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5*H*-[1,2,4]Oxadiazolo[5,4-*d*][1,5]benzothiazepines as anticonvulsant agents in DBA/2 mice

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Summary — A series of 3*a*,4-dihydro-5*H*-[1,2,4]oxadiazolo[5,4-*d*][1,5]benzothiazepines have been synthesized by 1,3-dipolar cycloaddition reaction of benzonitriloxide to the C=N double bond of 1,5-benzothiazepine derivatives, and the stereochemical features of compounds obtained have been determined by NMR spectroscopy. The results of evaluation of their activity in preventing seizures induced by audiogenic stimulation in DBA/2 mice are also reported and discussed. The 5-(4-bromophenyl)-1,3-diphenyl derivative **3b**, the most active compound of the series, is over 20 times more active than the parent benzothiazepine **1b** and shows an activity comparable to clobazam and better than desmethylclobazam.

[1,2,4]oxadiazolo[5,4-*d*][1,5]benzothiazepine / anticonvulsant activity

Introduction

A large number of people worldwide suffer from epilepsy and many of them still experience inadequate treatment. In fact the therapeutic efficacy of available antiepileptic drugs cannot be defined as totally satisfactory because at least 25% of people affected by epilepsy have seizures that are resistant to available therapies. Moreover, the most marketed anticonvulsants possess a broad range of undesirable side-effects. This warrants continuing research for anticonvulsant agents with more selective activity and lower toxicity to contribute to the improvement of life conditions of epilepsy sufferers whose sole hope is eventual development of more effective clinical strategies [1].

The choice to address our research towards cyclofunctionalized 1,5-benzothiazepine derivatives depended on the consideration that several annelated 1, 5-benzothiazepines exhibit anticonvulsant activity comparable to that of diazepam and show affinity for both peripheral and central benzodiazepine receptors (BZRs) [2–4].

In the present report, the synthesis of 5*H*-[1,2,4]-oxadiazolo[5,4-*d*][1,5]benzothiazepines **3** is described and the results of the evaluation of their activity in preventing seizures induced by acoustic stimulation in DBA/2 mice are also reported and discussed. This strain of mice is genetically susceptible to sound-induced seizures and is considered an excellent animal model for the study of certain kinds of human epilepsy and for testing new anticonvulsant agents [5].

For a better and wider definition of structure–activity relationships, derivatives **3a** and **3k–m**, previously synthesized by us [6], were also examined.

Chemistry

The synthesis of the compounds under study (**3b–j**, new compounds) was carried out according to the previously reported method [6]. The reaction between 1,5-benzothiazepine derivatives **1a–m** and benzonitril-oxide, generated *in situ* from benzohydroximinoyl chloride **2** and triethylamine, leads to 3*a*,4-dihydro-1-phenyl-5*H*-[1,2,4]oxadiazolo[5,4-*d*][1,5]benzothiazepines **3a–m** (scheme 1) in which an oxadiazole ring is fused at the 'd' edge of the heptatomic nucleus. The cycloaddition reaction has been found to be regiospecific and affords a single regioisomer, according to the FMO approach. The 6,6-dioxide derivative **4b** was obtained by oxidation of compound **3b** with hydrogen peroxide.

The stereochemistry of the synthesized compounds was unambiguously determined by NOE measure-

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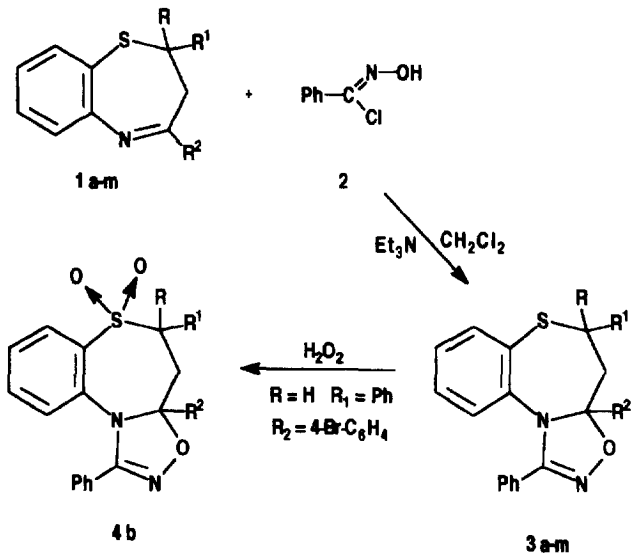

Compound	*R*	*R¹*	*R²*
a	H	C_6H_5	C_6H_5
b	H	4-BrC_6H_4	C_6H_5
c	H	4-ClC_6H_4	C_6H_5
d	H	4-CNC_6H_4	C_6H_5
e	H	4-FC_6H_4	C_6H_5
f	H	4-$MeOC_6H_4$	C_6H_5
g	H	4-MeC_6H_4	C_6H_5
h	H	4-$NO_2C_6H_4$	C_6H_5
i	H	4-$CF_3C_6H_4$	C_6H_5
j	H	2-Thienyl	C_6H_5
k	H	C_6H_5	CH_3
l	CH_3	CH_3	CH_3
m	H	CH_3	CH_3

Scheme 1.

ments in combination with analysis of proton coupling constants, which, according to our previous studies [6], allowed us to establish that the heptatomic ring adopts a twisted-boat conformation in compounds **3a–m** to avoid steric hindrance between the exocyclic substituents. The 5-substituent occupies a quasi-equatorial position in the predominant conformation and the substituent at C-3*a* occupies a nearly axial position.

Pharmacological results and discussion

The anticonvulsant effects of 1,5-benzothiazepine precursors **1a** and **1b**, 5*H*-[1,2,4]oxadiazolo[5,4-*d*]-[1,5]benzothiazepines **3a–m** and 6,6-dioxide derivative **4b** were studied after intraperitoneal administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures.

Table I reports the median effective dose (ED_{50}) values required to prevent tonic and clonic phases of seizures in audiogenic tests. The tested compounds showed anticonvulsant properties with the following rank order: **3b** > **4b** > **3f** > **3g** > **3k** > **3m** > **1a**, **b**, **3a**, **3c–e**, **3h–j** and **3l**.

It is remarkable to note that 3*a*,4-dihydro-5-(4'-bromophenyl)-1,3-diphenyl-5*H*-[1,2,4]oxadiazolo[5,4-*d*][1,5]benzothiazepine **3b**, the most active compound of the series, is over 20 times more active in preventing induced seizures in DBA/2 mice than the parent 1,5-benzothiazepine **1b** and shows a better anticonvulsant activity than that of desmethylclobazam and comparable to that of clobazam, two clinically useful anticonvulsant 1,5-benzodiazepines. Therefore compound **3b** may represent a valuable candidate for further therapeutic investigations.

The sulfone **4b**, a possible oxidative metabolite of compound **3b**, also shows a significant activity even if it is slightly lower to that of the parent compound **3b**. The time course study (fig 1) indicates that compound **3b** has a more intense and prolonged activity with respect to **4b**, but the graph shows a similar trend thus suggesting that *in vivo* compound **3b** could be converted into derivatives **4b**.

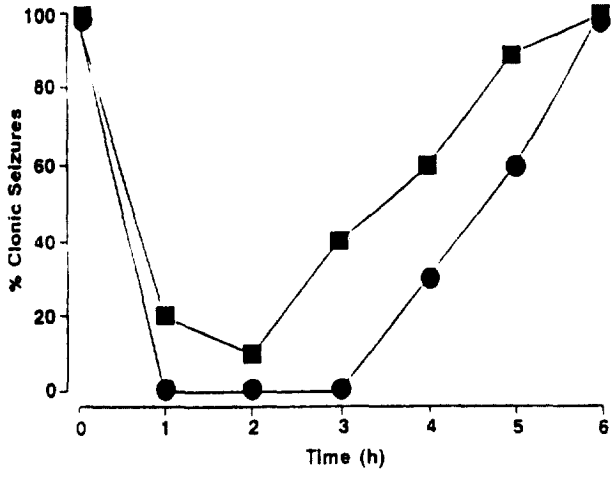

Fig 1. Anticonvulsant effects at a dose of 30 μmol/kg ip of compounds **3b** (circles) and **4b** (squares) against audiogenic seizures in DBA/2 mice. The ordinate shows percentage of response; the abscissa shows the time after intraperitoneal administration of drug in hours. Ten animals were used for the determination of each point.

Table I. Relative lipophilicity (R_m) and ED_{50} values (with 95% confidence limits) of various benzothiazepine derivatives against the clonic and tonic phases of the audiogenic seizures[a].

Compound	R_m	*Clonus*	*Tonus*
1a	0.308	> 200	> 200
1b	0.432	> 200	> 200
3a	0.368	> 200	> 200
3b	0.499	8.9 (5.8–13.8)	8.5 (5.7–12.7)
3c	0.475	> 200	> 200
3d	0.308	> 200	> 200
3e	0.450	> 200	> 200
3f	0.368	182 (68.9–481.7)	143 (59.5–344.1)
3g	0.337	63.8 (24.3–167.6)	45.9 (22.5–93.7)
3h	0.327	> 200	> 200
3i	0.479	> 200	> 200
3j	0.368	> 200	> 200
3k	0.213	119.1 (102.1–139)	104 (89.7–120.7)
3l	0.142	> 200	> 200
3m	0.101	109.3 (93–128.5)	104 (89.7–120.7)
4b	0.160	21.0 (17.2–25.8)	19.3 (15.4–24.1)
Clobazam		6.6 (4.43–9.38)	5.12 (4.20–6.24)
Desmethylclobazam		18.8 (9.56–36.82)	9.26 (4.67–18.39)

[a]All data are expressed as μmol/kg ip.

Regarding the correlation between structure and activity in the oxadiazolobenzothiazepine derivatives, some trends could be envisaged. Among 3*a*,5-diphenyl-substituted derivatives (**3a–i**) the introduction of methyl or methoxy groups, and especially a bromine atom, at position 4' of the phenyl ring at C-5 increases the activity with respect to that of the non-substituted derivative **3a**. Other substituents negatively influence the activity.

The presence of a methyl group at the 3*a* position in compounds **3k** and **3m** enhances the activity with respect to the corresponding 3*a*-phenyl-substituted derivative **3a**. Experiments on this group of compounds are in progress and will be detailed in future reports.

The varying degree of anticonvulsant activity exhibited by the derivatives reported here cannot be directly related to their lipophilicity. In fact, compounds possessing very similar lipophilicity (R_m) (table I), such as **3b** and **3i**, showed a different degree of anticonvulsant activity, suggesting the importance of other parameters. It is possible that the different potency is due to a different transport mechanism of diffusion through the blood–brain barrier, providing an easier access to the central nervous system for more active compounds. The mechanism of action of the tested compounds is currently under investigation.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a C Erba Model 1106 Elemental Analyzer and were found to be within ±0.4% of the theoretical values. Merck silica-gel F_{254} plates were used for TLC. Column chromatography was performed with Merck silica gel 60 (70–230 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian Gemini 300 in $CDCl_3$ with TMS as the internal standard; chemical shifts are in δ (ppm) and coupling constants (*J*) in hertz.

General procedure for the synthesis of 3a,4-dihydro-1-phenyl-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepines ***3b–j***

Benzohydroximinoyl chloride (230 mg, 1.5 mmol) was added under stirring to a solution of the appropriate 1,5-benzothiazepine derivative (1 mmol) in 30 ml of methylene chloride and a solution of triethylamine (150 mg, 1.5 mmol) in the same solvent (5 ml) was added dropwise. The reaction mixture was kept under stirring at room temperature for 24 h. After completion of the reaction, the solvent was evaporated off at reduced pressure, and ether was added to the residue and the triethylamine hydrochloride was filtered. After removal of the solvent, the residue was purified by treatment with ether and, for compound **3d**, by column chromatography eluting with diethyl ether/light petroleum 20:80. All compounds were recrystallized from ethanol.

3a,4-Dihydro-1,3a-diphenyl-5-(4-bromophenyl)-5H-[1, 2,4]-oxadiazolo[5,4-d][1,5]benzothiazepine ***3b***. Mp: 218–220°C (yield 45%). ^{1}H-NMR: 2.57 (dd, J = –14.27 and J = 13.73, 1H, H_{ax}-4), 2.91 (dd, J = –14.27 and J = 4.70, 1H, H_{eq}-4), 3.44 (dd, J = 4.70 and J = 13.73, 1H, H-5), 6.74–8.11 (m, 18H, ArH). ^{13}C-NMR: 39.02 (C-4), 45.16 (C-5), 99.97 (C-3*a*), 121.27 (C-Br), 122.10 (C-6*a*), 123.60, 125.31, 127.29, 127.72, 128.02, 128.13, 129.03, 130.25, 131.04, 131.80, 137.23 (aromatic CH), 126.37 (C-1'), 140.27 (C-10*a*), 142.94 and 145.22 (C-1" and C-1'") 157.72 (C-1). Anal $C_{28}H_{21}BrN_2OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-chlorophenyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine ***3c***. Mp: 190–192°C (yield 80%). ^{1}H-NMR: 2.58 (dd, J = –14.03 and J = 13.59, 1H, H_{ax}-4), 2.92 (dd, J = –14.03 and J = 4.42, 1H, H_{eq}-4), 3.46 (dd, J = 4.42 and J = 13.59, 1H, H-5), 6.75–8.10 (m, 18H, ArH). ^{13}C-NMR: 39.02 (C-4), 45.08 (C-5), 99.97 (C-3*a*), 122.10 (C-6*a*), 133.18 (C-Cl), 123.56, 125.30, 127.29, 127.70, 127.99, 128.83, 128.92, 129.02, 130.24, 131.03, 137.20, (aromatic CH), 126.35 (C-1'), 140.25 (C-10*a*), 142.41 and 145.19 (C-1" and C-1'"), 157.74 (C-1). Anal $C_{28}H_{21}ClN_2OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-cyanophenyl)-5H-[1,2,4]-oxadiazolo[5,4-d][1,5]benzothiazepine ***3d***. Mp: 245–247°C (yield 52%). ^{1}H-NMR: 2.58 (dd, J = –14.72 and J = 13.67, 1H, H_{ax}-4), 2.92 (dd, J = –14.72 and J = 4.83, 1H, H_{eq}-4), 3.57 (dd, J = 4.83 and J = 13.67, 1H, H-5), 6.75–8.10 (m, 18H, ArH). ^{13}C-NMR: 38.75 (C-4), 45.34 (C-5), 99.69 (C-3*a*), 111.18 (C-CN), 118.50, (CN), 121.49 (C-6*a*), 123.56, 125.30, 127.15, 127.60, 128.00, 128.80, 128.95, 129.02, 130.45, 131.02, 132.49, 137.00 (aromatic CH), 126.12 (C-1'), 139.92 (C-10*a*), 144.32 and 145.12 (C-1" and C-1'"), 157.43 (C-1). Anal $C_{29}H_{21}N_3OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-fluorophenyl)-5H-[1,2,4]-oxadiazolo[5,4-d][1,5]benzothiazepine ***3e***. Mp: 198–200°C (yield 37%). ^{1}H-NMR: 2.59 (dd, J = –14.45 and J = 13.49, 1H, H_{ax}-4), 2.92 (dd, J = –14.45 and J = 4.52, 1H, H_{eq}-4), 3.49 (dd, J = 4.52 and J = 13.49, 1H, H-5), 6.74–8.12 (m, 18H, ArH). ^{13}C-NMR: 38.49 (C-4), 45.15 (C-5), 100.04 (C-3*a*), 22.16 (C-6*a*), 115.32, 123.50, 125.20, 127.21, 127.64, 127.90, 128.00, 128.83, 128.93, 130.10, 130.94, 137.12 (aromatic CH), 126.32 (C-1'), 139.72 (C-10*a*), 140.23 and 145.10 (C-1" and C-1'"), 157.49 (C-l), 161.92 (C-F, J = 248.27). Anal $C_{28}H_{21}FN_2OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-methoxyphenyl)-5H-[1,2,4]-oxadiazolo[5,4-d][1,5]benzothiazepine ***3f***. Mp: 183–185°C (yield 48%). ^{1}H-NMR: 2.61 (dd, J = –14.56 and J = 13.89, 1H, H_{ax}-4), 2.91 (dd, J = –14.56 and J = 4.78, 1H, H_{eq}-4), 3.47 (dd, J = 4.78 and J = 13.89, 1H, H-5), 3.76 (s, 3H, CH_3), 6.75–8.12 (m, 18H, ArH). ^{13}C-NMR: 39.38 (C-4), 45.36 (C-5), 55.24 (CH_3), 100.13 (C-3*a*), 122.51 (C-6*a*), 113.93, 123.41, 125.08, 125.44, 127.23, 127.37, 127.62, 127.82, 128.71, 128.90, 130.86, 137.18 (aromatic CH), 126.37 (C-1'), 140.34 (C-10*a*), 136.10 and 145.05 (C-1" and C-1'") 157.46 (C-1) 158.75 (C-OCH_3). Anal $C_{29}H_{24}N_2O_2S$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-methylphenyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine ***3g***. Mp: 170–172°C (yield 50%). ^{1}H-NMR: 2.28 (s, 3H, CH_3), 2.62 (dd, J = –14.51 and J = 13.6, 1H, H_{ax}-4), 2.91 (dd, J = –14.51 and J = 4.42, 1H, H_{eq}-4), 3.46 (dd, J = 4.42 and J = 13.6, 1H, H-5), 6.74–8.11 (m, 18H, ArH). ^{13}C-NMR: 21.03 (CH_3), 39.20 (C-4), 45.60 (C-5), 100.12 (C-3*a*), 122.55 (C-6*a*), 123.40, 125.09, 126.17, 127.22, 127.63, 127.83, 128.72, 128.90, 129.28, 129.90, 130.87, 137.19, (aromatic CH), 126.38 (C-1'), 137.13 (C-CH_3), 140.36 (C-10*a*), 140.93 and 145.05 (C-1" and C-1'"), 157.48 (C-1). Anal $C_{29}H_{24}N_2OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-nitrophenyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine ***3h***. Mp: 244–246°C (yield 68%). ^{1}H-NMR: 2.64 (dd, J = –14.58 and J = 13.68, 1H, H_{ax}-4), 2.97 (dd, J = –14.58 and J = 4.36, 1H, H_{eq}-4), 3.46 (dd, J = 4.36 and J = 13.68, 1H, H-5), 6.78–8.13 (m, 18H, ArH). ^{13}C-NMR: 38.81 (C-4), 45.13 (C-5), 99.72 (C-3*a*), 121.44 (C-6*a*), 123.70, 125.52, 127.01, 127.36, 127.64, 128.90, 129.28, 129.00, 130.56, 131.38, 134.99, 137.08 (aromatic CH), 126.35 (C-1'), 139.98 (C-10*a*), 148.40 (C-NO_2), 145.24 and 147.07 (C-1" and C-1'") 157.43 (C-1). Anal $C_{28}H_{21}N_3O_3S$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(4-trifluoromethylphenyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine ***3i***. Mp: 200–203°C (yield 39%). ^{1}H-NMR 2.62 (dd, J = –14.53 and J = 13.55, 1H, H_{ax}-4), 2.94 (dd, J = –14.63 and J = 4.53, 1H, H_{eq}-4), 3.52 (dd, J = 4.53 and J = 13.55, 1H, H-5), 6.76–8.13 (m, 18H, ArH). ^{13}C-NMR: 39.09 (C-4), 45.38 (C-5), 99.93 (C-3*a*), 121.90 (C-6*a*), 123.68, 125.40, 125.70, 125.74, 126.81, 127.27, 127.72, 128.06, 129.04, 130.40, 131.02, 137.20 (aromatic CH), 124.5 (C-F, J = 272), 126.32 (C-1'), 130.05 (C-CF_3), 140.17 (C-10*a*), 145.23 and 147.72 (C-1" and C-1'"), 157.56 (C-1). Anal $C_{29}H_{21}F_3N_2OS$ (C, H, N).

3a,4-Dihydro-1,3a-diphenyl-5-(2-thienyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine ***3j***. Mp: 198–201°C (yield 39%). ^{1}H-NMR: 2.54 (dd, J = –14.69 and J = 13.57, 1H, H_{ax}-4), 3.02 (dd, J = –14.69 and J = 4.74, 1H, H_{eq}-4), 3.62 (dd, J = 4.74 and J = 13.57, 1H, H-5), 6.72–8.09 (m, 17H, ArH). ^{13}C-NMR: 38.79 (C-4), 41.20 (C-5), 100.06 (C-3*a*), 122.08 (C-6*a*), 120.09, 123.36, 125.09, 126.25, 127.33, 127.62, 127.83, 128.72, 128.90, 130.02, 130.89, 137.30 (aromatic CH), 140.13 (C-10*a*), 143.09 and 145.20 (C-1" and C-1'"), 157.37 (C-1). Anal $C_{26}H_{20}N_2OS_2$ (C, H, N).

Synthesis of 3a,4-dihydro-1,3a-diphenyl-5-(4-bromophenyl)-5H-[1,2,4]oxadiazolo[5,4-d][1,5]benzothiazepine-6,6-dioxide ***4b***

A solution of the **3b** (5 mmol) in 50 ml acetic acid was treated with 1.5 g of H_2O_2 27%. The mixture was refluxed for 5 h, cooled to room temperature and, after 24 h, afforded product **4b** which was recrystallized from $CHCl_3$. Mp: 240–243°C (yield 68%). ^{1}H-NMR: 3.15 (dd, J = –13.83 and J = 1.53, 1H, H_{eq}-4), 3.90 (dd, J = –13.83 and J = 12.60, 1H, H_{ax}-4), 4.38 (dd, J = 1.53 and J = 12.60, 1H, H-5), 6.93–8.03 (m, 18H, ArH). ^{13}C-NMR: 38.48 (C-4), 62.93 (C-5), 101.42 (C-3*a*), 123.93 (C-6*a*), 124.09 (C-Br), 127.27, 127.62, 128.70, 128.88, 129.01, 129.10, 129.80, 130.80, 131.38, 131.39, 134.13 (aromatic CH), 130.11, 134.66 and 135.64 (C-1', C-1" and C-1'"), 153.81 (C-1). Anal $C_{28}H_{21}BrN_2O_3S$ (C, H, N).

Lipophilicity measurements

The relative lipophilicity of the compounds was measured by reversed-phase thin-layer chromatography according to the previously described method [7]. Silanized silica-gel plates Merck 60 F_{254} were used as nonpolar stationary phase. The plates were dried at 105°C for 1 h before use. The polar mobile phase was a 2:1 v/v mixture of acetone and water. Each compound was dissolved in chloroform (3 mg/ml) and 5 µl of solution was applied onto the plate. The experiments were

repeated five times with different disposition of the compounds on the plate. The R_f values were expressed as the mean values of the five determinations. The R_m values were calculated from the experimental R_f values according to the formula $R_m = \log[(1/R_f) - 1]$. Higher R_m values indicate higher lipophilicity.

Pharmacology

The anticonvulsant properties of these derivatives were evaluated in DBA/2 mice, which are genetically susceptible to sound-induced seizures [4]. DBA/2 mice (6–12 g, 21–28 d old) were purchased from Charles River (Calco, Como, Italy) and were exposed to auditory stimulation, 45 min following intraperitoneal (ip) administration of vehicle or drugs. For systemic injections, the compounds were given intraperitoneally (0.1 ml/10 g body weight of the mouse) as a freshly prepared solution in 50% dimethylsulfoxide (DMSO) and 50% sterile saline (0.9% NaCl). Individual mice were placed under a hemispheric Perspex dome (diameter 58 cm) and 60 s was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 60 s or until tonic extension occurred. Seizure response was assessed on the characteristic sequence consisting of wild running, clonus, tonus and, frequently, respiratory arrest. The control and drug-treated mice were scored for latency to and incidence of the different phases of the seizures. These results were compared with the activity shown by clinically useful anticonvulsant 1,5-benzodiazepines such as clobazam and desmethylclobazam; ED_{50} values were calculated by the method of probits analysis [8].

Acknowledgments

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