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Original article

Synthesis and anticonvulsant activity of some substituted 1,2,4-thiadiazoles

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Abstract

A series of new substituted 1,2,4-thiadiazoles were synthesized by appropriate route and screened for anticonvulsant, neurotoxic and sedative-hypnotic activity. The structures of the synthesized compounds were confirmed by IR spectroscopy, ^{13}C NMR and elemental (nitrogen and sulphur) analysis. After i.p. injection of the compounds to mice or rate at doses of 30, 100, and 300 mg/kg, body weights were examined in the maximal electroshock-induced seizures (MES) and subcutaneous pentylenetetrazole (scPTZ)-induced seizure models after 0.5 and 4 h. Rotorod method and phenobarbitone-induced hypnosis potentiation study were employed to examine neurotoxicity and sedative-hypnotic activity, respectively. All the compounds except **4g** showed protection against MES screen after 0.5 h. Compounds **3a–c**, **4a–c** were active at 100 mg/kg dose i.p., whereas remaining compounds showed activity at 300 mg/kg. All 14 compounds except 3 g showed neurotoxicity at 100 and 300 mg/kg after 0.5 h. Compounds **3b** and **4b** showed NT after 4 h. Two compounds **3b** and **4g** showed significant ($p < 0.05$) percentage increase in sleeping time i.e. 67% and 59%, respectively. It may be concluded that the synthesized compounds were potent against MES-induced seizures than ScPTZ induced and showed low potency as sedative-hypnotic agent which is advantageous.

Keywords: 1,2,4-Thiadiazole; Anticonvulsant activity; Maximal electroshock-induced seizures; Subcutaneous pentylenetetrazole-induced seizure; Neurotoxicity

1. Introduction

Epilepsy is one of the most common diseases of the brain affecting at least 50 million persons worldwide. There is currently a need for improved agents for the treatment of seizures, since currently available drugs are effective only in 60–80% of epileptic patients [1]. Polytherapy with antiepileptic drugs (AEDs) is necessary in clinical practice because of the limited efficacy of monotherapy [2,3]. In recent years many new chemical entities (NCEs) have been designed that were structurally dissimilar from many common anticonvulsant containing dicarboximide function (CONRCO), which contributes to toxic side effects. During the past decade several new drugs were approved (Rufinamide, Retigabine, Pregabaline, Remacemide). However, none of the available antiepileptic drug is ideal as these can be associated with chronic and toxic side effects [4]. Thus the search for the new anticonvulsant drugs continue to be an active area of investigation in medicinal chemistry. The MES test is a proven method of generalized tonic–clonic seizures and identifies clinical candidates that prevent seizure spread [5,6]. At present scientists are working at various nucleus to explore suitable pharmacophore responsible for anticonvulsant potential. As a part of our ongoing research program [7,8] to find novel anticonvulsants, herein we describe synthesis, anticonvulsant, neurotoxicity and sedative-hypnotic activity of some new 1,2,4-thiadiazoles.

2. Chemistry

The synthesis of 1,2,4-thiadiazoline was accomplished as showed in Fig. 1. Aryl thiourea (1) was prepared by reacting

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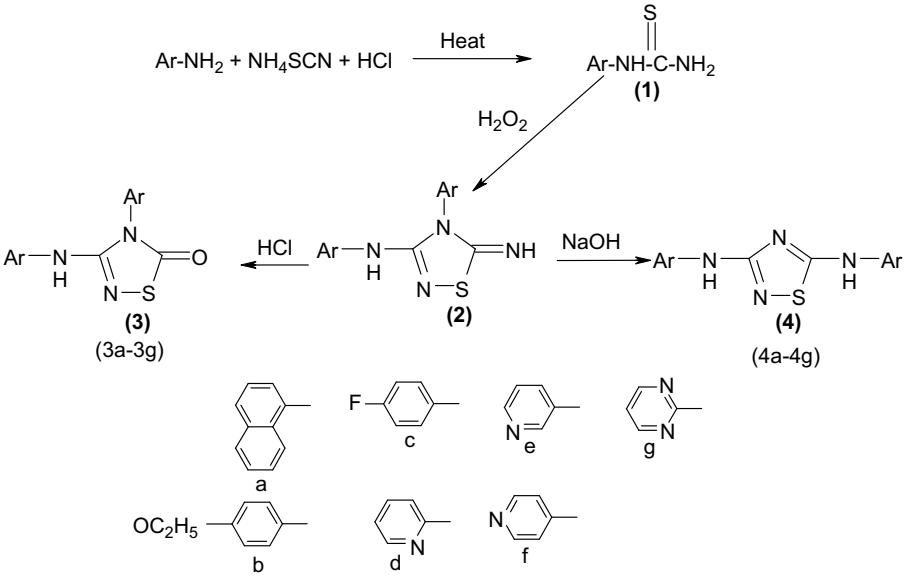


Fig. 1. Scheme for the synthesis of title compounds.

aromatic/hetero aromatic amines with ammonium thiocyanate in the presence of concentrated hydrochloric acid. Compounds **1** were oxidatively cyclized into 3-aryl amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline (2). Compounds **2** on refluxing with concentrated hydrochloric acid gave 3-aryl amino-4-aryl-5-oxo-Δ^2-1,2,4-thiadiazoline (**3a**–**g**). Compounds **2** isomerised to 3,5-diarylamino-1,2,4-thiadiazole in the presence of sodium hydroxide solution (**4a**–**g**).

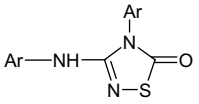
3. Pharmacology

Initial anticonvulsant evaluation was undertaken by the anticonvulsant drug development (ADD) program protocol. Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague–Dawley, 100–150 g) were used as experimental animals. Four animals constitute one group. The compounds were suspended in 0.5% methyl cellulose–water mixture or in polyethylene glycol (PEG).

4. Results and discussions

All synthesized compounds except **4g** showed protection against MES seizures after 0.5 h of drug administration. Compounds **3a**–**c** and **4a**–**c** were active at 100 mg/kg, whereas remaining compounds showed activity at 300 mg/kg. These synthesized compounds exhibited their ability to diminish the magnitude of tonic–clonic seizures. Only six compounds namely **3a**,**b** and **4a**–**c** exhibited prolonged duration of action at 300 mg/kg after 4 h. Mentioned five compounds showed rapid and long duration of action. Only one out 14 compounds (**3b**) showed protection against ScPTZ. All other compounds were inactive in ScPTZ screen which indicates their ineffectiveness against absence seizures. None of the compounds were active after 4 h in ScPTZ screen. NT screening revealed that all 14 compounds except **3g** showed NT at 100–300 mg/kg after 0.5 h. NT level of reference drugs phenytoin and chlorpromazine is 100 mg/kg at 0.5 h. Only **3b** and **4b** showed pronged NT level (after 4 h). Some of the synthesized compounds were challenged in phenobarbitone-induced hypnosis potentiation test to see their effect on general behavior. Compounds **3b** and **4g** showed significant percentage increase in sleep level i.e. 67 and 59.5%, respectively. Percentage increase in sleep by remaining compounds was insignificant. Above results and discussion indicates that the new compounds are more selective towards MES screen than ScPTZ screen. Present research will guide our future development of potent and selective anticonvulsant drugs.

Table 1
Physical constants of the synthesized 3-arylamino-4-aryl-5-oxo-Δ^2-1,2,4-thiadiazolines



Code	M.p. (°C)	Yield (%)	Molecular formula	Molecular weight	Element (found %/calculated %)[a]
3a	170	71	$C_{22}H_{15}N_3SO$	369.45	N (11.40/11.39) S (8.67/8.66)
3b	200	68	$C_{18}H_{19}N_3SO_3$	357.43	N (11.73/11.75) S (8.91/8.95)
3c	210	74	$C_{14}H_9N_3SOF_2$	305.11	N (13.83/13.75) S (10.53/10.48)
3d	190	60	$C_{12}H_9N_5SO$	271.3	N (25.85/25.80) S (11.80/11.79)
3e	184	69	$C_{12}H_9N_5SO$	271.3	N (25.75/25.80) S (11.75/11.79)
3f	180	79	$C_{12}H_9N_5SO$	271.3	N (25.85/25.80) S (11.75/11.79)
3g	174	43	$C_{10}H_7N_7SO$	273.28	N (35.86/35.70) S (11.70/11.80)

[a] Elemental analyses for N and S were within 0.4% of the theoretical values.

Table 2
IR and ^{13}C NMR spectroscopic data of the title compounds

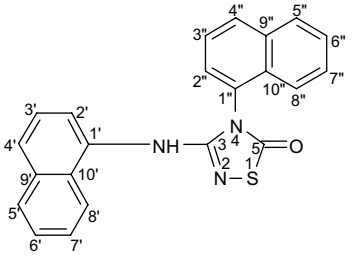
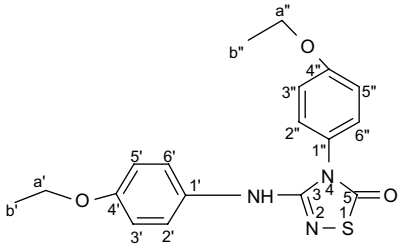
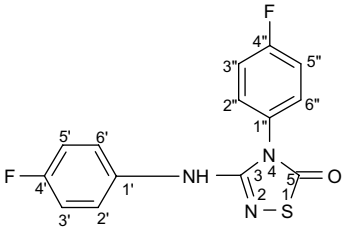
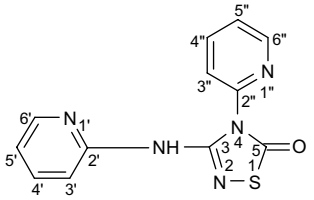
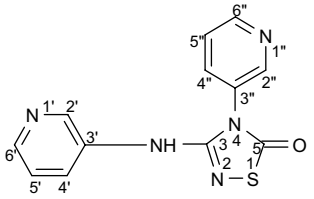
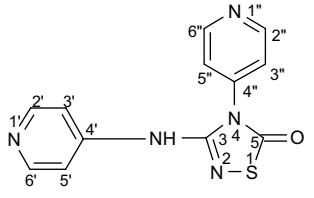
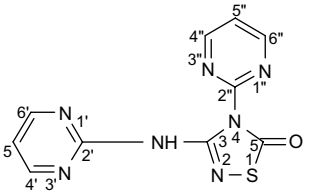
Code	Structure	IR	^{13}C NMR
3a		3366 (NH), 1722 (C=O), 1261 (CN), 1630 (C=C)	163 (C-3), 165 (C-5), 140.8 (C-1′), 109.4 (C-2′, C-2″), 126 (C-3′, C-6′, C-3″, C-6″), 119 (C-4′, C-4″), 128.6 (C-5′, C-5″), 125 (C-7′, C-7′), 121 (C-8′, C-8″), 134.3 (C-9′, C-9″), 124.7 (C-10′, C-10″)
3b		3432 (NH), 1713 (C=O), 1310 (CN), 683 (CS), 1624 (C=C)	163 (C-3), 165 (C-50), 136 (C-1′), 132.9 (C-1″), 116.9 (C-2′, C-6′, C-2″, C-6″), 115.2 (C-3′, C-5′, C-3″, C-5″), 147.5 (C-4′, C-4″), 14.8 (Ca′, Ca″), 64 (C-b′, C-b″)
3c		3424 (NH), 1711 (C=O), 1318 (CN), 733 (CS), 1643 (C=C), 1167 (CF)	163 (C-3), 165 (C-5), 140 (C-1), 117.9 (C-2′, C-6′), 116.3 (C-3′, C-5′), 152.9 (C-4′), 128.4 (C-1″), 123.2 (C-2″, C-6″), 115.7 (C-3″, C-5″), 158.5 (C-4″)
3d		3412 (NH), 1719 (C=O), 1421 (C=N), 763 (CS), 1310 (CN), 1623 (C=C)	163 (C-3), 165 (C-5), 158.6 (C-2′), 147.7 (C-2″), 109.9 (C-3′, C-3″), 138.3 (C-4′C-4″), 113.3 (C-5,C-5″), 148.2 (C-6′,C-6″)
3e		3428(NH), 1709 (C=O), 1421 (C=N), 1316 (CN),721 (CS),1624 (C=C)	163 (C-3), 165 (C-5), 137.6 (C-2′, C-2″), 134.4 (C-3′), 145.1 (C-3″), 122.8 (C-4′,C-4″), 124.7 (C-5′,C-5″), 138.9 (C-6′,C-6″)
3f		3446 (NH), 1732 (C=O), 1416 (C=N), 1302 (CN), 732 (CS), 1653 (C=C)	163 (C-3), 165 (C-5), 150.3 (C-2′, C-6′, C-2″, C-6″), 109.1 (C-3′, C-5′, C-3″, C-5″), 155.3 (C-4′, C-4″)

Table 2 (*continued*)

Code	Structure	IR	^{13}C NMR
3g		3426 (NH), 1744 (C=O), 1402 (C=N), 1284 (CN), 693 (CS), 1614 (C=C)	163 (C-3), 165 (C-5), 158 (C-2′), 169.3 (C-2″), 157.9 (C-4′C-4″), 110.3 (C-5′,C-5″), 157.9 (C-6′,C-6″)

5. Experimental protocol

5.1. Chemistry

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using silica gel glass plates and solvent system of benzene:ethanol (9:1). The spots were developed in iodine chamber and visualized under ultraviolet lamp. Infrared (IR) and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra were recorded for the compounds in Testcan Shimadzu FTIR 8000 (KBr) ^{13}C Avance Bruker 300 MHz spectrophotometer, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis (N and S) was undertaken with Perkin Elmer-2400 instrument and the measured values agreed within ±0.4% with the calculated. Mass spectra were obtained on Joel SX 102/M-6000 mass spectrometer applying FAB method.

5.1.1. Synthesis of aryl thiourea (*1*)

Aryl amine (0.1 mol) was taken in 250 mL of beaker containing 100 mL distilled water. HCl (10 mL) was added and the contents were warmed to dissolve the amine. Ammonium thiocyanate (7.6 g, 0.1 mol) was added to the amine solution and the mixture was heated. The mixture was poured on crushed ice, the precipitate thus obtained was filtered off by suction and recrystallized from ethanol. Spectral analysis, m.p. and elemental analysis confirm the formation of the corresponding thiourea.

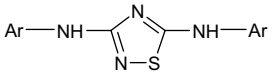
5.1.2. Synthesis of 3-amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline (*2a–g*)[9]

Corresponding aryl thiourea (**1a–g**, 0.5 mol) was taken in a conical flask equipped with separating funnel and condenser and was dissolved in a warm 10 mL of HCl. Hydrogen peroxide (60–70 mL) was added dropwise from the separating funnel with continuous stirring and the mixture was kept aside for 2 h. The oxidized mixture was then diluted with water and was neutralized with dilute ammonia. The precipitate thus obtained was collected and recrystallized from ethanol (95%).

5.1.3. Synthesis of 3-aryl amino-4-aryl-5-oxo-Δ^2-1,2,4-thiadiazolines (*3a–g*)

Required compound **2** (0.0l mol) was taken in a round-bottomed flask. After adding 10 mL of hydrochloric acid in 25 mL of ethanol, they were refluxed for an hour on water bath. On cooling the product, it was filtered and recrystallized from ethanol. Physico-chemical and spectral data of the compounds are presented in Tables 1 and 2, respectively.

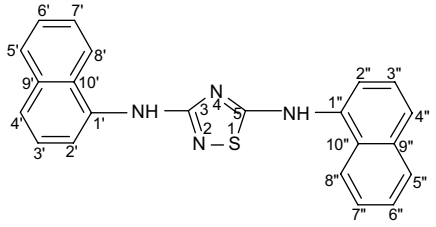
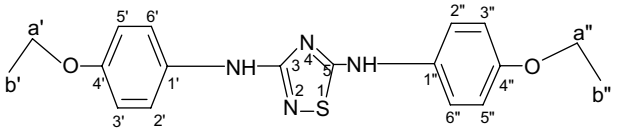
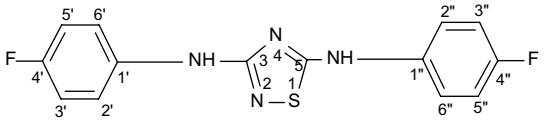
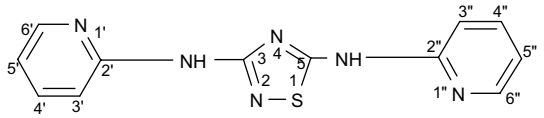
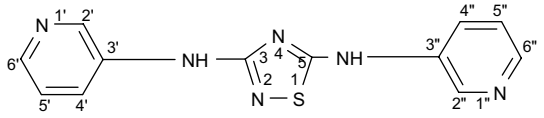
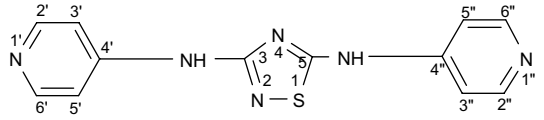
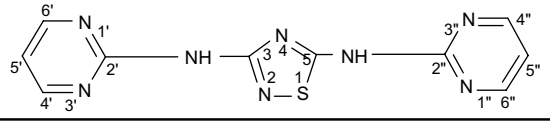
Table 3
Physical constants of the synthesized 3,5-diarylamino-1,2,4-thiadiazoles



Code	M.p. (°C)	% Yield	Molecular formula	Molecular weight	Element (found %/calculated %)[a]
4a	178	68	$C_{22}H_{16}N_4S$	368.48	N (15.21/15.19) S (8.73/8.59)
4b	145	51	$C_{18}H_{20}N_4SO_2$	356.4	N (15.74/15.71) S (8.89/8.85)
4c	150	73	$C_{14}H_{10}N_4SF_2$	304.21	N (18.43/18.40) S (10.67/10.51)
4d	240	54	$C_{12}H_{10}N_6S$	270.32	N (31.20/31.07) S (11.73/11.83)
4e	248	68	$C_{12}H_{10}N_6S$	270.32	N (31.28/31.07) S (11.73/11.83)
4f	270	85	$C_{12}H_{10}N_6S$	270.32	N (31.20/31.07) S (11.73/11.83)
4g	243	59	$C_{10}H_8N_8S$	272.29	N (41.09/41.13) S (11.70/11.75)

[a] Elemental analyses for N and S were within 0.4% of the theoretical values.

Table 4
IR and ^{13}C NMR spectroscopic data of the titled compounds

Code	Structure	IR	^{13}C NMR
4a		3410 (NH), 1243 (CN), 731 (CS), 1619 (C=C)	163 (C-3, C-5), 142 (C-1′, C-1″), 121 (C-2′, C-2″), 125 (C-3′, C-3″), 126 (C-4′, C-4″), 128.6 (C-5′, C-5″), 119 (C-6′, C-6″), 126.6 (C-7′, C-7″), 109.4 (C-8, C-8″), 134.3 (C-9, C-9″), 124.7 (C-10′, C-10″)
4b		3398 (NH), 1296 (CN), 743 (CS), 1627 (C=C), 1441 (C–O)	163 (C-3, C-5), 134.7 (C-1′, C-1″), 116.9 (C-2′, C-6′, C-2″, C-6″), 115.2 (C-3′, C-5′, C-3″, C-5″), 147.5 (C-4′, C-4″), 64.7 (C-a′, C-a″), 14.8 (C-b′, C-b″)
4c		3409 (NH), 1293 (CN), 738 (CS), 1611 (C=C), 1153 (CF)	163 (C-3, C-5), 138.7 (C-1′, C-1″), 117.9 (C-2′, C-6′, C-2″, C-6″), 116.3 (C-3′, C-5′, C-3″, C-5″), 152.2 (C-4′, C-4″)
4d		3427 (NH), 1477 (C=N), 1239 (CN), 723 (CS), 1624 (C=C)	163 (C-3, C-5), 154.7 (C-2′, C-2″), 109.9 (C-3′, C-3″), 138.3 (C-C-4′, C4″), 113.3 (C-5′, C-5″), 148.2 (C-6′, C-6″)
4e		3418 (NH), 1456 (C=N), 1326 (CN), 742 (CS), 1635 (C=C)	163 (C-3, C-5), 137.6 (C-2′, C-2″), 134.1 (C-3′, C-3″), 122.8 (C-4′, C-4″), 124.7 (C-5′, C-5″), 138.9 (C-6′, C-6″)
4f		3418 (NH), 1456 (C=N), 1326 (CN), 742 (CS), 1635 (C=C)	163 (C-3, C-5), 150.3 (C-2′, C-6′, C-2″, C-6″), 109.1 (C-3′, C-5′, C-3″, C-5″), 155.3 (C-4′, C-4″)
4g		3425 (NH), 1412 (C=N), 1298 (CN), 725 (CS), 1626 (C=C)	163 (C-3, C-5), 163.3 (C-2′, C-2″), 157.9 (C-4′, C-6′, C-4″, C-6″), 110.3 (C-5′, C-5″)

5.1.4. Synthesis of 5-diaryl amino-1,2,4-thiadiazole (**4a–g**)

Compound **2** (0.01 mol) was dissolved in warm ethanol (20 mL). Aqueous sodium hydroxide (10%, 10 mL) was added and mixed. The contents were refluxed for 4 h on water bath. On cooling the crystal of the product separated out. The crystals were filtered and recrystallized from ethanol. The melting point was determined. Physico-chemical and spectral data of the compounds are presented in Tables 3 and 4, respectively.

5.2. Pharmacology

5.2.1. Anticonvulsant screening

In the preliminary screening, each compound was administered an i.p. injection at three dose levels (30, 100 and 300 mg/kg) and the anticonvulsant activity was assessed after 30 min and 4 h interval of administrations [10,11]. The anticonvulsant efficacy was evaluated by the maximal electroshock-induced

Table 5
Anticonvulsant activity and minimal motor impairment of substituted-1,2,4-thiadiazoline

Code	Intraperitoneal injection in mice[a]					
	MES		ScPTZ		Neurotoxicity screen	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
3a	100	300	–	–	300	–
3b	100	300	300	–	300	300
3c	100	–	–	–	300	–
3d	300	–	–	–	100	–
3e	300	–	–	–	100	–
3f	300	–	–	–	100	–
3g	300	–	–	–	–	–
4a	100	300	–	–	300	–
4b	100	300	–	–	300	300
4c	100	300	–	–	100	–
4d	300	–	–	–	100	–
4e	300	–	–	–	100	–
4f	300	–	–	–	100	–
4g	–	–	–	–	300	–
Phenytoin	30	100	–	–	100	100
Carbamazepine	30	–	100	–	100	300

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals (number of animals = 4) were examined 0.5 and 4 h after injections were made. The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg).

seizure (MES) and subcutaneous pentylenetetrazole (scPTZ). The data are presented in Table 5.

5.2.2. Neurotoxicity screen

The test is used to evaluate the activity of drugs interfering with motor coordination. The skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions/min. The rod diameter was 3.2 cm. Trained animals were given i.p. injection of the test compounds in doses of 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

5.2.3. Phenobarbitone-induced hypnosis potentiation test

The drug solution in polyethylene glycol (PEG 100) was administered in a dose of 100 mg/kg to a group of six animals. The animals were grouped into groups of six animals each.

Table 6
Phenobarbitone-induced hypnosis potentiation test for some selected compounds

Code	Duration of sleep in minutes ± SEM	% Increase in sleep
3a	92 ± 4.80	16.4
3b	132 ± 5.30***	67.1
3c	93 ± 6.40	17.7
4a	97 ± 6.20*	22.7
4b	108 ± 4.20**	36.7
4g	126 ± 6.50***	59.5
PBS # only (control)	79 ± 4.87	–

PBS #: Phenobarbitone sodium.
*$p < 0.5$; **$p < 0.01$; ***$p < 0.005$.

Thirty minutes after drug administration, animals were injected phenobarbitone sodium. The animals fell asleep on their back. Sleeping time of each rat was taken as the interval between the loss and return of the righting reflex as indicated by inability or ability, respectively, of the rat to right itself in three successive trials when placed on its back. The time taken by animals to awake was noted. The results are reported in Table 6. A control was also performed after pre-treatment with test substance vehicle.

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