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Original article

Synthesis and anticonvulsant activity of a new 6-alkoxy-[1,2,4]triazolo[4,3-*b*]pyridazine

Li-Ping Guan [a,b,*], Xin Sui [b], Xian-Qing Deng [b], Ying-Chun Quan [b], Zhe-Shan Quan [b,*]

[a] School of Food, Drug & Medicine Zhejiang Ocean University, Zhoushan, Zhejiang Province 316004, P.R. China
[b] College of Pharmacy, Yanbian University, Yanji, Jili 133000, P.R. China

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ABSTRACT

A series of 6-alkoxy-[1,2,4]triazolo[4,3-*b*]pyridazine derivatives were synthesized. In initial screening and quantitative evaluation, compound **2r** was among the most active agents, exhibiting in the same time the lowest toxicity. In the anti-maximal electroshock test, it showed median effective dose (ED_{50}) of 17.3 mg/kg and median toxicity dose (TD_{50}) of 380.3 mg/kg, and the protective index (PI) of 22.0, which is much better than PI of the reference drugs. In a subsequent test, compound **2r** had median hypnotic dose (HD_{50}) of 746.6 mg/kg, thus demonstrating much better margin of safety compared to reference drugs. Compound **2r** also showed oral activity against MES-induced seizures and lower oral neurotoxicity. For explanation of the putative mechanism of action, compound **2r** was tested in chemical induced models.
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1. Introduction

Epilepsy is the most frequent neurologic infection characterized by excessive temporary neuronal discharge [1]. The overall prevalence of the disease is 1.0% of the population and up to 50 million people world wide [2]. Despite the development of several new anticonvulsants, the treatment of epilepsy remains still inadequate. About one third of patients do not respond well to currently available treatment, even if multiple drugs with complementary activities are used [3,4]. Furthermore, more than 50% of epilepsy patients experience unwanted side effects of drug treatment [5,6] and even life-threatening conditions [7]. Based on the reported observations, the aim of our study is to investigate novel potential antiepileptic drugs which are more potent and deprived of the most serious side effects than the existing ones.

In our previous work [8,9], several differently substituted quinoline derivatives exhibited anticonvulsant activities. Among these, 6-benzyloxy-3, 4-dihydro-2(1*H*)-quinoline (compound **I**) showed the strongest activity. Compound **I** had an ED_{50} of 29.6 mg/kg in the maximal electroshock seizure (MES) test, and a TD_{50} >300 mg/kg. Introduction of triazole ring to the first and second position of this 6-benzyloxy-3, 4-dihydro-2(1*H*)-quinoline caused a remarkable increase in anticonvulsant activity. This was observed in 7-benzyloxyl-4,5-dihydro-[1,2,4]tr-iazolo[4,3-*a*]quinoline (compound **II**), which showed an ED_{50} of 17.3 mg/kg in the MES test. Another derivative in the group of 7-hexyloxy-5-phenyl-1,2,4-triazolo[4, 3-*a*]quinoline (compound **III**) exhibited higher activity (ED_{50} = 6.5 mg/kg) and lower toxicity (TD_{50} = 228.2 mg/kg) in the MES test (Fig. 1) [10,11]. The hypothesis was that a triazole ring may have higher affinity for the receptor and enhance anticonvulsant activity, and there were some similar design reports [12,13]. Chau et al [14]. carried out a similar docking experiment with the imidazobenzodiazepine, alprazolam. Alprazolam is an agonist benzodiazepine, like flunitrazepam, but its activity was higher than that of flunitrazepam. With the introduction of a triazole ring to the first and second position of flunitrazepam, the physical space occupied by this imidazobenzodiazepine is similar to that found for flunitrazepam, but it has a significantly higher affinity for the recognition site. The imidazo-ring on the *a*-face is found to be in closer proximity to the important recognition determinant α_1 His 129 than is the case with flunitrazepam.

Pyridazinone and pyridazine analogs have been reported to possess anticonvulsant properties [15–17]. Compound **IV**(6-phenoxypyridazin-3(2*H*)-one) showed a slight positive anticonvulsant

* Corresponding author: School of Food, Drug & Medicine Zhejiang Ocean University, Zhoushan, Zhejiang Province 316004, P.R. China. Tel.: +86 580 2554536.
E-mail addresses: glp730@yahoo.com.cn (L.-P. Guan), zsquan@ybu.edu.cn (Z.-S. Quan).

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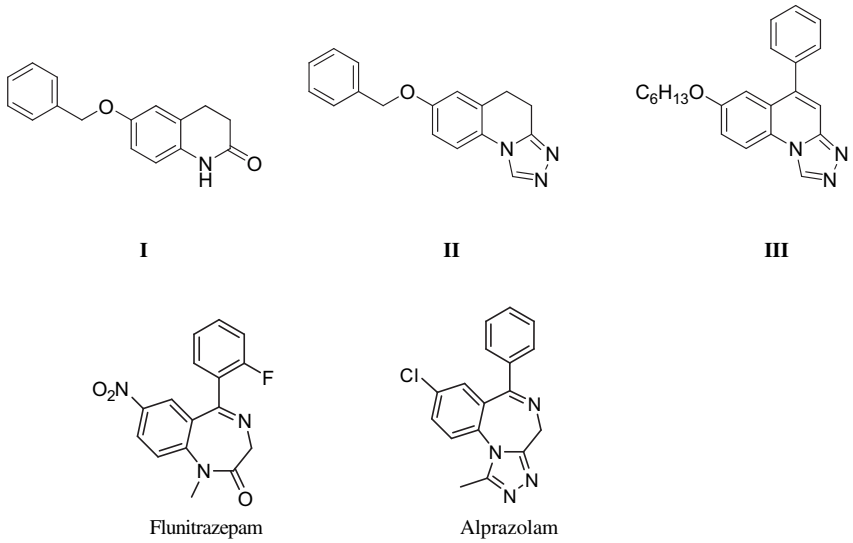


Fig 1. The structure of compounds I, II, III, flunitrazepam and alprazolam.

activity with an effective dose of 300 mg/kg in the anti-MES test. Keeping the above-mentioned in view, it was thought worthwhile to design the synthesis of title compounds wherein the biologically active triazole moiety is fused to a potent pyridazinone ring at 2, 3 positions. Compound **V** was designed and synthesized, with the aim of exploring effective compounds with lower neurotoxicity. The pharmacology test showed a weak anticonvulsant activity with an ED_{50} of 109.6 mg/kg in the MES test (Fig. 2).

To obtain compounds with better anticonvulsant activity, we synthesized 6-alkoxy-[1,2,4]triazolo[4,3-*b*]pyridazine derivatives (**2a**–**r**) using 6-phenoxy-[1,2,4]triazolo[4,3-*b*]pyridazine (compound **V**) as the leading compound to investigate the contribution of different alkoxyl groups at position 6 of the 1,2,4-triazolo[4,3-*b*]pyridazine to anticonvulsant activity. Via the conjugation effect, the electron cloud density at the triazole ring was increased because of the lone-pair electrons of the oxygen atom of 6-alkoxy. This increased the combination ability of compounds **2a**–**r** to the receptor. Their structures were characterized using IR, ^{1}H NMR, ^{13}CNMR and MS. Their anticonvulsant activity was evaluated using the MES test and is reported for the first time. Their neurotoxicity was evaluated using the rotarod test in mice. In this contribution, to explain the possible mechanism of action, compound **2r** was tested in the pentylenetetrazol (PTZ), isoniazid (ISN), 3-mercaptopropionic acid (3-MP) and thiosemicarbazide test. Under identical conditions, the anti-MES activity and neurotoxicity of the marketed agents phenobarbital and carbamazepine were evaluated as a comparison.

2. Results and discussion

2.1. Chemistry

Target compounds **2a**–**r** was prepared by two-step synthesis. As illustrated in Scheme 1, in the first step, the intermediates **1a**–**r** was obtained by two routes [18,19]. The first route started with 3, 6-dichloropyridazine, appropriate alcohol and sodium hydroxide (NaOH) in phase transfer catalyst (Tetrabutyl ammonium bromide) at room temperature to obtain derivatives **1a**–**g**. In the second route, intermediates **1h**–**r** were obtained from the condensation of various substituted phenols with starting material 3,6-dichloropyridazine and potassium carbonate (K_2CO_3) in DMF. In the second step, those intermediates were condensed with formyl hydrazine by the cyclization to furnish the title compounds. All the compounds were identified by spectral data. In general, IR spectra showed the C=C peak at 1585–1590 cm^{-1}, the C=N peak at 1620–1624 cm^{-1}, and the N–N peak at 1168–1175 cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed the triazolo-H proton as a singlet at 8.76–8.85 ppm.

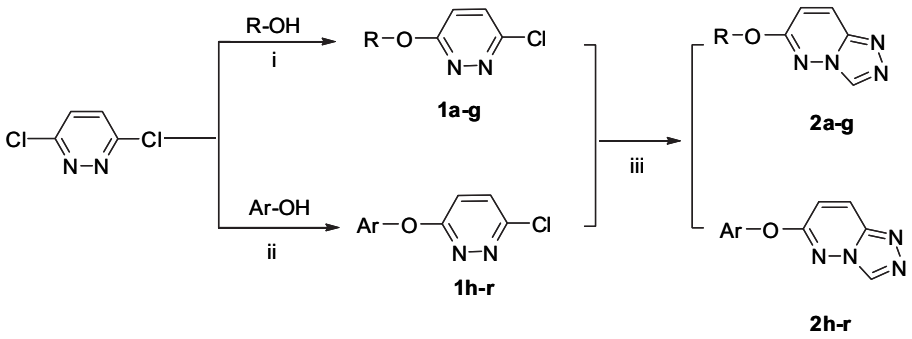
2.2. Pharmacology

The anticonvulsant activity and neurotoxicity of the synthesized compounds **2a**–**r** were evaluated by the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institute of Health (NIH) (Bethesda, MD, USA) using established procedures [20,21].

Phase-I studies involved two tests: MES and TOX. The TOX was measured by the rotorod test. Eighteen derivatives of pyridazine (**2a**–**r**) were subjected to anticonvulsant screens. Phase-I study was a qualitative assay involving three mice at common dose levels of 30, 100 and 300 mg/kg (Table 1). Protective activity in the MES test in mice after intraperitoneal (i.p.) administration was found for compounds **2a**–**r** (Table 1). All synthesized compounds exhibited anticonvulsant activity. Of these, seven compounds (including all three alkyl-substituted derivatives **2b**–**d** and four phenyl-substituted derivatives **2n**–**p**), **2r** possessed anticonvulsant activity against MES-induced seizure at 30 mg/kg. The remaining 11 compounds (**2a**, **2e**–**m** and **2o**) were active at 100 mg/kg. The rotarod toxicity test result indicated that four compounds (**2a**, **2e**–**g**) showed toxicity at 100 mg/kg.

In the phase-II pharmacology test, 18 compounds were quantitatively evaluated for anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) (Table 2) by intraperitoneal (i.p.) administration. Among these, 10 compounds (**2b**-**d**, **2h** and **2m**–**r**) were more active than the leading compound **V** in the MES test, and four compounds (**2o**–**r**) had lower neurotoxicity than the leading compound **V**. The remaining eight compounds (**2a**, **2e**–**g** and **2i**–**l**) did not show activity even at 100 mg/kg. We believe that the higher anticonvulsant activity of some compounds may be due to the triazole ring, which enhanced higher affinity of the pyridazine ring for the recognition site [14].

The length of the alkyl chain appeared to have a direct effect on the anticonvulsant activity of the 6-alkyloxyl derivatives (Table 2).



R
2a = *n*-C_4H_9 2e = *n*-C_8H_{17}
2b = *n*-C_5H_{11} 2f = *n*-C_9H_{19}
2c = *n*-C_6H_{13} 2g = *n*-$C_{10}H_{21}$
2d = *n*-C_7H_{15}

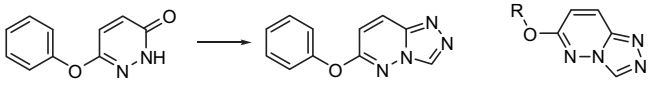
Ar
2h = 2-$C_6H_5CH_3$ 2l = 4-$C_6H_4OCH_3$ 2p = 4-C_6H_4Cl
2i = 3-$C_6H_4CH_3$ 2m = 2-C_6H_4Cl 2q = 4-C_6H_4Br
2j = 4-$C_6H_4CH_3$ 2n = 3-C_6H_4Cl 2r = 2,4-$C_6H_3Cl_2$
2k = 2-$C_6H_4OCH_3$ 2o = 4-C_6H_4F

Scheme 1. The synthesis route of compounds **2a**–**r**. Reagents:(i)NaOH, $(C_4H_9)N_4Br$, C_2H_5OH; (ii) K_2CO_3, DMF; (iii) $HCONHNH_2$, n-BuOH.

For compounds **2a**–**c**, as the length of the alkyl chain increased, ED_{50} gradually decreased, with compound **2c** (with the *n*-hexyl-substituted group) being the most active. This trend reversed, when the length of the alkyl chain increased from seven to ten carbon atoms.

The structure–activity relationship was obtained by analyzing the activities of synthesized compounds **2h–r** (Table 2). The pharmacology result revealed that when the substitution was a member of the halogen family, the activity order was 4-Cl > 4-F > 4-Br; the position of atomic Cl on the phenyl ring greatly influenced anticonvulsant activity, the activity order being 2,4-Cl_2 > 4-Cl > 3-Cl > 2-Cl. The 2,4-Cl-substituted derivative **2r** exhibited the strongest anticonvulsant activity, with an ED_{50} of 17.3 mg/kg (which was close to the value for the currently prescribed epilepsy drug phenobarbital). It also had very low neurotoxicity (TD_{50} = 380.3 mg/kg) and thus a high PI value of 22.0 was obtained. Comparing the influence of electron-donor group to anticonvulsant activity, only one compound (**2h**) exhibited anticonvulsant activity, with an ED_{50} of 54.8 mg/kg. The remaining four compounds (**2i**–**l**) did not show activity even at 100 mg/kg.

Compound **2r** was therefore chosen to be evaluated further in phase-III pharmacological tests. The toxicity profile of compound **2r** (Table 3) was determined in phase-III testing by administering the drug (i.p.) to mice at different doses ($1TD_{50}$, $2TD_{50}$, $4TD_{50}$). The toxicity induced by compound **2r** was characterized by reduced motor activity, ataxia, sedation, ptosis, muscular relaxation, loss of righting reflex, decreased respiration, and cyanosis. Mice given doses of $2TD_{50}$, and $4TD_{50}$ also experienced hypnosis, analgesia, and anesthesia. The median hypnotic dose (HD_{50}) for **2r** was found to be 746.6 mg/kg, which is almost twice the TD_{50} (380.3 mg/kg). Compound **2r** exhibited a greater margin of safety (HD_{50}/ED_{50} = 43.2) against MES-induced seizures than any of the prototype drugs whose HD_{50}/ED_{50} was <20. The toxicity of compound **2r** was clearly much lower than any of the prototype drugs.



IV V Compounds 2a–r

Fig 2. The structure of compounds IV,Vand **2a**–**r**.

As in phase-II tests involved the evaluation of ED_{50} and TD_{50} of compound **2r**, except that the candidate drug was administered orally (p.o.) rather than ip in mice. As shown in Table 2, the oral anticonvulsant time to peak effect (TPE) was 2 h, which was comparable with that for carbamazepine. A decrease in the anticonvulsant activity and neurotoxicity of compound **2r** administered p.o. compared with that by i.p. administration was clearly shown (Table 2). Nevertheless, protective index (PI) values were comparable for the two modes of drug delivery; the p.o. ED_{50}/i.p. ED_{50} ratios for compound **2r** were >2.64 and 1.43 calculated by the MES test and the rotarod test, respectively. These ratios suggested that compound **2r** was adequately absorbed in mice after oral administration. Neurotoxicity was not observed after oral administration at 545.5 mg/kg; the PI in p.o. administration was 12.0, which was better than for the prototype drugs.

Table 1
Phase-I evaluation of the anticonvulsant activity of various compounds after intraperitoneal administration in mice (i.p.).

Compound	R or Ar	Dosage (mg/kg)	MES[a]		Rotarod[b]	
			0.5 h	4 h	0.5 h	4 h
IV	–C_6H_5	100	2/3	0/3	2/3	0/3[c]
2a	*n*-C_4H_9	100	2/3	0/3	3/3	0/3
2b	*n*-C_5H_{11}	30	1/3	0/3	3/3	0/3
2c	*n*-C_6H_{13}	30	2/3	0/3	3/3	0/3
2d	*n*-C_7H_{15}	30	1/3	0/3	3/3	0/3
2e	*n*-C_8H_{17}	100	2/3	0/3	3/3	0/3
2f	*n*-C_9H_{19}	100	2/3	0/3	3/3	0/3
2g	*n*-$C_{10}H_{21}$	100	2/3	0/3	3/3	0/3
2h	2-$C_6H_4CH_3$	100	3/3	0/3	0/3	0/3
2i	3-$C_6H_4CH_3$	100	2/3	0/3	0/3	0/3
2j	4-$C_6H_4CH_3$	100	2/3	0/3	0/3	0/3
2k	2-$C_6H_4OCH_3$	100	2/3	0/3	0/3	0/3
2l	4-$C_6H_4OCH_3$	100	2/3	0/3	0/3	0/3
2m	2-C_6H_4Cl	100	3/3	0/3	0/3	0/3
2n	3-C_6H_4Cl	30	1/3	1/3	0/3	0/3
2o	4-C_6H_4F	30	1/3	0/3	0/3	0/3
2p	4-C_6H_4Cl	30	2/3	1/3	0/3	0/3
2q	4-C_6H_4Br	100	3/3	0/3	0/3	0/3
2r	2,4-$C_6H_3Cl_2$	30	3/3	1/3	0/3	0/3

[a] Maximal electroshock seizure test (number of animals protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested).
[c] compounds are metabolized/excreted at 4 h.

Table 2
Phase-II quantitative anticonvulsant data in mice (test drug administered via the intraperitoneal route).

Compound	R or Ar	MES, ED_{50}[a]	TD_{50}[b]	PI [c](TD_{50}/ED_{50})
IV	$-C_6H_5$	109.6 (93.4–128.6) [d]	273.9 (233.4–321.2)	2.5
2a	n-C_4H_9	>100	–[e]	–
2b	n-C_5H_{11}	39.4 (33.6–46.3)	70.4 (58.5–84.7)	1.79
2c	n-C_6H_{13}	32.9 (28.0–38.6)	60.8 (49.0–71.3)	1.8
2d	n-C_7H_{15}	42.4 (33.9–53.0)	73.0 (62.2–85.6)	1.7
2e	n-C_8H_{17}	>100	–	–
2f	n-C_9H_{19}	>100	–	–
2g	n-$C_{10}H_{21}$	>100	–	–
2h	2-$C_6H_4CH_3$	54.8 (46.7–64.3)	220.0 (182.9–264.6)	4.01
2i	3-$C_6H_4CH_3$	>100	–	–
2j	4-$C_6H_4CH_3$	>100	–	–
2k	2-$C_6H_4OCH_3$	>100	–	–
2l	4-$C_6H_4OCH_3$	>100	–	–
2m	2-C_6H_4Cl	47.3 (40.3–55.5)	190.1 (162.0–223.0)	4.0
2n	3-C_6H_4Cl	39.4 (33.6–46.2)	228.2 (194.5–267.7)	5.8
2o	4-C_6H_4F	38.0 (31.6–45.7)	317.2 (270.7–372.2)	8.3
2p	4-C_6H_4Cl	36.7 (31.5–42.7)	394.0 (335.8–462.3)	10.7
2q	4-C_6H_4Br	49.1 (40.9–58.9)	328.6 (280.1–385.5)	6.7
2r(**ip**)	2,4-$C_6H_3Cl_2$	17.3 (12.6–23.8)	380.3 (324.1–446.2)	22.0
2r(**po**)	2,4-$C_6H_3Cl_2$	45.6 (38.9–53.5)	545.5 (454.7–654.5)	12.0
Carbamazepine (i.p.)		8.8 (5.5–14.1)	71.6 (45.9–135)	8.1
Carbamazepine (p.o.)		15.4 (12.4–17.3)	217 (131.5–270.1)	14.1
Phenobarbital		21.8 (21.8–25.5)	69 (62.8–72.9)	3.2

[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.
[e] Not tested.

To further investigate the effects of anticonvulsant activity in several models, compound **2r** was tested against convulsions induced by chemical substances, i.e., PTZ, ISN, 3-MP, and thiosemicarbazide. Compound **2r** was administered (i.p.) into mice at 50 mg/kg (which was similar to their $3ED_{50}$, but far below their TD_{50}). The reference drug carbamazepine was administered (i.p.) at 50 mg/kg.

Compound **2r** and the reference drug carbamazepine did not inhibit the clonic seizures induced by sc-PTZ, but they inhibited tonic seizures and reduced lethality to a degree (Table 4). Carbamazepine inhibited clonic seizures, tonic seizures and death induced by ISN at values of 60%, 100% and 100%, respectively; compound **2r** inhibited clonic seizures, tonic seizures and death induced by ISN at values of 10%, 100% and 100%, respectively (Table 4). PTZ and ISN have been reported to produce seizures by inhibiting aminobutyric acid (GABA) neurotransmission [22,23]. GABA is the main inhibitory neurotransmitter in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures [24], whereas enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compounds **2r** may have inhibited or attenuated PTZ-induced and ISN-induced seizures in mice by enhancing GABAergic neurotransmission.

In the 3-MP-induced seizure model, carbamazepine inhibited clonic seizures, tonic seizures and death at the values of 20%, 100%, and 90%, respectively. Compound **2r** showed similar inhibition of clonic seizures, tonic seizures and death (20%, 100% and 60%, respectively) (Table 4). In thiosemicarbazide-induced convulsion, the effect was similar to that of the 3-MP-induced seizure model when compared with the reference drug; compound **2r** showed less inhibition of clonic seizures, stronger inhibition of tonic seizures, and comparable inhibition of death (10%, 100% and 60%, respectively) (Table 4). 3-MP and thiosemicarbazide were shown to be competitive inhibitors of an enzyme involved in GABA synthesis, glutamate decarboxylase (GAD); they inhibit GABA synthesis, resulting in a decreased level of GABA in the brain [25]. Compound **2r** showed moderate antagonism to 3-MP-induced seizures and thiosemicarbazide-induced seizures. This suggests that Compound **2r** may activate GAD or inhibit aminotransferase (GABA-T) in the brain.

In conclusion, the results of the present study demonstrated that 6-alkoxy-[1,2,4]triazolo[4,3-*b*]pyridazine derivatives have potent anticonvulsant activity. In particular, compound **2r** showed better anticonvulsant activity, but also much lower toxicity, than a benchmark marketed drug. Compound **2r** demonstrated antagonistic activity against seizures induced by PTZ, ISN, 3-MP and thiosemicarbazide. These experiments suggested that compound **2r** may activate GAD or inhibit GABA-T, thereby enhancing GABAergic neurotransmission.

Table 3
Phase -III quantitative toxicity profile of **2r** and prototype anticonvulsant drugs.

Compound	HD_{50}[a,b]	HD_{50}/ED_{50}
2r	746.6 (622.3–895.8)	43.2
Carbamazepine	172.0 (134.0–198.0)	19.5

[a] Median hypnoitic dose (HD_{50}) in milligrams per kilogram; determined by loss of righting reflex.
[b] 95% confidence intervals in parentheses.

3. Experimental section

3.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Switzerland), ^1H NMR and ^{13}C NMR spectra were measured on a AV-300 (Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). Elemental analysis (CHN) were performed on a Perkin Elmer 204Q CHN or a Heraeus CHN Rapid Analyzer" The major chemicals were

Table 4
Effect of compound **2r** on pentylenetetrazol, iaoniazid, 3-mercaptopropionic, and thiosemicarbazide-induced convulsion in mice.

chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
pentylenetetrazol	DMSO: CMC-Na	–	0.5	100	100	40
	Carbamazepine	50	0.5	100	0	0
	2r	50	0.5	100	10	0
isoniazid	DMSO: CMC-Na	–	0.5	100	100	75
	Carbamazepine	50	0.5	40	0	0
	2r	50	0.5	90	0	0
3-mercaptopropionic acid	DMSO: CMC-Na	–	0.5	100	100	100
	Carbamazepine	50	0.5	80	0	10
	2r	50	0.5	80	0	40
thiosemicarbazide	DMSO: CMC-Na	–	2.5	100	100	100
	Carbamazepine	50	2.5	90	0	0
	2r	50	2.5	90	0	40

purchased from Alderich Chemical Corporation. All other chemicals were the analytical grade.

3.2. Preparation of 3-chloro-6-(hexyloxy)pyridazine (*1c*)

3,6-Dichloropyridazine (1.50 g, 10 mmol), hexanol (1.15 g, 11 mmol), tetrabutylammonium bromide (1.0 g, 3 mmol), NaOH (0.6 g, 15 mmol) and 15 mL CH_2Cl_2 were placed in a 50 mL round-bottomed flask [18]. The mixture was stirred for 6–15 h in a nitrogen atmosphere. The organic layer was separated, the water layer was extracted twice with dichloromethane (30 mL × 2), then combined the organic layer and dried over anhydrous $MgSO_4$. The crude product was purified by recrystallization with petroleum, and a pure product was obtained in a yield of 78%. mp: 60.5–62 °C (lit.59–61 °C). ^{1}H NMR ($CDCl_3$) δ: 7.35 (1H, d, J = 9.18 Hz, pyridazine-H), 6.94 (1H, d, J = 9.18 Hz, pyridazine-H), 4.47 (2H, t, $-OCH_2$), 1.32–1.85 (8H, m, $(-CH_2)_4$), 0.90 (3H, t, $-CH_3$). MS (M + 1): 215.

3.3. Preparation of 3-chloro-6-(p-tolyloxy)pyridazine (*1k*)

3, 6-Dichloropyridazine (1.50 g, 10 mmol), 4-methyl phenol (1.11 g, 10 mmol), K_2CO_3 (1.40 g, 10.1 mmol) and DMF 15 mL was placed in a 50 mL round-bottomed flask [19]. The mixture was refluxed for 7 h in a nitrogen atmosphere. The reaction mass was cooled to room temperature, then added 30 mL of water and kept overnight. The separated solid was filtered. The crude product was purified by recrystallization in acetone and water, and a pure product was obtained in a yield of 90%. mp: 112–114.5 °C. ^{1}H NMR ($CDCl_3$) δ: 7.47 (1H, d, J = 9.18 Hz, pyridazine-H), 7.13 (1H, d, J = 9.18 Hz, pyridazine-H), 7.06–7.48 (4H, m, $-C_6H_4$), 2.37 (3H, s, $-CH_3$). MS (M + 1): 221.

3.4. General procedure for the synthesis of 6-alkoxy-[1,2,4]triazolo [4,3-b]pyridazine (*2a–r*)

6-Alkoxy-3-chloro-pyridazine (0.04 mol) and formoc hydrazine (2.4 g, 0.04 mol) were dissolved in *n*-butyl alcohol in a round-bottomed flask, and the mixture was refluxed for 10–20 h in a nitrogen atmosphere. Solvents were removed under reduced pressure, and the residue was extracted twice with dichloromethane 30 mL. The dichloromethane layer was washed three times with water (30 mL × 3) and dried over anhydrous $MgSO_4$. After removing the solvents, products was purified by silica gel column chromatography (dichloromethane–methanol = 20:1).

3.4.1. 6-Butoxy-[1,2,4]triazolo[4,3-b]pyridazine (*2a*)

Yield: 69%; m.p.: 63–65 °C. ^{1}H NMR ($CDCl_3$): δ 8.83 (1H, s, triazolo-H), 7.94 (1H, d, J = 9.8 Hz, pyridazine-H), 6.77 (1H, d, J = 9.8 Hz, pyridazine-H), 4.31(2H, t, J = 6.6 Hz, $-OCH_2$), 1.42–1.82 (4H, m, $-(CH_2)_2$), 0.83 (3H, t, J = 7.0 Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ14.09, 19.73, 31.91, 68.04, 116.96, 125.83, 138.54, 142.92, 160.86. IR (KBr) cm^{-1}: 1589 (C═C), 1623 (C═N), 1253, 1027 (C–O–C), 1171 (N–N). MS m/z: 193(M + 1). Anal. Calcd for $C_9H_{12}N_4O$: C, 56.24; H, 6.29; N, 29.15. Found: C, 56.11; H, 6.22; N, 29.07.

3.4.2. 6-Pentyloxy-[1,2,4]triazolo[4,3-b]pyridazine (*2b*)

Yield: 63%; oil. ^{1}H NMR ($CDCl_3$): δ 8.83 (1H, s, triazolo-H), 7.93 (1H, d, J = 9.8 Hz, pyridazine-H), 6.77 (1H, d, J = 9.8 Hz, pyridazine-H), 4.30 (2H, t, J = 6.6 Hz, $-OCH_2$), 1.39–1.83 (6H, m, $-(CH_2)_3$), 0.92 (3H, t, J = 7.1 Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 13.93, 22.46, 28.13, 29.66, 68.33, 116.99, 125.82, 138.54, 142.91, 160.75. IR (KBr) cm^{-1}: 1586 (C═C), 1624 (C═N), 1256, 1025 (C–O–C), 1173 (N–N). MS m/z: 207(M + 1). Anal. Calcd for $C_{10}H_{14}N_4O$: C, 58.24; H, 6.84; N, 27.17. Found: C, 58.15; H, 6.76; N, 27.02.

3.4.3. 6-Hexyloxy-[1,2,4]triazolo[4,3-b]pyridazine (*2c*)

Yield: 62.5%; m.p.: 77–78 °C. ^{1}H NMR ($CDCl_3$): δ 8.85 (1H, s, triazolo-H), 7.95 (1H, d, J = 9.8 Hz, pyridazine-H), 6.78 (1H, d, J = 9.8 Hz, pyridazine-H), 4.32 (2H, t, J = 6.6 Hz, $-OCH_2$), 1.35–1.87 (8H, m, $-(CH_2)_4$), 0.91 (3H, t, J = 6.8 Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 13.98, 22.53, 25.54, 28.41, 29.68, 31.42, 68.34, 116.97, 125.83, 128.39, 132.66, 138.54, 142.92, 160.85. IR (KBr) cm^{-1}: 1588 (C═C), 1621 (C═N), 1252, 1024 (C–O–C), 1173 (N–N). MS m/z: 221 (M + 1). Anal. Calcd for $C_{11}H_{16}N_4O$: C, 59.98; H, 7.32; N, 25.44. Found: C, 59.87; H, 7.26; N, 25.34.

3.4.4. 6-Heptyloxy-[1,2,4]triazolo[4,3-b]pyridazine (*2d*)

Yield: 62%; m.p.: 78–80 °C. ^{1}H NMR ($CDCl_3$): δ 8.82 (1H, s, triazolo-H), 7.93 (1H, d, J = 9.8 Hz, pyridazine-H), 6.76 (1H, d, J = 9.8 Hz, pyridazine-H), 4.29 (2H, t, J = 6.6 Hz, $-OCH_2$), 1.29–1.82 (10H, m, $-(CH_2)_5$), 0.87 (3H, t, J = 6.5 Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ14.03, 22.55, 25.82, 28.89, 29.67, 31.68, 68.34, 116.98, 125.53, 138.53, 142.67, 160.87. IR (KBr) cm^{-1}: 1586 (C═C), 1621 (C═N), 1255, 1023 (C–O–C), 1174 (N–N). MS m/z: 235 (M + 1). Anal. Calcd for $C_{12}H_{18}N_4O$: C, 61.52; H, 7.74; N, 23.91. Found: C, 61.45; H, 7.66; N, 23.84.

3.4.5. 6-Octyloxy-[1,2,4]triazolo[4,3-b]pyridazine (*2e*)

Yield: 64%; oil. ^{1}H NMR ($CDCl_3$): δ 8.83 (1H, s, triazolo-H), 7.93 (1H, d, J = 9.8 Hz, pyridazine-H), 6.77 (1H, d, J = 9.8 Hz, pyridazine-H), 4.30 (2H, t, J = 6.6 Hz, $-OCH_2$), 1.23–1.82 (12H, m, $-(CH_2)_6$), 0.87 (3H, t, J = 6.8 Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 14.06, 22.61, 25.86, 29.19, 29.67, 31.75, 68.34, 109.47, 116.98, 125.82, 138.53, 160.85. IR (KBr) cm^{-1}: 1588 (C═C), 1622 (C═N), 1251, 1025 (C–O–C), 1169 (N–N). MS m/z: 249(M + 1). Anal. Calcd for $C_{13}H_{20}N_4O$: C, 62.88; H, 8.12; N, 22.56. Found: C, 62.78; H, 8.06; N, 22.45.

*3.4.6. 6-Nonyloxy-[1,2,4]triazolo[4,3-b]pyridazine (**2f**)*

Yield: 58%; oil. ^{1}H NMR ($CDCl_3$): δ 8.82 (1H, s, triazolo-H), 7.93 (1H, d, $J = 9.8$ Hz, pyridazine-H), 6.76 (1H, d, $J = 9.8$ Hz, pyridazine-H), 4.29 (2H, t, $J = 6.5$ Hz, $-OCH_2$), 1.25–1.82 (14H, m, $-(CH_2)_7$), 0.85 (3H, t, $J = 6.7$ Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 14.05, 22.53, 26.06, 29.57, 29.81, 30.19, 30.44, 30.78, 68.61, 117.29, 125.65, 138.43, 142.82, 160.89 . IR (KBr) cm^{-1}: 1587 (C=C), 1624 (C=N), 1251, 1024 (C–O–C), 1170 (N–N). MS *m/z*: 263(M + 1). Anal. Calcd for $C_{14}H_{22}N_4O$: C, 64.09; H, 8.45; N, 21.36. Found: C, 63.89; H, 8.32; N, 21.34.

*3.4.7. 6-Decyloxy-[1,2,4]triazolo[4,3-b]pyridazine (**2g**)*

Yield: 60%; m.p.: 79–81 °C. ^{1}H NMR ($CDCl_3$): δ 8.85 (1H, s, triazolo-H), 7.95 (1H, d, $J = 9.8$ Hz, pyridazine-H), 6.78 (1H, d, $J = 9.8$ Hz, pyridazine-H), 4.32 (2H, t, $J = 6.5$ Hz, $-OCH_2$), 1.28–1.85 (16H, m, $-(CH_2)_8$), 0.88 (3H, t, $J = 6.7$ Hz, $-CH_3$). ^{13}C NMR ($CDCl_3$): δ 14.09, 22.65, 25.87, 28.45, 29.24, 29.50, 29.68, 30.15, 31.86, 68.35, 116.98, 125.82, 138.53, 142.85, 160.78. IR (KBr) cm^{-1}: 1587 (C=C), 1622 (C=N), 1251, 1026 (C–O–C), 1168 (N–N). MS *m/z*: 277(M + 1). Anal. Calcd for $C_{15}H_{24}N_4O$: C, 65.19; H, 8.75; N, 20.27. Found: C, 65.05; H, 8.76; N, 20.14.

*3.4.8. 6-(2-Tolyloxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2h**)*

Yield: 68.3%; m.p.: 108–110 °C. ^{1}H NMR ($CDCl_3$): δ 8.76 (1H, s, triazolo-H), 8.11 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.07 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.11–7.33 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 16.18, 101.21, 115.99, 121.48, 125.61, 126.56, 127.07, 127.36, 131.64, 138.71, 142.92, 167.77. IR (KBr) cm^{-1}: 1590 (C=C), 1620 (C=N), 1251, 1024 (C–O–C), 1174 (N–N). MS *m/z*: 227 (M + 1). Anal. Calcd for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76. Found: C, 63.65; H, 4.37; N, 24.67.

*3.4.9. 6-(3-Tolyloxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2i**)*

Yield: 71%; m.p.: 131–133 °C. ^{1}H NMR ($CDCl_3$): δ 8.81 (1H, s, triazolo-H), 8.11 (1H, d, $J = 9.7$ Hz, pyridazine-H), 7.03 (1H, d, $J = 9.7$ Hz, pyridazine-H), 7.14–7.39 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 21.38, 116.38, 118.11, 121.71, 126.91, 127.12, 128.48, 129.60, 138.75, 142.89, 153.75, 160.45. IR (KBr) cm^{-1}: 1585 (C=C), 1621 (C=N), 1254, 1028 (C–O–C), 1169 (N–N). MS *m/z*: 227 (M + 1). Anal. Calcd for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76. Found: C, 63.60; H, 4.31; N, 24.59.

*3.4.10. 6-(4-Tolyloxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2j**)*

Yield: 73.6%; m.p.: 127–129 °C. ^{1}H NMR ($CDCl_3$): δ 8.79 (1H, s, triazolo-H), 8.09 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.02 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.08–7.24 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$):δ 20.95, 116.40, 120.90, 122.89, 126.25, 126.88, 128.17, 130.40, 138.75, 142.56, 149.86, 161.13. IR (KBr) cm^{-1}: 1588 (C=C), 1623 (C=N), 1254, 1026 (C–O–C), 1172 (N–N). MS *m/z*: 227(M + 1). Anal. Calcd for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76. Found: C, 63.63; H, 4.39; N, 24.69.

*3.4.11. 6-(2-Methoxyphenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2k**)*

Yield: 69.5%; oil. H NMR ($CDCl_3$): δ 8.77 (1H, s, triazolo-H), 8.09 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.07 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.04–7.32 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 55.82, 112.77, 116.07, 121.04, 122.59, 126.62, 127.41, 138.65, 142.79, 145.13, 160.70, 165.54. IR (KBr) cm^{-1}: 1587(C=C), 1621 (C=N), 1252, 1026 (C–O–C), 1170 (N–N). MS *m/z*: 243 (M + 1). Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.45; H, 4.04; N, 23.01.

*3.4.12. 6-(4-Methoxyphenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2l**)*

Yield: 72%; m.p.: 186–188 °C. ^{1}H NMR ($CDCl_3$): δ 8.79 (1H, s, triazolo-H), 8.10 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.02 (1H, d, $J = 9.8$ Hz, pyridazine-H), 6.96–7.16 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 55.62, 114.83, 116.34, 122.11, 126.86, 138.74, 142.89, 147.52, 154.16, 160.85. IR (KBr) cm^{-1}: 1587 (C=C), 1620 (C=N), 1251, 1028 (C–O–C), 1175 (N–N). MS *m/z*: 243(M + 1). Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.40; H, 3.98; N, 23.04.

*3.4.13. 6-(2-Chlorophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2m**)*

Yield: 60.5%; m.p.: 150–153 °C. ^{1}H NMR ($CDCl_3$): δ 8.77 (1H, s, triazolo-H), 8.15 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.08 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.13–7.55 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 115.73, 123.50, 127.27, 128.19, 129.56, 130.85, 138.66, 142.95, 148.17, 160.19, 165.03. IR (KBr) cm^{-1}: 1586 (C=C), 1621 (C=N), 1252, 1029 (C–O–C), 1174 (N–N). MS *m/z*: 247(M + 1). Anal. Calcd for $C_{11}H_7ClN_4O$: C, 53.56; H, 2.86; N, 22.71. Found: C, 53.43; H, 2.78; N, 22.67.

*3.4.14. 6-(3-Chlorophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2n**)*

Yield: 58.4%; m.p.: 142–144 °C. ^{1}H NMR ($CDCl_3$): δ 8.82 (1H, s, triazolo-H), 8.14 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.04 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.13–7.45 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 116.05, 119.62, 121.98, 122.90, 126.24, 126.66, 127.30, 130.65, 138.72, 142.83, 160.57. IR (KBr) cm^{-1}: 1588 (C=C), 1621 (C=N), 1250, 1026 (C–O–C), 1169 (N–N). MS *m/z*: 247 (M + 1). Anal. Calcd for $C_{11}H_7ClN_4O$: C, 53.56; H, 2.86; N, 22.71. Found: C, 53.41; H, 2.70; N, 22.62.

*3.4.15. 6-(4-Fluorophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2o**)*

Yield: 58%; m.p.: 201–203 °C. ^{1}H NMR ($CDCl_3$): δ 8.79 (1H, s, triazolo-H), 8.13 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.04 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.13–7.20 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 116.14, 116.46, 116.78, 122.75, 122.86, 127.14, 138.70, 142.86, 162.08. IR (KBr) cm^{-1}: 1589 (C=C), 1620 (C=N), 1252, 1026 (C–O–C), 1170 (N–N). MS *m/z*: 231 (M + 1). Anal. Calcd for $C_{11}H_7FN_4O$: C, 57.39; H, 3.07; N, 24.34. Found: C, 57.25; H, 2.91; N, 24.17.

*3.4.16. 6-(4-Chlorophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2p**)*

Yield: 61%; m.p.: 189–191 °C. ^{1}H NMR ($CDCl_3$): δ 8.80 (1H, s, triazolo-H), 8.13 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.04 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.17–7.46 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ 116.13, 122.69, 126.24, 127.23, 130.00, 138.68, 142.57, 160.73. 165.14. IR (KBr) cm^{-1}: 1587 (C=C), 1622 (C=N), 1254, 1029 (C–O–C), 1173 (N–N). MS *m/z*: 247(M + 1). Anal. Calcd for $C_{11}H_7ClN_4O$: C, 53.56; H, 2.86; N, 22.71. Found: C, 53.49; H, 2.76; N, 22.69.

*3.4.17. 6-(4-bromophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2q**)*

Yield: 72.1%; m.p.: 190–193 °C. ^{1}H NMR ($CDCl_3$): δ 8.79 (1H, s, triazolo-H), 8.13 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.04 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.11–7.60 (4H, m, $-C_6H_4$). ^{13}C NMR ($CDCl_3$): δ116.13, 119.44, 122.90, 127.24, 132.25, 132.99, 138.68, 142.84, 160.66. IR (KBr) cm^{-1}: 1588 (C=C), 1620 (C=N), 1251, 1028 (C–O–C), 1173 (N–N). MS *m/z*: 291(M + 1). Anal. Calcd for $C_{11}H_7BrN_4O$: C, 45.39; H, 2.42; N, 19.25. Found: C, 45.21; H, 2.32; N, 19.04.

*3.4.18. 6-(2,4-Dichlorophenoxy)-[1,2,4]triazolo[4,3-b]pyridazine (**2r**)*

Yield: 59%; m.p.: 192–194 °C. ^{1}H NMR ($CDCl_3$): δ 8.78 (1H, s, triazolo-H), 8.16 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.11 (1H, d, $J = 9.8$ Hz, pyridazine-H), 7.22–7.54 (3H, m, $-C_6H_3$). ^{13}C NMR ($CDCl_3$): δ 115.50, 124.39, 127.51, 128.42, 130.65, 131.12, 131.54, 132.58, 138.61, 143.15, 160.59. IR (KBr) cm^{-1}: 1587 (C=C), 1624 (C=N), 1251, 1028 (C–O–C), 1168 (N–N). MS *m/z*: 281 (M + 1). Anal. Calcd for $C_{11}H_6Cl_2N_4O$: C, 47.00; H, 2.15; N, 19.93. Found: C, 47.12; H, 2.19; N, 20.14.

3.5. Pharmacology

The MES test and the rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA) [20,21]. All compounds were tested for anticonvulsant activities with KunMing mice in the 18–25 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds dissolved in polyethylene glycol-400.

In phase-I screening (Table 1), each compound was administered at the dose levels of 30, 100, and 300 mg/kg for evaluating the anticonvulsant activity, and its neurotoxicity was measured at 30 min and 4 h intervals after administration. Anticonvulsant efficacy was measured in the MES 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. The 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. Anticonvulsant drug-induced neurologic deficit was detected in mice by using the rotorod ataxia test.

The pharmacologic parameters estimated in phase-I screening were quantified for compounds **1a–r** 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 tested compound until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plot of this 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.

In phase-III testing, the general behavior of mice was assessed at regular time intervals up 24 h following i.p. 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 (Table 3) using the procedure described previously for evaluation of the ED_{50} and TD_{50} values.

In chemically induced seizures (Table 4), mice were given doses of convulsant drugs that could induce seizures at least 97% of animals. The doses used were: PTZ, 85 mg/kg; ISN, 250 mg/kg; 3-MP, 40 mg/kg and thiosemicarbazide, 50 mg/kg. The test compounds and standard AEDs were administered i.p. in a volume of 50 mg/kg to groups of 10 mice 30 min before either i.p. administration of INS and thiosemicarbazide or s.c. injection of PTZ, 3-MP. The mice were placed in individual cages and observed for 30 min, the number of clonic seizures, tonic seizures and the lethality were recorded [26–29]. Thiosemicarbazide also was administered i.p., and the test compounds were administered i.p. to mice 30 min before thiosemicarbazide. The mice were placed in individual cages and observed for 2 h 30 min, the number of clonic seizures, tonic seizures and the lethality were noted [30].

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