Med Chem Res (2012) 21:3428–3434
DOI 10.1007/s00044-011-9869-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In vivo screening of diarylimidazoles as anticonvulsant agents

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Received: 22 July 2011 / Accepted: 31 October 2011 / Published online: 19 November 2011

Abstract In this study, the anticonvulsant activity evaluation of a series of new 2,4(1*H*)-diarylimidazoles, characterized by the presence of different substituents on the aryl rings, was described. The anticonvulsant activity profile of those compounds was determined by Maximal Electroshock Seizure (MES), subcutaneous Metrazol Seizure (scMet) tests, and 6-Hz seizure model, whereas their neurotoxicity was examined using rotarod test. The trifluoromethoxy derivative, obtained starting from phenylglyoxal, *p*-OCF_3-benzaldehyde, and ammonium acetate in methanol (r.t.), exhibited a great ability to prevent the seizures induced in the 6-Hz test, becoming a new promising molecule to develop for the treatment of therapy-resistant partial seizures.

Keywords Epilepsy · Diarylimidazoles · In vivo evaluations · MES · 6 Hz · Toxicity

Introduction

Epilepsy is a very common neurological disease that affect about 0.5–1.0% of worldwide population (Sander, 2003). The major numbers of cases is detected among children, or people over 65 years; however, it can occur at any time. There are different types of epilepsy: in generalized seizures, for example, the origin is in both hemispheres, while focal seizures involve only a portion of the brain, in particular structures located in the temporal and frontal lobes (McCormick and Contreras, 2001). Currently, many treatments are available that can successfully prevent seizures; however, approximately one-third of all cases of seizures cannot be controlled (Kwan and Brodie, 2000; Dodson, 2004; Zuliani *et al.*, 2009). Moreover, many marketed antiepileptic drugs (AEDs) cause several side effects and drug interactions, which limit their clinical use (Baker *et al.*, 1997; Carrenom *et al.*, 2008; Worthen *et al.*, 2009). These evidences suggest an urgent need for more effective and better tolerated AEDs. Previous studies published from our group have identified analogues of the 2,4(1*H*)-diarylimidazole as being potent $hNa_V1.2$ sodium channel blockers able to provide protection against seizures induced in the maximal electroshock seizure (MES) model (Rivara *et al.*, 2008; Fantini *et al.*, 2009; Zuliani *et al.*, 2010). In this study, we decided to expand this class of interesting molecules, introducing different substituents on the aryl rings, with the aim to evaluate the ability of these new diarylimidazoles to inhibit seizures in various in vivo tests.

Results and discussion

Chemistry

The molecules here presented were synthesized through parallel synthesis employing a synthetic protocol previously described by us (Zuliani *et al.*, 2007). In particular, compounds **1–7** were prepared through parallel synthesis, starting from the appropriate phenylglyoxal **a**, benzaldehyde **b**, ammonium acetate as ammonia source, and a polar protic solvent, such as methanol, at room temperature (Scheme 1). The structures of the compounds were proved by their ^{1}H-NMR spectroscopic data and elemental analysis data (CHN).

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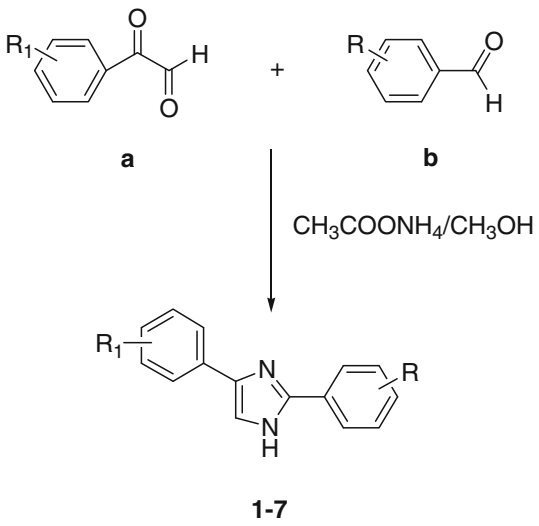


Scheme 1 Synthesis of the 2,4(1*H*)-diarylimidazoles **1–7**. *Reagents and conditions* 1 equiv. **a** in 3.8 ml CH_3OH; 1 equiv. **b** and 4 equiv. CH_3COONH_4 in 3.5 ml CH_3OH. Overnight at r.t

Pharmacology

The compounds here presented were tested at the National Institute of Neurological Disorders and Stroke (NINDS), via the Anticonvulsant Screening Program (ASP). The in vivo anticonvulsant activity evaluation was performed following the procedures proposed by the NINDS, via the ASP. In particular, various electrical seizures models were used: MES test, subcutaneous Metrazol Seizure (scMet) test, and 6-Hz minimal clonic seizure model. All the compounds synthesized were tested i.p to determine their ability to prevent seizures at different times and at different doses. For the best molecules, the ED_{50} was also measured. The in vivo evaluation involved the assessment of toxicity using the standardized rotarod test, which determines the minimal motor impairment of the animals, which are not genetically modified and predisposed to seizures (Dunham and Miya, 1957).

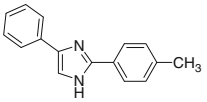
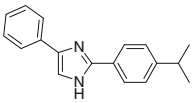
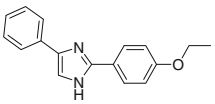
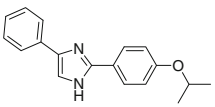
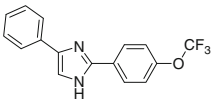
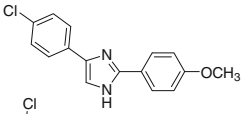
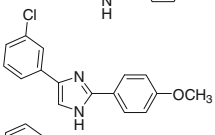
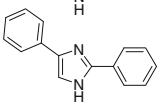
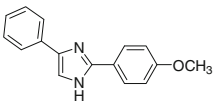
Maximal electroshock seizure model produces the spread of seizure similar to grand mal epilepsy, resulting useful for finding drugs to treat human generalized tonic-clonic seizures. Instead, scMet model identifies those compounds which raise seizure threshold, being useful for finding drugs to treat human generalized myoclonic seizures (White *et al.*, 2002). Both these tests are widely used for the first screening and early identification of new AEDs, but sometimes they can fail and do not identify molecules able to treat different types of seizures. For example, this has happened with levetiracetam, a very potent anticonvulsant drug useful for the treatment of therapy-resistant partial seizures. For this reason, all the molecules synthesized were submitted to an additional test, named 6-Hz seizure model, to have a complete view of their possible action. In fact, in this model, a low-frequency (6 Hz), long-duration (3 s) stimulus is delivered through corneal electrodes, and the ability of the molecules to prevent the raising of the seizures so induced is tested (Chen *et al.*, 2007).

The results are reported in Tables 1 and 2 and they are expressed as the number of animals protected out of the number of animals tested over time. Compounds **8** and **9** were added for comparison and they have been previously reported (Zuliani *et al.*, 2010).

In the Phase I, preliminary anticonvulsant screening all the compounds administered at 30, 100, and 300 mg/kg doses showed some degree of protection in MES test, whereas they were virtually inactive in the scMet screen. In the neurotoxicity screening, some molecules were completely devoid of minimal motor impairment (**1**, **2**, **6**, **7**), and the others showed only low toxicity, usually at high doses (300 mg/kg). Considering the results showed in Table 1, it is possible to observe an interesting behavior. In fact, the introduction of a chlorine on the phenyl ring in the 4 position on the imidazole is detrimental for the anticonvulsant activity at medium doses (100 mg/kg) and 0.5 h after administration of the drug, regardless of the position of the chlorine substituent, as demonstrated comparing compounds **6** and **7** with **8**. Nevertheless, considering a different time interval (4 h), the presence of the chlorine did not modify the activity (cf. **7** and **9**) or increased it (cf. **6** and **9**). These compounds, active at 100 and 300 mg/kg, after 4 h, have quick onset and long duration of action at relatively higher dose. It is also very important to consider that compounds **6** and **7** are completely devoid of toxicity, also at high doses, unlike the non-chlorine compound **8**. Another interesting observation is that the introduction of an electron releasing substituent similar to $–OCH_3$, as $–OCF_3$ is detrimental for the anticonvulsant activity, in the MES test, as can be evidenced by comparison of the diarylimidazole **5** with the reference molecules **8** and **9**.

The synthesized compounds were also tested in the 6-Hz model to identify their activity at five different time points, i.e., 0.25, 0.5, 1.0, 2.0, and 4 h. All the compounds administered at 100 mg/kg showed some activity in this test but, surprisingly, the best molecule is the trifluoromethoxy derivative **5**. It displayed 100% protection against induced convulsions at 0.25, 0.5, and 1 h at the dose of 100 mg/kg, and also had moderate activity at 2 and 4 h after drug administration, being more active that the reference compound **8**. The most informative compounds (**6** and **7**) were evaluated quantitatively in phase II screening (MES and scMet) in which the ED_{50} and TD_{50} were determined. Moreover, for compounds **1**, **5**, and **8**, the ED_{50} in the 6-Hz test were determined. The results

Table 1 Anticonvulsant activity and toxicity of compounds **1–9** administered intraperitoneally to mice

Comp.	Structure	Dose (mg/kg)	MES[a]		scMet[b]		Tox[c]	
			0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]	0.5 h[d]	4 h[d]
1		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	2/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
2		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
3		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	2/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	0/1	0/1	1/5	0/4	0/2
4		30	1/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	0/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	4/4	1/2
5		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	1/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	1/1	0/1	4/4	0/2
6		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	1/3	3/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
7		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	0/3	2/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	0/1	0/1	0/4	0/2
8[e]		30	1/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	0/3	0/1	0/1	1/8	0/4
		300	1/1	1/1	0/1	0/1	4/4	1/2
9[e]		30	0/1	0/1	0/1	0/1	0/4	0/2
		100	3/3	2/3	0/1	0/1	0/8	0/4
		300	1/1	1/1	1/1	0/1	4/4	2/2

[a] Maximal electroshock test (number of animals protected/number of animals tested)

[b] Subcutaneous metrazol test (number of animals protected/number of animals tested)

[c] Neurotoxicity (number of animals protected/number of animals tested)

[d] Time after drug administration

[e] Fantini *et al.* (2009)

obtained are reported in Tables 3 and 4, respectively, along with the data on the standard drug phenytoin.

The data reported in Table 3 showed that the newly synthesized compounds **6** and **7** exhibited similar activity to the lead compounds **8** and **9** in the MES test, but compound **7** had lower neurotoxicity and a PI value superior to that of **8** and **9**. Interestingly, the PI value of **7** in the scMet test is much higher than that of the reference molecules. Table 4 reports the quantitative anticonvulsant activity of compounds **1**, **5**, and **8** in the 6-Hz screen. The results obtained are very interesting, in fact the new molecule **5** ($-OCF_3$), less active of the parent unsubstituted compound **8** in the qualitative MES test, is absolutely more active in the 6 Hz, with a protective index higher than **8** and than the standard drug phenytoin.

In conclusion, in this study, as a continuation of our research on AEDs, the anticonvulsant activity evaluation of a series of new 2,4(1*H*)-diarylimidazoles have been described. The data obtained revealed that almost all of the derivatives synthesized were effective in the MES screen and some of the active compounds are devoid of toxicity. The most interesting result concerns compound **5**, characterized by the presence of an electron donor trifluoromethoxy group introduced instead of the methoxy one of the reference compound **9**. In fact, **5** showed a lower activity in the MES screen, but a great ability to prevent the

Table 2 Anticonvulsant activity by psychomotor seizure test of compounds **1–9** (6-Hz, dose of 100 mg/kg)

Comp.	0.25 h[a]	0.5 h[a]	1.0 h[a]	2.0 h[a]	4.0 h[a]
1	3/4	2/4	2/4	1/4	0/4
2	1/4	1/4	0/4	1/4	1/4
3	2/4	1/4	0/4	0/4	0/4
4	2/4	1/4	1/4	0/4	0/4
5	4/4	4/4	4/4	1/4	1/4
6	1/4	3/4	2/4	0/4	0/4
7	3/4	3/4	0/4	1/4	0/4
8	4/4	4/4	4/4	0/4	0/4
9	2/4	2/4	1/4	0/4	0/4

Number of animals active over the number of animals tested

[a] Time after drug administration

Table 4 Quantitative anticonvulsant activity (6 Hz) and toxicity in mice of compounds **1**, **5**, and **8**

Comp.	Test	ED_{50}	PI[a]
1	6-Hz	62.4	3.0
	TOX	188.0	
5	6-Hz	28.2	4.8
	TOX	135.8	
8	6-Hz	47.2	2.7
	TOX	126.8	
Phe[b]	6-Hz	27.5	1.3
	TOX	35.6	

Phe phenytoin

[a] Protective index ($PI = TD_{50}/ED_{50}$)

[b] Lenkowski *et al.* (2007)

seizures induced in the 6-Hz test compared to the unsubstituted **8** and to the drug phenytoin. Considering that this test has been validated as a model of therapy-resistant epilepsy, compound **5** can be considered as a new promising molecule to develop for the treatment of therapy-resistant partial seizures.

Experimental

Synthesis

Melting points are not corrected and were determined using a Gallenkamp melting point apparatus. Final compounds were synthesized in parallel using Büchi Syncore® Reactor and were analyzed on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer, for C, H, and N. The percentages recorded were within ±0.4% of the theoretical values. The ^{1}H-NMR spectra were recorded on a Bruker 300 Avance spectrometer (300 MHz); chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. Selective isolation of either non-basic or basic compounds was obtained using ISOLUTE® SCX-2 columns (Particles size: 30–90 μM). Reactions were monitored by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck).

General procedure for the synthesis of 2,4(*1H*)-diarylimidazoles **1–7**

A solution of phenylglyoxal monohydrate (0.70 mmol) in methanol (3.8 ml) was added dropwise to a stirred suspension of arylaldehyde (0.70 mmol) and ammonium acetate (3.41 mmol) in methanol (3.5 ml). The reaction mixture was stirred overnight at room temperature, then the solvent was evaporated and the residue was partitioned between saturated aqueous $NaHCO_3$ solution (20 ml) and methylene chloride (20 ml). The organic phase was dried

Table 3 Quantitative anticonvulsant activity (MES and scMet) and toxicity in mice (i.p.; tested at 0.25 h) of compounds **6–9**

Comp.	MES[a] ED_{50}^{b}	PI[c]	scMet[d] ED_{50}^{b}	PI[e]	$TD_{50}^{b,f}$
6	68.8 (56.1–88.4)	3.5	158.1 (134.4–189.5)	1.5	238.0 (209.8–258.6)
7	82.1 (73.1–88.8)	4.7	102.7 (69.8–133.6)	3.8	390.3 (333.6–518.2)
8[g]	61.7 (51.9–71.4)	2.1	160.9 (138.6–187.0)	0.8	126.8 (107.6–140.2)
9[g]	46.8 (44.0–52.0)	4.3	142.2 (103.6–203.4)	1.4	200.8 (163.0–243.6)
Phe[h]	9.5 (8.1–10.4)	6.9	–	–	65.5

Phe phenytoin

[a] Maximal electroshock test

[b] The interval in parentheses stands for the 95% confidence interval

[c] Protective index ($PI = TD_{50}/ED_{50}$) in the MES test

[d] Subcutaneous metrazol test

[e] Protective index ($PI = TD_{50}/ED_{50}$) in the scMet test

[f] Neurotoxicity

[g] Fantini *et al.* (2009)

[h] Cui *et al.* (2009) and Lenkowski *et al.* (2007)

over Na_2SO_4 and the solvent was removed in vacuo. The isolation of the target compounds from the crude reaction mixture was obtained using SCX-2 column (2 g, 30–90 μm, loading 0.4 meq/g). The column was pre-washed with DCM:methanol = 1:1 (10 ml), the side products were eluted with methanol (10 ml), and then the desired 2,4(5)-arylimidazoles were eluted with a methanolic ammonia 5% w/w solution (10 ml). All the products were then crystallized as the hydrochloride salt from absolute ethanol/diethyl ether.

2-(4-Methylphenyl)-4(5)-phenylimidazole (**1**)

79% yield, mp. >250°C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (s, 1H), 8.14 (d, 2H, J = 8.3 Hz), 7.99 (d, 2H, J = 8.5 Hz), 7.45–7.56 (m, 5H), 2.42 (s, 3H). Anal. Calcd. for $C_{16}H_{14}N_2 \cdot HCl \cdot H_2O$: C, 66.55; H, 5.93; N, 9.70. Found: C, 66.68; H, 5.96; N, 9.69.

2-(4-Isopropylphenyl)-4(5)-phenylimidazole (**2**)

72% yield, mp. 229–232°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.25 (d, 3H, J = 8.4 Hz), 8.00 (d, 2H, J = 7.5 Hz), 7.42–7.55 (m, 5H), 2.98–3.02 (m, 1H), 1.25 (d, 6H, J = 7.2 Hz). Anal. Calcd. for $C_{18}H_{18}N_2 \cdot HCl \cdot 1/2C_2H_5OH$: C, 70.91; H, 6.89; N, 8.70. Found: C, 71.15; H, 6.75; N, 8.60.

2-(4-Ethoxyphenyl)-4(5)-phenylimidazole (**3**)

82% yield, mp. 228–234°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (d, 3H. J = 9.9 Hz), 7.97 (d, 2H, J = 7.5 Hz), 7.45–7.56 (m, 3H), 7.21 (d, 2H, J = 8.7 Hz), 4.16 (q, 2H), 1.37 (t, 3H, J = 6.9 Hz). Anal. Calcd. for $C_{16}H_{17}N_2O \cdot HCl$: C, 67.88; H, 5.70; N, 9.31. Found: C, 67.88; H, 5.65; N, 9.10.

2-(4-Isopropoxyphenyl)-4(5)-phenylimidazole (**4**)

81% yield, mp. 222–225°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (d, 3H, J = 8.8 Hz), 8.00 (d, 2H, J = 8.5), 7.55–7.43 (m, 3H), 7.19 (d, 2H, J = 8.9 Hz), 4.76–4.84 (m, 1H), 1.31 (d, 6H, J = 6.0 Hz). Anal. Calcd. for $C_{18}H_{18}N_2O \cdot HCl$: C, 68.67; H, 6.08; N, 8.90. Found: C, 68.96; H, 6.34; N, 8.86.

2-(4-Trifluoromethoxyphenyl)-4(5)-phenylimidazole (**5**)

75% yield, mp. 223°C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.40 (d, 2H, J = 6.6 Hz), 8.27 (s, 1H), 8.01 (d, 2H, J = 5.7 Hz), 7.68 (d, 2H, J = 6.5 Hz), 7.53 (t, 2H, J = 5.7 Hz), 7.44 (t, 1H, J = 5.5 Hz). Anal. Calcd. for $C_{16}H_{11}N_2OF_3 \cdot HCl$: C, 56.40; H, 3.55; N, 8.22. Found: C, 56.21; H, 3.80; N, 8.09.

2-(4-Methoxyphenyl)-4(5)-(4-chlorophenyl)imidazole (**6**)

76% yield, mp. >270°C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.25 (s, 1H), 8.19 (d, 2H, J = 8.9 Hz), 8.02 (d, 2H, J = 6.7 Hz), 7.63 (d, 2H, J = 8.7 Hz), 7.23 (d, 2H, J = 8.9 Hz), 3.88 (s, 3H). Anal. Calcd. for $C_{16}H_{13}N_2OCl \cdot HCl$: C, 59.83; H, 4.39; N, 8.72. Found: C, 59.44; H, 4.37; N, 8.59.

2-(4-Methoxyphenyl)-4(5)-(3-chlorophenyl)imidazole (**7**)

70% yield, mp. 238–240°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32 (s, 1H), 8.23 (d, 2H, J = 8.9 Hz), 8.16 (t, 1H, J = 1.7 Hz), 7.99 (d, 1H, J = 7.7 Hz), 7.53 (t, 1H, J = 7.8 Hz), 7.48 (d, 1H, J = 8.1 Hz), 7.22 (d, 2H, J = 9.0 Hz), 3.88 (s, 3H). Anal. Calcd. for $C_{16}H_{13}N_2OCl \cdot HCl$: C, 59.83; H, 4.39; N, 8.72. Found: C, 60.13; H, 4.41; N, 8.75.

Description of animal testing methods for MES, scMet, 6-Hz and toxicity evaluations

Animals utilized in these experiments were male albino CF1 mice (18–25 g, Charles River, Portage, MI) experimental animals. All animals were allowed free access to both food (Prolab RMH 3000) and water except when they were removed from their cages for testing. Animals were used only once. All mice were housed, fed, and handled in a manner consistent with the recommendations in the National Research Council Publication, "Guide for the Care and Use of Laboratory Animals." No insecticides capable of altering hepatic drug metabolism enzymes were used in the animal facilities. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals. Compounds were administered in 0.5% methylcellulose intraperitoneally in a volume of 0.01 ml/g body weight. In vivo anticonvulsant activity was established using both an electrical and chemoconvulsant seizure test which have been described previously. The electrical tests used were the MES test and the 6-Hz test, whereas the scMet was used as the chemical test.

MES test

The MES is a model for generalized tonic-clonic seizures and provides an indication of a compound's ability to prevent seizure spread when all neuronal circuits in the

brain are maximally active. These seizures are highly reproducible and are electrophysiologically consistent with human seizures. For the MES, a drop of anesthetic/electrolyte solution (0.5% tetracaine hydrochloride in 0.9% saline) was applied to the eyes of each animal prior to placement of the corneal electrodes. The electrical stimulus in the MES test was 50 mA, 60 Hz, for mice, delivered for 0.2 s by an apparatus similar to that originally described by Woodbury and Davenport (Woodbury and Davenport, 1952; Swinyard *et al.*, 1989; White *et al.*, 1995). Abolition of the hindleg tonic extensor component of the seizure was used as the endpoint. Mice are initially tested at various intervals following doses of 30, 100, and 300 mg/kg of test compound given by i.p. injection are initially screened at a fixed dose of 30 mg/kg.

Subcutaneous Metrazol seizure threshold test (scMET)

Subcutaneous injection of the convulsant Metrazol produces clonic seizures in laboratory animals. The scMET test detects the ability of a test compound to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals are pretreated with various doses of the test compound given by i.p. injection. At the previously determined TPE of the test compound, the dose of Metrazol which will induce convulsions in 97% of animals (CD_{97}: 85 mg/kg mice) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress and observed for the next 30 min for the presence or absence of a seizure (Swinyard *et al.*, 1961). An episode of clonic spasms, approximately 3–5 s, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected.

The 6-Hz seizure test

It is an alternative electroshock paradigm that uses low-frequency (6 Hz), long-duration (3 s) electrical stimulation. Corneal stimulation (0.2 ms-duration monopolar rectangular pulses at 6 Hz for 3 s) was delivered by a constant-current device. During the stimulation, mice were manually restrained and released into the observation cage immediately after the current application. The seizures manifest in 'stunned' posture associated with rearing, forelimb, automatic movements, and clonus, twitching of the vibrissae and Straub-tail. The duration of the seizure activity ranges from 60 to 120 s in untreated animals. At the end of the seizure, animals resume their normal exploratory behavior. The experimental end point is protection against the seizure. The animal is considered to be protected if it resumes its normal exploratory behavior within 10 s from the stimulation (Barton *et al.*, 2001; Kaminski *et al.*, 2004).

Acute toxicity—minimal motor impairment

To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotarod procedure is used to disclose minimal muscular (MMI) or neurological impairment (Dunham and Miya, 1957). When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered toxic if it falls off this rotating rod three times during a 1 min period. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response, and changes in muscle tone.

Determination of median effective (ED_{50}) or behavioral toxic dose (TD_{50})

All quantitative in vivo anticonvulsant efficacy/toxicity studies were conducted at the previously determined TPE. Groups of at least eight mice were tested with various doses of the candidate drug until at least two points were established between the limits of 100% protection or minimal toxicity and 0% protection or minimal toxicity. The dose of drug required to produce the desired endpoint in 50% of animals (ED_{50} or TD_{50}) in each test, the 95% confidence interval, the slope of the regression line, and the SEM of the slope were then calculated by a computer program based on the method described by Finney (Finney, 1971).

Acknowledgments We are grateful to the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) for anticonvulsant evaluation. We are grateful to the Centro Interpartimentale Misure of the University of Parma for providing the NMR instrumentation.

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