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Original article

Synthesis and anticonvulsant activity of some novel 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline

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Abstract

A series of 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline have been synthesized using an appropriate synthetic route and characterized by elemental analyses and spectral data. The anticonvulsant activity of all the synthesized compounds was evaluated against maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (ScPTZ) induced seizure models in mice. The neurotoxicity was assessed using the rotorod method. All the test compounds were administered at doses of 30, 100, and 300 mg/kg body weight and the anticonvulsant activity was noted at 0.5 and 4 h time intervals after the drug administration. Some of the compounds were evaluated for the Phenobarbitone induced hypnosis potentiation test. Among the compounds tested, all except **2h** showed protection from MES seizures, whereas only **3b** was found to be active in the ScPTZ test.
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Keywords: Thiadiazoline; Anticonvulsant; Neurotoxicity

1. Introduction

Epilepsy is a central nervous system (CNS) malfunction that leads either to generalized hyperactivity involving essentially all parts of the brain or hyperactivity of only a portion of the brain [1–3]. It has been estimated that adequate control of seizures could not be obtained in up to 20% of the patients with epilepsy using first generation of antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, sodium valproate and diazepam). A group of new drugs including felbamate, gabapentin, lamotrigine, oxacarbazepine, topiramate, milacemide, vigabatrin and zonisamide is entering into clinical practice. The convulsions of approximately 25% of epileptics are adequately controlled by current clinically available drugs. Current drug therapy is accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism and megaloblastic anemia [4,5]. The past decade has witnessed a continuous interest in the development of anticonvulsant drugs.

In continuation of our earlier work [6,7], the present paper report on the synthesis, anticonvulsant and neurotoxicity evaluation of some new 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline.

2. Chemistry

The synthesis of 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline was accomplished as showed in Fig. 1. Aryl thiourea (**1**) was prepared by reacting aromatic/hetero aromatic amines with ammonium thiocyanate in the presence of concentrated hydrochloric acid. Aryl thiourea (**1**) and thiourea were oxidatively cyclised to yield 3-aryl amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline whereas two moles of aryl thiourea were oxidatively cyclised into 3-amino-4–aryl-5-imino-Δ^2-1,2,4-thiadiazoline.

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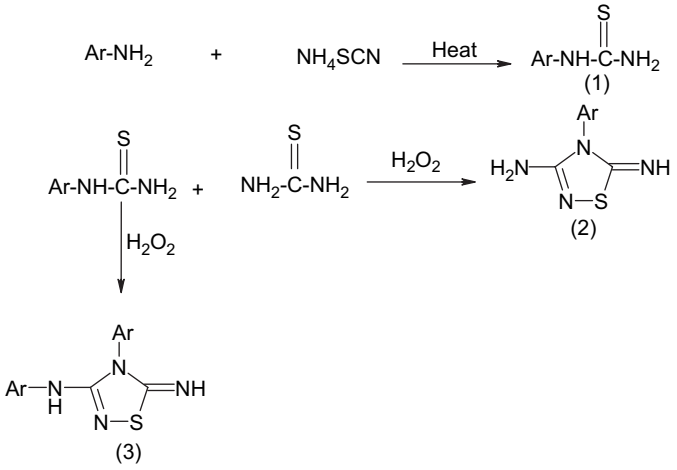


Fig. 1. Scheme for the synthesis of title compounds.

3. Pharmacology

All the compounds were injected intraperitoneally into the mice and evaluated in the initial anticonvulsant screening with at least three dose levels (30, 100 and 300 mg/kg), following the anticonvulsant drug development (ADD) program protocol [8,9]. The profile of anticonvulsant activity was established by MES pattern test and ScPTZ seizure threshold test. Minimal motor impairment was measured by the rotorod (neurotoxicity, NT) test. Sedative-hypnotic activity was established by Phenobarbitone induced hypnosis potentiation test.

4. Results and discussion

Discovery and development of new chemical entity for treatment of epilepsy rely heavily on the use of predictable animal models. Presently, there are three in vivo models that are routinely used by most AED discovery programs. These include the maximal electroshock seizures, the ScPTZ and kindling models. Of these the MES and ScPTZ models represent the two animal seizures models most widely used in the search for new AEDs.

The anticonvulsant and neurotoxicity test results for the titled compounds are reported in, along with the data of clinically used drugs. All the 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazolines except **2h** were active in the MES screen, indicating their ability to prevent seizure spread. At a dose of 100 mg/kg, compounds that showed protection were **2c**, **2d**, **3a** and **3g** at 0.5 h. Compound **3b** was found to be active in the ScPTZ test, a test used to identify compound that elevate seizures' threshold. Only **2h** exhibited no activity at maximum administered dose. Compounds **2d**, **3a**–**3c** showed rapid onset of action and prolonged duration, on the other hand remaining compounds acted very fast but with short duration of activity. It should be noted that only few standard anticonvulsants exhibited broad spectrum of activity by showing activity in both models. Our synthesized compounds showed preferably only MES seizures' protection. Out of seventeen compounds only four (**2d**, **3a**–**3c**) showed prolonged activity. Three compounds **2b**, **3a** and **3b** exhibited potent anticonvulsant activity at 30 mg/kg at 0.5 h (Table 1).

In the neurotoxicity test, compounds **2i** and **2j** did not show toxicity at the highest administered dose (300 mg/kg), whereas all remaining compounds showed toxicity at 100 mg/kg or 300 mg/kg doses. Compounds **2b** and **3d** exhibited prolonged neurotoxicity. Some selected compounds (Table 5) were evaluated for sedative-hypnotic effect using Phenobarbitone induced hypnosis potentiation test. Compounds **2c**, **2d** and **3b** showed significant ($p < 0.05$) percentage increase in sleeping time of the animals. These results indicate that synthesized compounds exhibited potent anticonvulsant activity without significantly inducing sleep (except **2c**, **2d** and **3b**).

In conclusion the present results have revealed that a number of 3-aryl amino/amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazolines exhibit a range of activities in anticonvulsant screen and some of the compounds showed significant increase in sleeping time. Compounds **2b**, **3a** and **3b** exhibited potent anticonvulsant activity in MES screen at 30 mg/kg. Prepared compounds are more selective for MES screen than ScPTZ test.

Table 1
Anticonvulsant activity and minimal motor impairment of compounds **2a**–**2j** and **3a**–**3g**

Code	Intraperitoneal injection in mice[a]					
	MES		ScPTZ		Neurotoxicity screen[b]	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
2a	300	–	–	–	100	–
2b	30	–	–	–	300	300
2c	100	–	–	–	100	–
2d	100	300	–	–	100	–
2e	300	–	–	–	100	–
2f	300	–	–	–	100	–
2g	300	–	–	–	100	–
2h	–	–	–	–	100	–
2i	300	–	–	–	00	–
2j	300	–	–	–	–	–
3a	30	300	–	–	300	–
3b	30	300	300	–	300	–
3c	100	300	–	–	300	–
3d	300	–	–	–	100	300
3e	300	–	–	–	100	–
3f	300	–	–	–	100	–
3g	100	–	–	–	100	–
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 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).

[b] Neurotoxicity at 100 mg/kg (0.25 h and 1 h).

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 ultra violet lamp. Infrared (IR) and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra were recorded for the compounds in test in Shimadzu FTIR 8000 (KBr) and ^{13}C Advance Bruker 300 MHz spectrophotometers, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Elemental analysis (C, H, N) was undertaken with Perkin Elemer-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 contents were warmed to dissolve the amine. Ammonium thiocynate (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 recrystallised from ethanol. Spectral analysis, melting point and elemental analysis confirm the formation of the corresponding thiourea.

5.1.2. Synthesis of 4-(4-chloro-phenyl)-5-imino-4,5-dihydro-[1,2,4] thiadiazole-3-ylamine (*2a–2j*)

Corresponding aryl thiourea (**1a–1g**, 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. The mixture was kept aside for 2 h. The oxidized mixture was then diluted with water and neutralized with dilute ammonia. The precipitate thus obtained was collected and recrystallised from ethanol (95%). Physico-chemical data of the compounds (**2a–2j**) are presented in Table 2.

5.1.3. Synthesis of (aryl)-[4-(aryl)-5-imino-4,5-dihydro-3H-[1,2,4] thiadiazol-3-yl]-amine (*3a–3g*)

Compounds **1** were oxidatively cyclised into 3-aryl amino-4-aryl-5-imino-Δ^2-1,2,4-thiadiazoline. The product was recrystallised from hot ethanol. Physico-chemical and spectroscopic data of the compounds (**3a–3g**) are presented in Tables 3 and 4, respectively.

5.2. Pharmacological evaluation

The anticonvulsant evaluation was carried out using reported procedures. Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague–Dawley, 100–150 g) were used as experimental animals. The compounds were suspended in 0.5% methyl cellulose–water mixture or in polyethylene glycol (PEG).

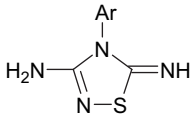
5.2.1. Anticonvulsant screening

In the preliminary screening, each compound was administered by 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 intervals of administration. The anticonvulsant efficacy was evaluated by the maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (ScPTZ). The data are presented in Table 1.

5.2.2. Neurotoxicity screen

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.

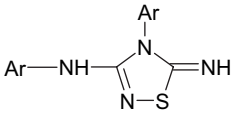
Table 2
Physical constants of the synthesized compounds **2a–2j**

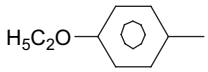
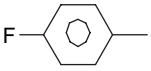
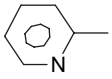
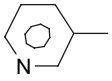
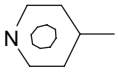
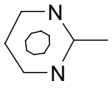


Code	M.p. (°C)	Yield (%)	Mol. formula	Mol. wt.	Element (found (%)/calculated (%))[a]
2a	63	69	$C_{12}H_{10}N_4S$	242.30	N (23.07/23.11), S (13.27/13.20)
2b	248	65	$C_9H_{10}N_4SO$	222.27	N (25.23/25.19), S (14.54/14.41)
2c	108	73	$C_{10}H_{12}N_4SO$	236.30	N (23.73/23.69), S (13.55/13.54)
2d	98	74	$C_8H_7N_4SF$	210.23	N (26.65/26.71), S (15.38/15.22)
2e	160	68	$C_8H_7N_4SCl$	226.5	N (24.93/24.72), S (14.07/14.12)
2f	107	74	$C_8H_7N_4SBr$	271.14	N (20.63/20.65), S (11.74/11.83)
2g	84	65	$C_7H_7N_5S$	193.23	N (36.17/36.22), S (16.80/16.56)
2h	89	65	$C_7H_7N_5S$	193.23	N (36.73/36.22), S (16.08/16.56)
2i	210	67	$C_7H_7N_5S$	193.23	N (36.65/36.22), S (16.87/16.56)
2j	103	75	$C_6H_6N_6S$	194.22	N (44.00/43.80), S (16.53/16.47)

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

Table 3
Physical constants of the synthesized compounds (aryl)-[4-(aryl)-5-imino-4,5-dihydro-3*H*-[1,2,4]thiadiazol-3-yl]-amine



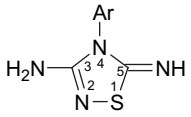
Code	Ar	M.p. (°C)	Yield (%)	Mol. formula	Mol. wt.	Element (found (%)/calculated (%))[a]
3a	 1′-Naphthyl	162	81	$C_{22}H_{16}N_4S$	368.96	N (15.06/15.17), S (8.64/8.6)
3b	 4′-Ethoxyphenyl	143	73	$C_{18}H_{20}N_4SO_2$	356.42	N (15.61/15.71), S (8.87/8.97)
3c	 4′-Fluorophenyl	141	85	$C_{14}H_{10}N_4SF_2$	304.32	N (16.46/18.40), S (10.51/10.51)
3d	 2′-Pyridyl	293	43	$C_{12}H_{10}N_6S$	270.32	N (31.16/31.07), S (11.89/11.83)
3e	 3′-Pyridyl	313	64	$C_{12}H_{10}N_6S$	270.2	N (31.84/31.07), S (11.39/11.83)
3f	 4′-Pyridyl	309	70	$C_{12}H_{10}N_6S$	270.32	N (31.18/31.07), S (11.81/11.83)
3g	 2′-Pyrimidyl	210	90	$C_{10}H_8N_8S$	272.29	N (41.24/41.13), S (11.77/11.75)

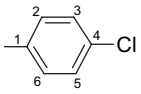
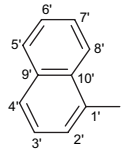
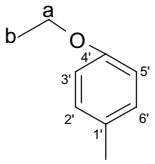
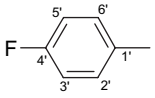
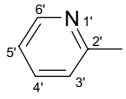
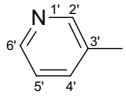
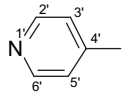
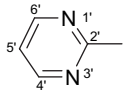
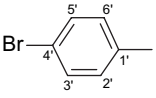
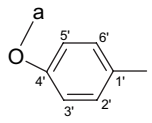
[a] Elemental analyses for N and S were within 0.4% of the theoretical values.

5.2.3. Phenobarbitone induced hypnosis potentiation test

The drug solution in polyethylene glycol (PEG 100) was administered at a dose of 100 mg/kg to a group of six animals. The animals were grouped into six each. 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 and 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 5. A control was also performed after pre-treatment with test substance vehicle.

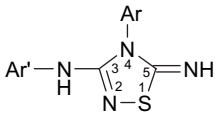
Table 4
IR and ^{13}C NMR spectroscopic data of the titled compounds



Code	Ar	IR	^{13}C NMR; ^{1}H NMR
2a		3415 (NH), 1578 (C=N), 1299 (CN), 725 (CS), 1618 (C=C)	163 (C-3, C-5), 139.4 (C-1′), 117.7 (C-2, C-6), 129.7 (C-3′, C-5′), 124.3 (C-4′); 6.4–7.02 (m, 4H, Ar-H), 2 (s, 2H, NH_2)
2b		3411 (NH), 1568 (C=N), 1285 (CN), 636 (CS), 1616 (C=C)	163 (C-3, C-5), 141.2 (C-1′), 109.4 (C-2), 126.6 (C-3′, C-6′), 119 (C-4′), 128.6 (C-5′), 125 (C-7′), 121 (C-8′), 134.3 (C-9′, C-9″), 124.7 (C-10′); 6.55–7.61 (m, 7H, naphthalene), 2 (s, 2H, NH_2)
2c		3402 (NH), 1589 (C=N), 1295 (CN), 723 (CS), 1624 (C=C)	163 (C-3, C-5), 132.9 (C-1′), 116.9 (C-2′, C-6′), 115.2 (C-3′, C-5′), 147.5 (C-4′), 64.7 (Ca), 14.8 (Cb); 1.33 (m, 3H, CH_3), 3.98 (m, 2H, CH_2), 6.4–7.02 (m, 4H, Ar-H), 2 (s, 2H, NH_2)
2d		3398 (NH), 1590 (C=N), 1208 (CN), 709 (CS), 1617 (C=C), 1153 (CF)	163 (C-3, C-5), 116.3 (C-3′, C-5′), 136.9 (C-1′), 117.9 (C-2′, C-6), 152.9 (C-4′); 6.4–7.02 (m, 4H, Ar-H), 2 (s, 2H, NH_2)
2e		3410 (NH), 1576 (C=N), 1282 (CN), 780 (CS), 1615 (C=C)	163 (C-3, C-5), 147.7 (C-2′), 109.9 (C-3′), 138.3 (C-4′), 113.3 (C-5′), 148.2 (C-6′); 6.60–8.11 (m, 4H, pyridine ring), 2 (s, 2H, NH_2)
2f		3406 (NH), 1583 (C=N), 1322 (CN), 732 (CS), 1680 (C=C)	163 (C-3, C-5), 137.6 (C-2′), 145.1 (C-3′), 122.8 (C-4′), 124.7 (C-5′), 138.9 (C-6′); 7.26–8.53 (m, 4H, pyridine ring), 2 (s, 2H, NH_2)
2g		3366 (NH), 1581 (C=N), 1240 (CN), 747 (CS), 1636 (C=C)	163 (C-3, C-5), 150.3 (C-2′, C-6′), 109.1 (C-3′, C-5′), 155.3 (C-4′); 6.64–8.44 (m, 4H, pyridine ring), 2 (s, 2H, NH_2)
2h		3403 (NH), 1558 (C=N), 1339 (CN), 731 (CS), 1621 (C=C)	163 (C-3, C-5), 169.3 (C-2′), 157.9 (C-4′, C-6′), 110.3 (C-5′); 6.58–8.38 (m, 3H, pyridine ring), 2 (s, 2H, NH_2)
2i		3407 (NH), 1576 (C=N), 1281 (CN), 701 (CS), 1618 (C=C)	163 (C-3, C-5), 140.3 (C-1′), 118.5 (C-2′, C-6′), 132.5 (C-3′, C-5′); 6.4–7.02 (m, 4H, Ar-H), 2 (s, 2H, NH_2), 113.1 (C-4′)
2j		3410 (NH), 1556 (C=N), 1282 (CN), 60 (CS), 1616 (C=C), 1217 (C–O)	163 (C-3, C-5), 133.6 (C-1′), 117.3 (C-2′, C-6′), 115.1 (C-3′, C-5′), 150.7 (C-4′), 55.9(Ca); 6.4–7.02 (m, 4H, Ar-H), 3.73 (m, 3H, CH_3)

(*continued on next page*)

Table 4 (*continued*)



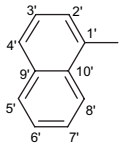
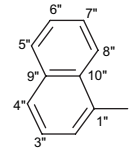
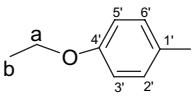
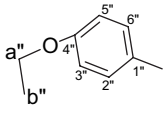
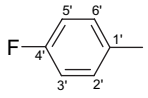
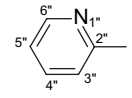
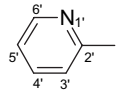
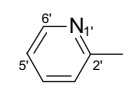
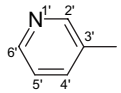
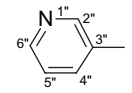
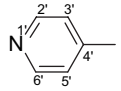
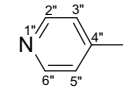
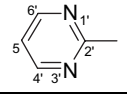
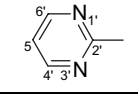
Code	Ar′	Ar	IR	^{13}C NMR
3a			3453 (NH), 1571 (C═N), 1261 (CN), 704 (CS), 1606 (C═C)	163 (C-3, C-5), 140.8 (C-1′), 141.2 (C-1″), 109.4 (C-2′, C-2″), 126 (C-3′, C-4′, C-3″, C-4″), 119 (C-4′, C-4″), 128.6 (C-5′, C-5″), 125 (C-7′, C-7″), 121 (C-8′, C-8″), 134.3 (C-9′, C-9″), 124.7 (C-10′, C-10″)
3b			3419 (NH), 1576 (C═N), 1290 (CN), 720 (CS), 1667 (C═C), 1242(C–O)	163 (C-3, C-5), 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″)
3c			3417 (NH), 1575 (C═N), 1297 (CN), 728 (CS), 1648 (C═C), 1161 (CF)	163 (C-3, C-5), 140 (C-1′), 136.9 (C-1″), 117.9 (C-2′, C-6′, C-2″, C-6″), 116.3 (C-3′, C-5′, C-3″, C-5″), 152.9 (C-4′, C-4″)
3d			3417 (NH), 1542 (C═N), 1324 (CN), 723 (CS), 1658 (C═C)	163 (C-3, C-5), 158.6 (C-1′), 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			3411 (NH), 1565 (C═N), 1289 (CN), 7 68 (CS), 1614 (C═C)	163 (C-3, C-5), 137.6 (C-2′, C-2″),134.4 (C-2′), 145.1 (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			3332 (NH), 1582 (C═N), 1240 (C–N), 747 (CS), 1636 (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″), 1155.3 (C-4′, C-4″)
3g			3427 (NH), 1568 (C═N), 2312 (CN), 722 (CS), 1643 (C═C)	163 (C-3, 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″)

Table 5
Phenobarbitone induced hypnosis potentiation test for some selected compounds

Code	Duration of sleep in minutes ± SEM	% Increase in sleep
2b	86 ± 5.85	8.8
2c	116 ± 4.33	46.8*
2d	95 ± 4.76	20.2
3a	92 ± 5.31	16.4
3b	123 ± 5.54	55.6*
3c	83 ± 4.38	5.1
3g	88 ± 5.48	11.4
Phosphate buffer solution (control)	79 ± 4.87	–

*Significant at the level of $p < 0.05$ at dose of 100 mg/kg; number of animals taken for the study $n = 6$.

Acknowledgements

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