a] Maximal electroshock test (number of animals protected/number of animals tested).

Table 2
Antidepressant activities of the compounds in forced swimming tests.

Compound	R	Antidepressant activities[a]	
		Duration of immobility(s) (mean ± S.E.M.)[b]	Change from control (%)
5a	$-C_6H_4$(*o*-Cl)	73.7 ± 17.51**	−40.17
5b	$-C_6H_4$(*p*-Cl)	79.3 ± 14.24**	−35.63
5c	$-C_6H_4$(*m*-Cl)	104.2 ± 13.72	−15.42
5d	α-Naphthalene	111.8 ± 25.97	−9.25
5e	$-C_6H_4$(*p*-CF_3)	92.2 ± 15.49	−25.16
5f	$-C_6H_4$(*o*-NH_2)	95.1 ± 17.34*	−22.80
5g	$-C_6H_4$(*m*-NH_2)	89.2 ± 14.99*	−27.59
5h	8-Quinoline	94.1 ± 16.02**	−23.62
5i	$-C_6H_5$	90.6 ± 18.23*	−26.46
5j	$-C_6H_4$(*p*-Br)	110.8 ± 19.38	−10.06
5k	$-C_6H_4$(*p*-CH_3)	78.1 ± 21.59**	−36.60
5l	$-C_6H_4$(*m*-CH_3)	75.1 ± 21.46**	−39.04
5m	$-C_6H_4$(*o*-CH_3)	78.3 ± 15.73**	−36.44
5n	$-C_6H_4$(*p*-F)	100.8 ± 25.01*	−18.18
5o	$-C_6H_4$(*o*-F)	111.7 ± 20.55	−9.33
5p	$-C_6H_4$(*m*-F)	91.5 ± 17.96*	−25.73
5q	$-C_6H_4$(*p*-OCH_3)	66.3 ± 12.44**	−46.18
5r	$-C_6H_4$(*o*-OCH_3)	91.1 ± 26.46*	−26.05
5s	$-C_6H_4$(*p*-OCH_3)	77.1 ± 15.53**	−37.41
5t	$-C_6H_3$(2,4-Cl)	59.6 ± 10.63**	−51.62
Fluoxetine	–	51.6 ± 13.60**	−58.09
Control	–	123.2 ± 18.42	–

*Significantly compared to control ($0.01 < p < 0.05$).
**Very significantly compared to control ($p < 0.01$).
[a] Compounds prepared were administered at 100 mg/kg, fluoxetine was administered at 100 mg/kg.
[b] Values represent the mean ± S.E.M. ($n = 8$).

4. Experimental

4.1. Chemistry

Melting points were determined in open capillary-tubes and were uncorrected. Infrared spectra were recorded (in KBr) using a FTIR1730 spectrometer (Perkin–Elmer, Waltham, MA, USA). ^{1}H NMR spectra were recorded using an AV-300 spectrometer (Bruker, Switzerland), and all chemical shifts were given in parts per million relative to tetramethylsilane. Mass spectra were measured using an HP1100 LC spectrometer (Agilent Technologies, Santa Clara, USA). Elemental analyses were performed using a 204Q CHN instrument (Perkin Elmer). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer (Heraeus, Hanau, Germany). The main chemicals were purchased from Aldrich Chemical Corporation (St Louis, MO, USA).

4.1.1. Synthesis of thieno[3,2-d]pyrimidine-2,4(1H,3H)-dione (**1**)

3-Amino-2-thiophenecarboxylic acid methyl ester (1 g, 6.36 mmol) and urea (3 g, 50 mmol) were placed in a round-bottomed flask, and the mixture was heated at 200 °C for 1.5 h. After the reaction was complete, the residue was dissolved in 40 mL of 20% NaOH, and a solution of 10% HCl was added to adjust the pH to 5–6. The precipitate was filtered and washed with water to produce a white solid. Yield: 79%, mp: 311–314 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.89 (t, 1H, J = 3.00 Hz, S–C=C–H), 8.02 (t, 1H, J = 3.00 Hz, S–C–H), 11.19 (s, 1H, –CONH), 11.54 (s, 1H, –CONHCO–). MS-EI m/z 169 (M + 1).

4.1.2. Synthesis of 2,4-dichlorothieno[3,2-d]pyrimidine (**2**)

Compound **1** (1 g, 5.9 mmol) was dissolved in phosphorus oxychloride (15 mL) and stirred under reflux for 5 h. Two-thirds of the solvent was removed under a vacuum. The mixture was poured into ice-water (50 mL). The precipitate that separated was collected by filtration, washed with water, and dried to give compound **2** as a white solid. Yield: 74%, mp: 140–143 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.55 (d, 1H, J = 6.00 Hz, S–C=C–H), 8.13 (d, 1H, J = 6.00 Hz, S–C–H). MS-EI m/z 205 (M + 1).

4.1.3. Synthesis of 1-(2-chlorothieno[3,2-d]pyrimidin-4-yl) hydrazine (**3**)

A solution of compound **2** (1 g, 4.9 mmol) in methanol (30 mL) was added dropwise to a solution of hydrazine hydrate (0.6 mL) in methanol (5 mL) at room temperature. The mixture was stirred and heated at 50 °C for 1 h, and then half of the solvent was removed under reduced pressure, and the solution was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether; the compounds obtained were pure enough for the

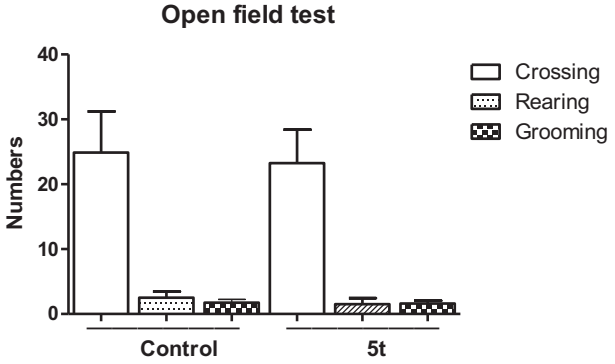


Fig. 2. Exploratory activity (counts) in the open-field test. The behavioral parameters were recorded for 3 min. Locomotion: number of line crossings; rearing: number of times seen standing on hind legs; grooming: number of modifications; **5t** was administered 60 min before the test. The values represent the mean ± SEM (n = 15).

subsequent step. Yield: 84%, mp: 314–316 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.01 (s, 2H, $-NH_2$), 7.23 (d, 1H, J = 6.00 Hz, S–C=C–H), 8.13 (d, 1H, J = 6.00 Hz, S–C–H), 9.48 (s, 1H, –NH). MS-EI m/z 201 (M + 1).

*4.1.4. Synthesis of 5-chlorotetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**4**)*

Compound **3** (0.8 g, 4.0 mmol) was dissolved in 20 mL of 30% HCl. A solution of $NaNO_2$ (0.28 g, 4.0 mmol) in 5 mL of water was added dropwise to the mixture in an ice-bath, ensuring that the reaction temperature was below 5 °C. The mixture was then stirred at room temperature for 2 h. After the reaction was complete, the precipitate was filtered and washed with water to produce **4** as a white solid. Yield: 81%, mp: 160–162 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.47 (d, 1H, J = 6.00 Hz, S–C=C–H), 7.99 (d, 1H, J = 6.00 Hz, S–C–H). MS-EI m/z 212 (M + 1).

*4.1.5. General procedure for the syntheses of 5-alkoxytetrazolo[1,5-c]thieno[2,3-e]pyrimidine derivatives (**5a**–**5t**)*

A mixture of an appropriately substituted phenol (5.1 mmol) and K_2CO_3 (5.1 mmol) was placed in a round-bottomed flask with 15 mL of ethyl acetate. The mixture was stirred and heated at 100 °C for 0.5 h, and then compound **4** (1 g, 4.7 mmol) was added to the mixture. After the reaction was complete, the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (30 mL), washed with water (10 mL × 3), dried over anhydrous $MgSO_4$, and purified using silica-gel column chromatography with methanol:dichloromethane (1:60) to give a white solid. The yields and melting point data of each compound are given below.

*4.1.5.1. 5-(2-Chloro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5a**).* Yield: 69%, mp: 137–141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.23–7.51 (m, 4H, Ar-H), 7.30 (d, 1H, J = 5.40 Hz, S–C=C–H), 7.89(d, 1H, J = 5.40 Hz, S–C–H). IR (KBr) cm^{-1}: 1200, 1256 (C–O–C), 1570 (C=N). MS-EI m/z 304 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSCl$: C, 47.45; H, 1.99; N, 23.06. Found: C, 47.66; H, 1.82; N, 22.89.

*4.1.5.2. 5-(4-Chloro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5b**).* Yield: 80%, mp: 139–141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.19–7.40 (m, 4H, Ar-H), 7.31 (d, 1H, J = 5.40 Hz, S–C=C–H), 7.90 (d, 1H, J = 5.40 Hz, S–C–H). IR (KBr) cm^{-1}: 1202, 1257 (C–O–C), 1570 (C=N). MS-EI m/z 304 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSCl$: C, 47.45; H, 1.99; N, 23.06. Found: C, 47.64; H, 1.83; N, 22.86.

*4.1.5.3. 5-(3-Chloro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5c**).* Yield: 76%, mp: 138–141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.17–7.39 (m, 4H, Ar-H), 7.31 (d, 1H, J = 5.37 Hz, S–C=C–H), 7.91 (d, 1H, J = 5.37 Hz, S–C–H). ^{13}C NMR ($CDCl_3$-d_6) δ 115.2, 120.0, 122.3, 123.9, 125.5, 130.2, 134.6, 137.0, 153.5, 158.9, 162.6, 164.3. IR (KBr) cm^{-1}: 1199, 1255 (C–O–C), 1570 (C=N). MS-EI m/z 304 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSCl$: C, 47.45; H, 1.99; N, 23.06. Found: C, 47.61; H, 1.80; N, 22.91.

*4.1.5.4. 5-(Naphthalen-1-yloxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5d**).* Yield: 65%, mp: 128–134 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.24 (d, 1H, J = 6.0 Hz, S–C=C–H), 7.37–8.03 (m, 7H, Ar-H), 7.85 (d, 1H, J = 6.0 Hz, S–C–H). ^{13}C NMR ($CDCl_3$-d_6) δ 114.9, 118.0, 121.9, 124.0, 124.3, 125.5, 126.2, 126.3, 127.4, 127.9, 134.8, 136.7, 149.1, 158.8, 163.6, 164.6. IR (KBr) cm^{-1}: 1195, 1256 (C–O–C), 1570 (C=N). MS-EI m/z 320 (M + 1). *Anal.* Calcd for $C_{16}H_9N_5OS$: C, 60.18; H, 2.84; N, 21.93. Found: C, 60.35; H, 2.65; N, 21.73.

*4.1.5.5. 5-[4-(Trifluoromethyl)phenoxy]tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5e**).* Yield: 57%, mp: 140–142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.33 (d, 1H, J = 6.00 Hz, S–C=C–H), 7.37–7.72 (m, 4H, Ar-H), 7.93 (d, 1H, J = 6.00 Hz, S–C–H). IR (KBr) cm^{-1}: 1195, 1257 (C–O–C), 1573 (C=N). MS-EI m/z 338 (M + 1). *Anal.* Calcd for $C_{13}H_6N_5OSF_3$: C, 46.29; H, 1.79; N, 20.76. Found: C, 46.43; H, 1.58; N, 20.91.

*4.1.5.6. 2-(Tetrazolo[1,5-c]thieno[2,3-e]pyrimidin-5-yloxy)aniline (**5f**).* Yield: 84%, mp: 190–194 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.81 (s, 2H, $-NH_2$), 6.78–7.15 (m, 4H, Ar-H), 7.32 (d, 1H, J = 5.31 Hz, S–C=C–H), 7.88 (d, 1H, J = 5.31 Hz, S–C–H). IR (KBr) cm^{-1}: 1195, 1252 (C–O–C), 1573 (C=N). MS-EI m/z 285 (M + 1). *Anal.* Calcd for $C_{12}H_8N_6OSCl$: C, 50.70; H, 2.84; N, 29.56. Found: C, 50.85; H, 2.64; N, 29.45.

*4.1.5.7. 3-(Tetrazolo[1,5-c]thieno[2,3-e]pyrimidin-5-yloxy)aniline (**5g**).* Yield: 80%, mp: 162–164 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.90 (s, 2H, $-NH_2$), 6.38–6.97 (m, 4H, Ar-H), 7.14 (d, 1H, J = 6.0 Hz, S–C=C–H), 7.88 (d, 1H, J = 6.0 Hz, S–C–H). IR (KBr) cm^{-1}: 1197, 1258 (C–O–C), 1573 (C=N). MS-EI m/z 286 (M + 1). *Anal.* Calcd for $C_{12}H_8N_6OSCl$: C, 50.70; H, 2.84; N, 29.56 Found: C, 50.87; H, 2.66; N, 29.43.

*4.1.5.8. 5-(Quinolin-8-yloxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5h**).* Yield: 47%, mp: 140–142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.16 (d, 1H, J = 6.00 Hz, S–C=C–H), 7.37–8.80 (m, 6H, Ar-H), 7.80 (d, 1H, J = 6.00 Hz, S–C–H). ^{13}C NMR ($CDCl_3$-d_6) δ 121.3, 121.5, 121.7, 123.9, 125.4, 126.4, 129.7, 135.9, 136.3, 141.6, 149.3, 150.1, 140.5, 158.7, 164.5. IR (KBr) cm^{-1}: 1192, 1253 (C–O–C), 1570 (C=N). MS-EI m/z 321 (M + 1). *Anal.* Calcd for $C_{15}H_8N_6OS$: C, 56.24; H, 2.52; N, 26.24. Found: C, 56.04; H, 2.38; N, 26.01.

*4.1.5.9. 5-Phenoxy-tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5i**).* Yield: 62%, mp: 168–170 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.24–7.44 (m, 5H, Ar-H), 7.31 (d, 1H, J = 5.40 Hz, S–C=C–H), 7.88 (d, 1H, J = 5.40 Hz, S–C–H). IR (KBr) cm^{-1}: 1197, 1253 (C–O–C), 1573 (C=N). MS-EI m/z 270 (M + 1). *Anal.* Calcd for $C_{12}H_7N_5OS$: C, 53.52; H, 2.62; N, 26.01. Found: C, 53.38; H, 2.45; N, 26.25.

*4.1.5.10. 5-(4-Bromo-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5j**).* Yield: 67%, mp: 138–144 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.14–7.55 (m, 4H, Ar-H), 7.32 (d, 1H, J = 5.40 Hz, S–C=C–H), 7.91 (d, 1H, J = 5.40 Hz, S–C–H). IR (KBr) cm^{-1}: 1194, 1248 (C–O–C), 1570 (C=N). MS-EI m/z 348 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSBr$: C, 41.40; H, 1.74; N, 20.11. Found: C, 41.38; H, 1.56; N, 20.30.

*4.1.5.11. 5-(4-Methyl-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5k**).* Yield: 52%, mp: 138–142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.39 (s, 3H, $-CH_3$), 7.13–7.24 (m, 4H, Ar-H), 7.31 (d, 1H, J = 5.43 Hz, S–C=C–H), 7.87 (d, 1H, J = 5.43 Hz, S–C–H). IR (KBr) cm^{-1}: 1195, 1252 (C–O–C), 1573 (C=N). MS-EI m/z 284 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5OS$: C, 55.11; H, 3.20; N, 24.72. Found: C, 55.29; H, 3.32; N, 24.50.

*4.1.5.12. 5-(3-Methyl-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5l**).* Yield: 72%, mp: 158–160 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.39 (s, 3H, $-CH_3$), 7.04–7.08 (m, 4H, Ar-H), 7.33 (d, 1H, J = 5.43 Hz, S–C=C–H), 7.88 (d, 1H, J = 5.43 Hz, S–C–H). IR (KBr) cm^{-1}: 1197, 1252 (C–O–C), 1573 (C=N). MS-EI m/z 284 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5OS$: C, 55.11; H, 3.20; N, 24.72. Found: C, 55.30; H, 3.32; N, 24.52.

*4.1.5.13. 5-(2-Methyl-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5m**).* Yield: 70%, mp: 132–135 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.24 (s, 3H, $-CH_3$), 7.14–7.29 (m, 4H, Ar-H), 7.30 (d, 1H, J = 5.37 Hz, S–C=C–H), 7.87 (d, 1H, J = 5.37 Hz, S–C–H). IR (KBr)

cm^{-1}: 1199, 1255 (C–O–C), 1573 (C=N). MS-EI *m/z* 284 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5OS$: C, 55.11; H, 3.20; N, 24.72. Found: C, 55.31; H, 3.34; N, 24.57.

*4.1.5.14. 5-(4-Fluoro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5n**).* Yield: 81%, mp: 163–164 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.08–7.24 (m, 4H, Ar-H), 7.31 (d, 1H, *J* = 5.37 Hz, S–C=C–H), 7.90 (d, 1H, *J* = 5.37 Hz, S–C–H). IR (KBr) cm^{-1}: 1200, 1254 (C–O–C), 1570 (C=N). MS-EI *m/z* 288 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSF$: C, 50.17; H, 2.11; N, 24.38. Found: C, 50.31; H, 2.26; N, 24.52.

*4.1.5.15. 5-(3-Fluoro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5o**).* Yield: 73%, mp: 156–161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.98–7.08 (m, 4H, Ar-H), 7.33 (d, 1H, *J* = 6.0 Hz, S–C=C–H), 7.37-7.40 (m, 1H, Ar-H), 7.92 (d, 1H, *J* = 6.0 Hz, S–C–H). IR (KBr) cm^{-1}: 1197, 1251 (C–O–C), 1570 (C=N). MS-EI *m/z* 288 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSF$: C, 50.17; H, 2.11; N, 24.38. Found: C, 50.30; H, 2.28; N, 24.54.

*4.1.5.16. 5-(2-Fluoro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5p**).* Yield: 78%, mp: 138–144 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.20–7.25 (m, 4H, Ar-H), 7.31 (d, 1H, *J* = 6.0 Hz, S–C=C–H), 7.90 (d, 1H, *J* = 6.0 Hz, S–C–H). IR (KBr) cm^{-1}: 1196, 1255 (C–O–C), 1570 (C=N). MS-EI *m/z* 288 (M + 1). *Anal.* Calcd for $C_{12}H_6N_5OSF$: C, 50.17; H, 2.11; N, 24.38. Found: C, 50.32; H, 2.30; N, 24.52.

*4.1.5.17. 5-(4-Methoxy-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5q**).* Yield: 66%, mp: 175–179 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.84 (s, 3H, $-OCH_3$), 6.93–7.20 (m, 4H, Ar-H), 7.31 (d, 1H, *J* = 5.37 Hz, S–C=C–H), 7.87 (d, 1H, *J* = 5.37 Hz, S–C–H). IR (KBr) cm^{-1}: 1201, 1259 (C–O–C), 1573 (C=N). MS-EI *m/z* 300 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5O_2S$: C, 52.17; H, 3.03; N, 23.40. Found: C, 52.34; H, 3.17; N, 23.55.

*4.1.5.18. 5-(2-Methoxy-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5r**).* Yield: 74%, mp: 166–169 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.76 (s, 3H, $-OCH_3$), 7.01–7.25 (m, 4H, Ar-H), 7.28 (d, 1H, *J* = 5.40 Hz, S–C=C–H), 7.87 (d, 1H, *J* = 5.40 Hz, S–C–H). IR (KBr) cm^{-1}: 1197, 1254 (C–O–C), 1573 (C=N). MS-EI *m/z* 300 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5O_2S$: C, 52.17; H, 3.03; N, 23.40. Found: C, 52.36; H, 3.17; N, 23.60.

*4.1.5.19. 5-(3-Methoxy-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5s**).* Yield: 60%, mp: 168–171 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.82 (s, 3H, $-OCH_3$), 6.85–7.33 (m, 4H, Ar-H), 7.32 (d, 1H, *J* = 6.0 Hz, S–C=C–H), 7.89 (d, 1H, *J* = 6.0 Hz, S–C–H). ^{13}C NMR ($CDCl_3$-d_6) δ 55.4, 107.6, 111.0, 111.0, 113.8, 124.0, 129.7, 130.0, 136.7, 154.0, 158.1, 160.5, 164.1. IR (KBr) cm^{-1}: 1199, 1255 (C–O–C), 1573 (C=N). MS-EI *m/z* 300 (M + 1). *Anal.* Calcd for $C_{13}H_9N_5O_2S$: C, 52.17; H, 3.03; N, 23.40. Found: C, 52.35; H, 3.15; N, 23.58.

*4.1.5.20. 5-(2,4-Dichloro-phenoxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine (**5t**).* Yield: 72%, mp: 159–162 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.25–7.31 (m, 3H, Ar-H), 7.50 (d, 1H, *J* = 5.40 Hz, S–C=C–H), 7.91 (d, 1H, *J* = 5.40 Hz, S–C–H). ^{13}C NMR ($CDCl_3$-d_6) δ 123.9, 124.7, 128.0, 128.4, 129.1, 130.2, 131.3, 137.0, 147.8, 158.9, 162.6, 164.4. IR (KBr) cm^{-1}: 1199, 1253 (C–O–C), 1570 (C=N). MS-EI *m/z* 338 (M + 1). *Anal.* Calcd for $C_{12}H_5N_5OSCl_2$: C, 42.62; H, 1.49; N, 20.71. Found: C, 42.48; H, 1.35; N, 20.89.

4.2. Pharmacology

The MES tests were carried out according to the phase-I tests of the antiepileptic drug development program [24,25]. Antidepressant activities were evaluated using the FST [26]. All compounds were tested for anticonvulsant activities and antidepressant activities using Kun-Ming mice in the weight range 20–24 g purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University, China. The mice were maintained on a 12-h light/dark cycle in a temperature-controlled (23 ± 2 °C) laboratory. Food and water were available ad libitum. The tested compounds were prepared as suspensions in aqueous Tween 80 (3% v/v, 0.9% NaCl), and injected intraperitoneally in a standard volume of 0.05 mL/20 g body weight.

4.2.1. Maximal electroshock (MES) seizure test

Seizures were elicited in mice using a 60-Hz 50-mA alternating current. 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.

4.2.2. Forced swimming test (FST)

Male Kun-Ming mice (22 ± 2 g) were used in the FSTs. On the test day, mice were assigned to different groups (*n* = 8 for each group). The synthesized compounds and the standard drug fluoxetine were administered as intraperitoneal injections. Control animals received a 3% aqueous solution of Tween 80. After 30 min, the mice were dropped one at a time into a Plexiglas cylinder (height 25 cm, diameter 10 cm, containing water to a height of 10 cm at 23–25 °C) and observed for 6 min. After the first 2 min of vigorous struggling, the animals were immobile. A mouse was judged immobile if it floated in the water in an upright position and made only slight movements to prevent sinking. The total duration of immobility was recorded during the last 4 min of the 6-min test.

4.2.3. Open-field test

Open-field tests were used to evaluate the exploratory activity of the animal [20]. The investigated compound (**5t** suspended in aqueous Tween 80) was administered 60 min before the experiment. The study was carried out on mice according to Archer's method [27], with slight modifications. Each mouse was placed individually in the center of the open-field apparatus, and the locomotor activity was assessed. The open-field apparatus was a non-transparent plastic container (80 cm × 60 cm × 30 cm), with the underside divided into 48 units of size 10 cm × 10 cm, without walls. The animals were gently placed in the center of the platform and were allowed to explore their surroundings. Hand-operated counters were used to score locomotion (ambulation, numbers of crossing lines with all four paws) and rearing frequencies (number of times an animal stood on its hind legs) for 3 min. The researchers, who did not know which groups had been treated, scored the behaviors in the open field. The experiments were performed in a dark room, and the apparatus was illuminated by a 60-W bulb giving a yellowish light, positioned 1 m above the center of the apparatus.

4.3. Statistical analysis

The results are expressed as the mean ± SEM.; *n* represents the number of animals. Data obtained from pharmacological experiments were analyzed using one-way analysis of variance, followed by Dunnett's post hoc test, using Pharmacologic Calculation System Version 4.1 (Microcomputer Specialists). A *P*-value of less than 0.05 was considered statistically significant.

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References

[1] F. Borsini, A. Meli, Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 94 (1988) 147–160.
[2] D. Mochizucki, Serotonin and noradrenaline reuptake inhibitors in animal models of pain, Hum. Psychopharmacol. 19 (2004) S15–S19.
[3] D.A. Williams, T.L. Lemke, Foye's Principles of Medicinal Chemistry, fifth ed., Lippincott Williams & Wilkins, New York, 2002, pp. 384–403.
[4] M.A. Rogawski, Diverse mechanisms of antiepileptic drugs in the development pipeline, Epilepsy Res. 69 (2006) 273–294.
[5] A.M. Kanner, J.C. Nieto, Depressive disorders in epilepsy, Neurology 53 (1999) S26–S32.
[6] K.U. Kühn, B.B. Quednow, M. Thiel, P. Falkai, W. Maier, C.E. Elger, Antidepressive treatment in patients with temporal lobe epilepsy and major depression: a prospective study with three different antidepressants, Epilepsy Behav. 4 (2003) 674–679.
[7] J.A. Cramer, I.E. Leppik, K.D. Rue, P. Edrich, G. Krämer, Tolerability of levetiracetam in elderly patients with CNS disorders, Epilepsy Res. 56 (2003) 135–145.
[8] J.A. Cramer, D. Blum, K. Fanning, M. Reed, The impact of comorbid depression on health resource utilization in a community sample of people with epilepsy, Epilepsy Behav. 5 (2004) 337–342.
[9] X.Y. Sun, C.X. Wei, X.Q. Deng, Z.G. Sun, Z.S. Quan, Evaluation of the anticonvulsant activity of 6-(4-chlorophenyoxy)-tetrazolo[5,1-a]phthalazine in various experimental seizure models in mice, Pharmacol. Rep. 62 (2010) 273–277.
[10] X.Y. Sun, C.X. Wei, X.Q. Deng, Z.G. Sun, Z.S. Quan, Synthesis and primary anticonvulsant activity evaluation of 6-alkyoxyl-tetrazolo [5,1-a]phthalazine derivatives, Arzneimittel-Forschung 60 (2010) 289–292.
[11] C. Alhano, A. Cupello, P. Mainardi, S. Scarrone, E. Favale, Successful treatment of epilepsy with serotonin reuptake inhibitors: proposed mechanism, Neurochem. Res. 31 (2006) 509–514.
[12] S. Chitra, N. Paul, S. Muthusubramanian, P. Manisankar, P. Yogeeswari, D. Sriram, Synthesis of 3-heteroarylthioquinoline derivatives and their in vitro antituberculosis and cytotoxicity studies, Eur. J. Med. Chem. 46 (2011) 4897–4903.
[13] D.R. Armour, M. Congreve, M. Evans, S. Guntrip, T. Hubbard, C. Kay, D. Middlemiss, et al., Tetrazole NK_1 receptor antagonists: the identification of an exceptionally potent orally active antiemetic compound, Bioorg. Med. Chem. Lett. 6 (1996) 1015–1020.
[14] M.Y. Wani, A.R. Bhat, A. Azam, L. Choi, F. Athar, Probing the antiamoebic and cytotoxicity potency of novel tetrazole and triazine derivatives, Eur. J. Med. Chem. 48 (2012) 313–320.
[15] R. Pellicciari, B.D. Giacomo, D. Coletta, B. Natalini, M.H. Ni, A new synthesis of carboxyterfenadine (fexofenadine) and its bioisosteric tetrazole analogs, Farmaco 54 (1999) 600–610.
[16] E. Nicolaï, G. Curé, J. Goyard, M. Kirchner, J.M. Teulonl, A. Versignyz, et al., Synthesis and angiotensin II receptor antagonist activity of C-linked pyrimidine derivatives, Eur. J. Med. Chem. 30 (1995) 365–375.
[17] X.Q. Deng, C.X. Wei, M.X. Song, K.Y. Chai, Z.G. Sun, Z.S. Quan, Synthesis and studies on anticonvulsant and antidepressant activities of 5-alkoxy-tetrazolo [1,5-a]quinolines, Bull. Korean Chem. Soc. 31 (2010) 447–452.
[18] H.J. Wang, C.X. Wei, X.Q. Deng, F.N. Li, Z.S. Quan, Synthesis and evaluation on anticonvulsant and antidepressant activities of 5-alkoxy-tetrazolo[1,5-a]quinazolines, Arch. Pharm. Chem. Life Sci. 342 (2009) 671–675.
[19] E. Beghi, M. Roncolato, G. Visona, Depression and altered quality of life in women with epilepsy of childbearing age, Epilepsia 45 (2004) 64–70.
[20] P.J. Elliott, J. Chan, Y.M. Parker, Behavioral effects of neurotensin in the open field: structure–activity studies, Brain Res. 381 (1986) 259–265.
[21] M.P. Kaster, A.O. Rosa, M.M. Rosso, E.C. Goulart, A.R. Santos, A.L. Rodrigues, Adenosine administration produces an antidepressant-like effect in mice: evidence for the involvement of A_1 and A_{2A} receptors, Neurosci. Lett. 355 (2004) 21–24.
[22] P.M. Galdino, M.V. Nascimento, B.L. Sampaio, R.N. Ferreira, J.R. Paula, E.A. Costa, Antidepressant-like effect of *Lafoensia pacari* A. St.-Hil. ethanolic extract and fractions in mice, J. Ethnopharmacol. 124 (2009) 581–585.
[23] H. Sakakibara, K. Ishida, O. Grundmann, J. Nakajima, S. Seo, V. Butterweck, Y. Minami, S. Saito, Y. Kawai, Y. Nakaya, J. Terao, Antidepressant effect of extracts from Ginkgo biloba leaves in behavioral models, Biol. Pharm. Bull. 29 (2006) 1767–1770.
[24] R.L. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyard, Synthesis of 8-alkoxy-4,5-dihydro-[1,2,4]triazole[4,3-a]quinoline-1-ones and evaluation of their anticonvulsant properties, Epilepsia 19 (1978) 409–428.
[25] R.J. Porter, R.J. Cereghino, G.D. Gladding, B.J. Hessie, H.J. Kupferberg, B. Scoville, B.G. White, Antiepileptic drug development program, Cleve Clin. Q. 51 (1984) 293–295.
[26] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–336.
[27] J. Archer, Tests for emotionality in rats and mice: a review, Anim. Behav. 21 (1973) 205–235.