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Antibacterial, Antifungal and Anticonvulsant Evaluation of Novel Newly Synthesized 1-[2-(1*H*-Tetrazol-5-yl)ethyl]-1*H*-benzo[d][1, 2,3]triazoles

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Several novel 1-[2-(1*H*-tetrazol-5-yl) ethyl]-1*H*-benzo[d][1,2,3]triazoles (**3a-h)** have been synthesized by the condensation of 1-[2-(1*H*-tetrazol-5-yl)-ethyl]-1*H*-benzotriazole (**2**) and appropriate acid chlorides. 1-[2-(1*H*-tetrazol-5-yl)-ethyl]-1*H*-benzotriazole (**2**) was synthesized by reacting 3-(1*H*-benzo[d][1,2,3]triazol-1-yl)propanenitrile with sodium azide and ammonium chloride in the presence of dimethylformamide. The synthesized compounds were characterized by IR and PMR analysis. The titled compounds were evaluated for their *in vitro* antibacterial and antifungal activity by the cup plate method and anticonvulsant activity evaluated by the maximal electroshock induced convulsion method in mice. All synthesized compounds exhibited moderate antibacterial activity against *Bacillus subtilis* and moderate antifungal activity against *Candida albicans*. Compounds 5-(2-(1 *H*-benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(4-aminophenyl)methanone **3d** and 5-(2-(1 *H*-benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(2-aminophenyl)methanone **3e** elicited excellent anticonvulsant activity.

Key words: Synthesis, 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1H-benzo[d][1,2,3]triazoles, Antibacterial activity, Antifungal activity, Anticonvulsant activity

INTRODUCTION

A tetrazole is a five-membered ring structure composed of four nitrogen atoms and one carbon atom and used as an intermediate in the synthesis of pharmaceuticals, especially cephalosporin's and other organic compounds. Tetrazole and its derivatives reportedly have antinociceptive (Rajasekaran *et al.*, 2005), anti-inflammatory (Rajasekaran *et al.*, 2003), antimicrobial (Upadhayaya *et al.*, 2005), (De Souza *et al.*, 2005), (Adamec *et al.*, 2005) and anticonvulsant properties (Rajasekaran *et al.*, 2004).

The benztriazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities e.g. anti-inflammatory (Habib *et al.*, 2000), analgesic (Calvino *et al.*, 1980), anticonvulsant (Rajasekaran *et al.*, 2004), antioxidant (Lin *et al.*, 2002), anti-emetic (Yoshikawa *et al.*, 2003) and antimicrobial (Borowski *et al.*, 2003).

Tetrazole derivatives of benzotriazole have been investigated and reported in the literature, but only for their immunosuppressive activities (Kacamarek *et al.*, 2002), (Zubrzak *et al.*, 2001).

Our research has focused on the incorporation of the benzotriazole moiety into tetrazoles based upon the hypothesis that this modification would improve efficacy, since both moieties are noted for their antimicrobial and anticonvulsant properties. Thus, a series of novel 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1*H*-benzo[d][1,2,3]triazoles were synthesized and tested for their antimicrobial and anticonvulsant properties.

MATERIALS AND METHODS

Melting points were determined using a melting point apparatus (Veego, VMP III, India) and were not corrected. Infrared spectra were obtained on a FTIR spectrophotometer (Perkin Elmer, 1600 series) using potassium bromide discs. Nuclear magnetic resonance (NMR) spectra were recorded on a Brucker 400 MHz spectrophotometer. Chemical shifts are reported in parts per million (δ) units

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relative to an internal standard of tetramethylsilane. Mass spectra were recorded on a Mass spectrophotometer (Jeol JMS-DX 303 and Finnigan MAT 8230). Elemental analysis determinations were performed on a Heraeus Carlo Erba 1108 and the analyses, indicted by the symbols of the elements, were determined to be within ± 0.4% of theoretical values.

Synthesis of the 3-(1*H*-Benzo[d][1,2,3]triazol-1-yl) propanenitrile (1)

Benzotriazole (50 mmol) was mixed with acrylonitrile (12.5 mL) and cooled in an ice bath. A crystal of resorcinol was added to prevent polymerization. Triton B (2 mL, 40% v/v) was added drop wise with shaking and a vigorous reaction observed. After this event was allowed to subside the mixture was heated to reflux on a steam bath for 2 h. The solution was cooled, extracted with ethylene dichloride and dried over anhydrous sodium sulphate. The dried nitrile was recrystallized from ether. The desired 3-(1*H*-benzo[d][1,2,3]triazol-1-yl)propanenitrile (**1**) was obtained as a solid with a 73.4% overall yield: m.p. 89-92 °C. IR: 2958 (C-H), 2254 (C≡N), 1456 (C-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.7 (2H, t, *J*=7.1 Hz, CH_2), 4.4 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_9H_8N_4$: C, 62.87; H, 4.68; N, 32.54. Found: C,62.62; H, 4.64; N, 32.42.

Synthesis of the 1-[2-(1*H*-Tetrazol-5-yl)-ethyl]-1*H*-benzotriazole (2)

Equimolar quantities of compound **1**, sodium azide, dimethylformamide (10 mL) and ammonium chloride were heated in an oil bath for 7 h at 125°C. The solvent was removed under reduced pressure, the residue dissolved in 100 mL of water and carefully acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5°C in an ice bath. Compound **2** was recrystallized from aqueous methanol (yield 78.40%) as a solid: m.p. 68°C-72°C; IR: 3462 (N-H), 2926 (C-H), 2853 (C-H), 1639 (C=N), 1458 (C-H), 1286 (N-N=N-), 1197 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*= 7.1 Hz, CH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_9H_9N_7$: C, 50.23; H, 4.22; N, 45.56. Found: C, 50.39; H, 4.14; N, 43.52.

General procedure for 1-[2-(1*H*-Tetrazol-5-yl)ethyl]-1*H*-benzo[d][1,2,3]triazoles (3a-h)

Compound **2** was treated with an equimolar amount of aromatic acid chlorides in 10 mL of 10% sodium bicarbonate solution. The mixture was shaken vigorously until the odor of aromatic acid chloride had disappeared. The solids separated out and were filtered and dried. Recrystallization of the dried compounds from aq.ethanol yielded compounds **3a-h**.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(phenyl)methanone (3a)

Yield 75.58%; m.p. 95-102°C. IR: 2930 (C-H), 1695 (C=O), 1437 (C-H), 1282 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.3 (9H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}N_7O$: C, 60.18; H, 4.10; N, 30.70. Found: C, 60.34; H, 4.42; N, 30.68.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(4-nitrophenyl)methanone (3b)

Yield 76.20%; m.p. 80-85°C. IR: 2887 (C-H), 1774 (C=O), 1576 (NO_2), 1457 & 1367 (C-H), 1283 (N-N=N-), 1110 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) d 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-8.1 (8H, m, Ar-H). Anal. Calcd for $C_{16}H_{12}N_8O_3$: C, 52.75; H, 3.32; N, 30.76. Found: C, 52.64; H, 3.42; N, 30.58.

1-(2-(1-Tosyl-1*H*-tetrazol-5-yl)ethyl)-1*H*-benzo[d][1,2,3] triazole (3c)

Yield 72.60%; m.p. 180-182°C. IR: 2930 (C-H), 1774 (C=O), 1618 (C=N), 1457 (C-H), 1283 (N-N=N-), 1108 and 1133 (tetrazole ring), 1044 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.1 (3H, s, CH_3), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 6.8-7.7 (8H, m, Ar-H). Anal. Calcd for: $C_{16}H_{15}N_7O_2S$ C, 52.02; H, 4.09; N, 26.54. Found: C, 52.34; H, 4.14; N, 26.78.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(4-aminophenyl)methanone (3d)

Yield 74.66%; m.p. 220-226°C. IR: 3464 (N-H), 2798 (C-H), 1666 (C=O), 1457 (C-H), 1301 (N-N=N-), 1105 and 1162 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 4.5 (2H, s, NH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{16}H_{14}N_8O$: C, 57.48; H, 4.22; N, 33.52. Found: C, 57.34; H, 4.33; N, 33.68.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(2-aminophenyl)methanone (3e)

Yield 64.44%; m.p. 140-146°C. IR: 3463 (N-H), 2798 (C-H), 1666 (C=O), 1486 (C-H), 1301 (N-N=N-), 1105 and 1162 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 4.5 (2H, s, NH_2), 6.8-7.3 (8H, m, Ar-H). Anal. Calcd for $C_{16}H_{14}N_8O$: C, 57.48; H, 4.22; N, 33.52. Found: C, 57.34; H, 4.14; N, 33.21.

4-5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl sulfonyl)benzenamine (3f)

Yield 71. 88%; m.p. 242-245°C. IR: 3383 (N-H), 1434 (C-H), 1282 (N-N=N-), 1132 (tetrazole ring), 1031 (SO_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H,

t, *J*=7.1 Hz, CH_2), 4.5 (2H, s, NH_2), 6.8-7.7 (8H, m, Ar-H). Anal. Calcd for $C_{15}H_{14}N_8O_2S$: C, 48.64; H, 3.81; N, 30.25. Found: C, 48.49; H, 3.62; N, 30.43.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(2-hydroxyphenyl)methanone (3g)

Yield 66.96%; m.p. 167-170°C. IR: 3372 (O-H), 1774 (C=O), 1457 (C-H), 1295 (N-N=N-), 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.1 (3H, s, CH_3), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 5 (1H, s, OH), 6.8-7.6 (8H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}N_7O_2$: C, 57.31; H, 3.91; N, 29.24. Found: C, 57.34; H, 4.05; N, 29.28.

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(4-hydroxyphenyl)methanone (3h)

Yield 65. 76%; m.p. 234-237°C. IR: 3371 (O-H), 1744 (C=O), 1458 (C-H), 1283 (N-N=N-), 1108 and 1138 (tetrazole ring) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.1 (3H, s, CH_3), 2.8 (2H, t, *J*=7.1 Hz, CH_2), 4.3 (2H, t, *J*=7.1 Hz, CH_2), 5 (1H, s, OH), 6.8-7.9 (8H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}N_7O_2$: C, 57.31; H, 3.91; N, 29.24. Found: C, 57.44; H, 4.08; N, 29.38.

Antibacterial *in vitro* susceptibility testing

Antibacterial activity was evaluated by the agar cup plate method of Mukerjee (1996). Nutrient agar medium was prepared by dissolving peptone (10 g), beef extract (10 g), and sodium chloride in purified water (1,000 mL); the pH of the media was adjusted to 8.4 with 5M sodium hydroxide solution. Agar (20 g) was added to this solution, boiled and stirred thoroughly until the agar was dissolved. Twenty mL of this nutrient agar medium was transferred into each boiling tube and plugged with non-absorbent cotton. The tubes containing the nutrient agar medium were sterilized by a pressure controlled heat sterilization technique using an autoclave set at 15 lbs and 115°C for 20 min. After sterilization the nutrient agar medium was melted, cooled and inoculated with two Gram positive organisms: Bacillus subtilis (*Bacillus subtilis* NCIM 2063), and Staphylococcus aureus (*Staphylococcus aureus* NCIM 2079), and two Gram negative organisms: Escherichia coli (*Escherichia coli* NCIM 2065) and Shigella niger (*Shigella niger* 2028). The inoculated media was poured into sterile Petri dishes to a uniform thickness of approximately 6 mm. Cups were created in the plates using sterile a cork borer (6 mm dia). The standard antibacterial agent ciprofloxacin (250 mg/mL), a solvent control (2% v/v Tween 80) and the synthesized compounds (in a concentration of 250 mg/mL) were added using sterile micropipettes into each cup. The plates were kept in the refrigerator for 6 h and then incubated at 37°C for 24 h; the diameters of the zone(s) of inhibition were measured and are presented in Table I.

Antifungal *in vitro* susceptibility testing

Antifungal activity was evaluated by the agar cup plate method of Mukerjee (1996). The antifungal activity was tested in a modified Sabouraud dextrose broth against *Candida albicans*. After sterilization, the medium was cooled, inoculated with *Candida albicans* and poured into sterile Petri dishes to yield a uniform thickness of approximately 6 mm. The cups were created in the plate by using a sterile cork borer (6 mm dia). The standard antifungal agent fluconazole (250 mg/mL), a solvent control (2% v/v Tween 80) and the synthesized compounds (in a concentration of 250 mg/mL) were added using sterile micropipettes into each cup. The plates were then incubated at 37°C for 24 h and the diameter of the zone(s) of inhibition measured and presented in Table I.

Neurotoxicity screening

Minimal motor impairment was measured in Swiss albino mice by using the Rota rod test (Kulkarni, 2004) and by the actophotometer (Kulkarni, 2004) method. The mice were trained to remain on an accelerating Rota rod (3.2 cm diam.) rotating at 10 rpm. Trained animals were administered (i.p.) the test compounds in a dose of 5 mg to 40 mg/kg. Neurotoxicity was indicated by the reduced ability of an animal to maintain equilibrium by remaining on the rod for at least 1 min in each of three trials. The data was analyzed statistically by students "t" test and are presented in Table II.

Anticonvulsant activity

Anti-convulsant activity was evaluated by the maximal electroshock induced seizure (MES) method of Misra *et al.* (1973) as modified by Loscher *et al.* (1994). Swiss albino mice weighing 25 to 30 g and regardless sex were separated into 10 groups each of six animals. All mice were administered their injections by the i.p. route. A

Table I. Antibacterial and antifungal activities of the synthesized compounds

Compound	Zone of inhibition (mm)				
	B. subtilis	*S. aureus*	*S. niger*	*E. coli*	*C. albicans*
3a	07	00	00	00	06
3b	08	00	00	00	08
3c	06	00	00	00	09
3d	12	00	00	00	11
3e	13	00	00	00	12
3f	16	00	00	00	15
3g	08	00	00	00	06
3h	13	00	00	00	12
Ciprofloxacin	20	19	21	23	22
Fluconazole	--	--	--	--	23

Table II. Effect of synthesized compounds on the performance of mice (Rota rod and actophotometer testing)

Behaviour	Effect 30 min after administration (mean ± SEM) (i.p.)					
	10% Tween 80 suspension	5 mg kg^{-1}	10 mg kg^{-1}	20 mg kg^{-1}	30 mg kg^{-1}	40 mg kg^{-1}
Grip test	No effect	No effect	No effect	No effect	No effect	48±0.54**
Motor activity	No effect	No effect	No effect	No effect	No effect	98±3.46**

** $P < 0.01$ indicates the significant difference when compared with control groups

Table III. Anticonvulsant activity data of the synthesized compounds

S.No	Compound	Duration (Mean ± SEM, sec)			
		Extensor	Clonus	Stupor	Recovery /Death
1)	Control	18.5 ± 0.43	18.5 ± 0.43	18.5 ± 0.43	Death
2)	Phenobarbitone	5.67 ± 0.46**	2.17 ± 0.31**	3.33 ± 0.42**	Recovery
3)	**3a**	12.33 ± 0.67*	14.66 ± 0.66*	13.64 ± 0.62*	Recovery
4)	**3b**	10.26 ± 0.38*	12.83 ± 0.60*	15.17 ± 0.88*	Recovery
5)	**3c**	14.17 ± 0.37*	10.23 ± 0.41*	10.33 ± 0.42*	Recovery
6)	**3d**	8.17 ± 0.80**	7.33 ± 0.36**	9.50 ± 0.48**	Recovery
7)	**3e**	9.88 ± 0.24**	10.02 ± 0.18**	9.68 ± 0.66**	Recovery
8)	**3f**	11.44 ± 0.38**	11.64 ± 0.64**	10.47 ± 0.36**	Recovery
9)	**3g**	15.50 ± 0.42*	15.33 ± 0.21*	14.33 ± 0.24*	Recovery
10)	**3h**	13.86 ± 0.62**	12.98 ± 0.44**	13.42 ± 0.92**	Recovery

Dose 18 mg kg^{-1} for all the test compounds and 20 mg kg^{-1} for phenobarbitone.
** $P < 0.001$ vs. Control highly significant
* $P < 0.01$ vs. Control significant

control group of animals was received a 0.5%v/v Tween 80 (0.5) mL suspension and a second group received phenobarbitone (Nicholas Primal) as standard and at a dose of 20 mg/kg. Tween 80 suspensions (0.5% v/v) of the test compounds were similarly administered at a dose of 20 mg/kg test preparation. At 1 h following the administration of the test compounds, standard and control, animals received electroshock (150 mA for 0.2 sec) by electroconvulsiometer (Techno, India) through ear electrodes. The onset times for tonic, flexion, extension and clonic phases were noted and recorded. The protective index was observed as the reduction time of the tonic extensor phase (Table III).

RESULTS AND DISCUSSION

Antibacterial and antifungal activities

All of the 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1*H*-benzo[d][1,2,3] triazoles that were synthesized and tested demonstrated growth inhibitory activities against *Bacillus subtilis* and the pathogenic fungus *Candida albicans*. All of the titled compounds failed to show a zone of inhibition for the gram positive organism *Staphylococcus aureus* and the gram negative organisms *Shigella niger* and *Escherichia coli*. The standard antibacterial agent ciprofloxacin and standard antifungal agent fluconazole both showed zones of inhibition against the entire span of test microorganisms. As indicated in Table I, the most potential antibacterial and antifungal of the 1-[2-(1*H*-tetrazol-5-yl)ethyl]-1*H*-benzo[d] [1,2,3]triazoles, was found to be 4-5-(2-(1*H*-Benzo[d][1, 2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl sulfonyl) benzenamine **3f**.

Neurotoxicity screening

Neurotoxicity screening indicated that all of the synthesized compounds did not affect any behavioral changes in mice at doses of up to 30 mg/mL.

Anticonvulsant activities

The MES method was used for the evaluation of anticonvulsant activity because this is the most widely employed seizure models for the early identification and high throughput screening of investigational anticonvulsant compounds. All of the compounds that were produced and tested exhibited an anticonvulsant activity in the MES test when administered i.p. at the doses of 18 mg/kg. Compounds 5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(4-aminophenyl) methanone **3d** and 5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl)(2-aminophenyl)methanone **3e** showed significant protection from seizures in the mice. Among the compounds tested, all compounds, except **3c** and **3g**, provided moderate protection from seizures. The compounds 1-(2-(1-Tosyl-1*H*-tetrazol-5-yl)ethyl)-1*H*-benzo[d][1,2,3]triazole **3c** and

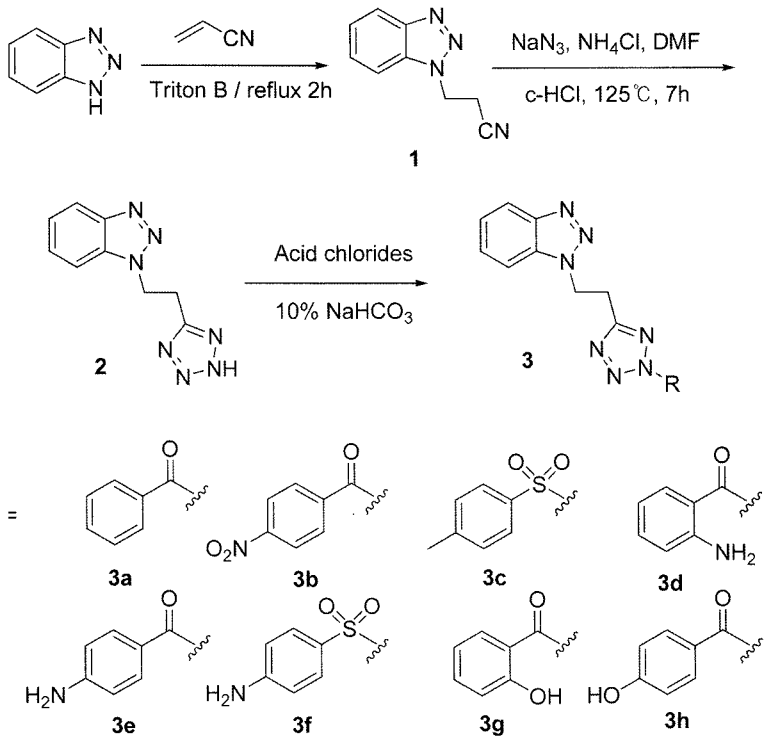

Fig. 1. Synthetic scheme of titled compounds

5-(2-(1*H*-Benzo[d][1,2,3]triazo-1-yl)ethyl)-1*H*-tetrazol-1-yl) (2-hydroxyphenyl)methanone **3g** elicited mild anticonvulsant activity. The standard drug phenobarbitone at a dose of 20 mg/kg produced the most drastic reduction in the duration of the stimulated extensor phase.

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