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Synthesis and anticonvulsant properties of new *N*-[(4-arylpiperazin-1-yl)-methyl] derivatives of 3-aryl pyrrolidine-2,5-dione and 2-aza-spiro[4.4]nonane-1,3-dione☆

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Abstract

A series of *N*-[(4-arylpiperazin-1-yl)-methyl] derivatives of 3-arylpyrrolidine-2,5-dione and 2-aza-spiro[4.4]nonane-1,3-dione were synthesized and tested for anticonvulsant activity in the maximum electroshock seizure (MES) and metrazole seizure threshold (sc.MET) tests. The most potent in the series were *N*-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione ($ED_{50} = 14.18$ mg/kg) and *N*-[{4-(2-methoxyphenyl)-piperazin-1-yl}-methyl]-3-(3-bromophenyl)-pyrrolidine-2,5-dione ($ED_{50} = 33.64$ mg/kg). Structures of the novel compounds were confirmed by elemental and spectral analyses.

Keywords: Pyrrolidine-2,5-dione; 2-Aza-spiro[4.4]nonane-1,3-dione; Spirosuccinimides; Anticonvulsant activity

1. Introduction

Approximately 1% of the world population suffer from epilepsy. Clinically available anticonvulsant drugs produce satisfactory seizure control in 60–70% of patients. In the past decade, several new drugs e.g., felbamate, lamotrigine, gabapentin, tiagabine, topiramate have been approved. Further compounds, e.g. levetiracetam, rufinamide, losigamone, remacemide, pregabalin, retiagabine, seretolide are presently in the clinical trials [1]. Although these drugs have been shown to be effective in epileptic syndromes in a number of patients, their efficacy does not appear to be superior to that of the established antiepileptic drugs. Therefore there is a need for new antiepileptic drugs with novel therapeutic targets, enhanced efficacy and minimal side effects. It is well known that numerous derivatives with anticonvulsant activity contain 5-, 6-heterocycles rings, one or two carbonyl groups, as well as an aromatic system [2–5]. Following these findings, our attention has been focused on a group of 3-substituted pyrrolidine-2,5-diones with different substituents at the nitrogen atom. Recently we have shown that a great number of 3-phenylpyrrolidine-2,5-dione derivatives with pyridyl-, aryl- and aminophenyl-moiety at the nitrogen atom [6–9] exhibits anticonvulsant activity, especially in the maximal electroshock (MES) test. Further studies [10,11] with that group of compounds led to the synthesis of new derivatives of 3-arylpyrrolidine-2,5-dione containing a 4-arylpiperazin-1-yl-alkyl moiety at the nitrogen atom. Pharmacological studies showed an anticonvulsant activity of some compounds of that group. To carry on our research, we have presently synthesized a series of new 1-[(4-arylpiperazin-1-yl)-methyl] derivatives of 3-arylpyrrolidine-2,5-dione (**3a**–**h**).

On the other hand, in a study with spirosuccinimide derivatives, Scott et al. [12–14] found that when the spiro nucleus was introduced into the 3-position of the pyrrolidine-2,5-dione ring, it enhanced the anticonvulsant activity. Following the latter studies, in our experiment we replaced the phenyl moiety at the 3-position of pyrrolidine-2,5-dione with a cyclopentyl ring connected with it by spiro carbon (**6*a**–**f**). Such

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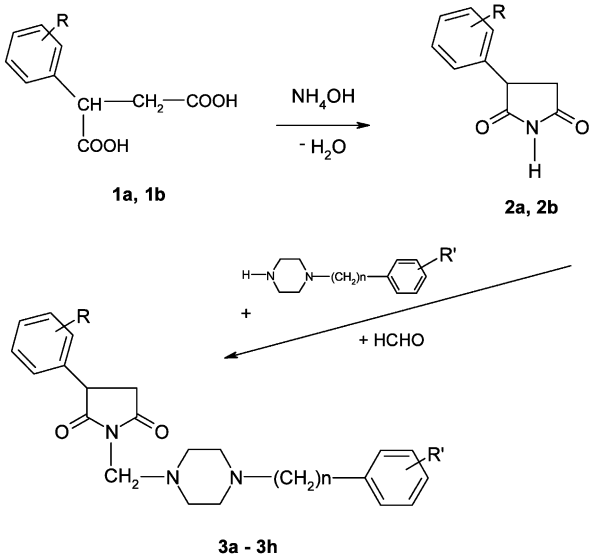
modification should have improved anticonvulsant potency. * Compound **6a** is reported in a US Patent (ggo) 7 pp. 7. 57,226.

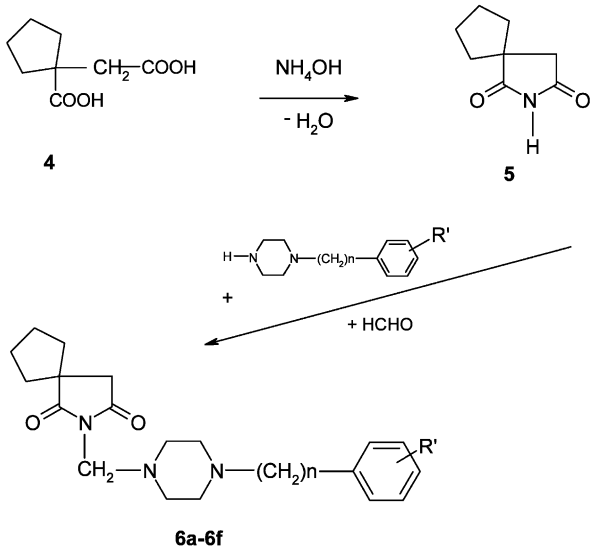
2. Chemistry

The synthesis of compounds **3a**–**h** and **6a**–**f** is shown in Schemes 1 and 2. The starting 3-(2-chlorophenyl)-, 3-(3-bromophenyl)-succinic acids **1a**, **1b** and cyclopentane-1-carboxy-1-acetic acid (**4**) were prepared according to the described procedure [13,15]. The methods of synthesis of 3-(2-chlorophenyl)-pyrrolidine-2,5-dione (**2a**), 3-(3-bromophenyl)-pyrrolidine-2,5-dione (**2b**) and 2-aza-spiro-[4.4]nonane-1,3-dione (**5**) have been described in a separate publication [11]. Compounds **3a**–**h** and **6a**–**f** were prepared in a Mannich-type reaction from the appropriately 3-substituted pyrrolidine-2,5-dione **2a**, **2b** or **5**, formaldehyde and the corresponding 4-phenylpiperazine to obtain designed compounds **3a**–**h** and **6a**–**f**. The reaction was carried out in ethanol at a room temperature for ca. 3–6 h and was eventually refluxed for 30 min. Compounds **3d** and **3g** were isolated as the hydrochloride salts.



Comp.	3a	3b	3c	3d	3e	3f	3g	3h
n	0	1	0	0	0	1	0	0
R	2-Cl	2-Cl	2-Cl	2-Cl	3-Br	3-Br	3-Br	3-Br
R'	H	H	3-Cl	2-OCH_3	H	H	3-Cl	2-OCH_3

Scheme 1. Synthesis of compounds **3a**–**h**.



Comp.	6a	6b	6c	6d	6e	6f
n	0	1	0	0	0	0
R'	H	H	3-Cl	2-OCH_3	2-F	4-F

Scheme 2. Synthesis of compounds **6a**–**f**.

3. Experimental

3.1. Chemistry

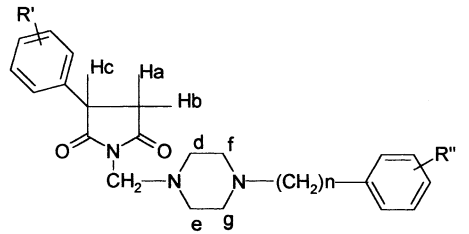
Melting points (m.p.) were determined in a electrothermal digital m.p. apparatus and are uncorrected. The chemical structures of the obtained compounds were confirmed by elemental and spectral analyses. ^{1}H NMR spectra were obtained in a Varian Mercury spectrometer operating at 300 MHz. Chemical shifts were reported as parts per million (ppm) from $(CH_3)_4Si$ (TMS) as an internal standard. Signal multiplicities are represented by the following abbreviations: s (singlet), d (doublet), dd (double doublet), q (quartet), m (multiplet). The elemental analyses for C, H, N were within $\pm$0.4% of the theoretical values.

The purity of the compounds was checked by the thin-layer chromatography (TLC) performed on Merck silica gel GF_{254} aluminium sheets, using the developing systems: (A) benzene:ethyl acetate:acetone (10:5:1), (B) acetone:isopropanol:25% ammonia (9:11:2). Spots were detected by their absorption under UV light and by visualisation with 0.05 mol I_2 in 10% HCl.

3.1.1. General procedure for the preparation of the N-[(4-arylpiperazin-1-yl)-methyl]-3-arylpyrrolidine-2,5-dione (**3a**–**h**) and N-[(4-arylpiperazin-1-yl)-methyl]-2-azaspiro[4.4]nonane-1,3-dione derivatives (**6a**–**f**)

To a suspension of 3-(2-chlorophenyl)-, 3-(3-bromophenyl)-pyrrolidine-2,5-diones (**2a**, **2b**) or 2-aza-spiro [4,4]nonane-1,3-dione (**5**) (0.02 mol) in a 96% ethanol (25 ml) there was added a 35% solution of formaldehyde

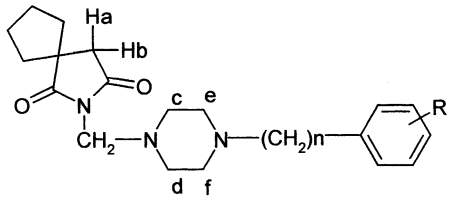
Table 1
^{1}H NMR data of synthesized compounds **3a**–**h**



Comp	3a	3b	3c	3d	3e	3f	3g	3h
n	0	1	0	0	0	1	0	0
R'	2-Cl	2-Cl	2-Cl	2-Cl	3-Br	3-Br	3-Br	3-Br
R"	H	H	3-Cl	2-OCH_3	H	H	3-Cl	2-OCH_3

Comp.	^{1}H NMR (ppm)
3a	2.79–2.86 (m, 4H, H-d,e), 2.88–2.91 (d, 1H, H-b, $J = 6.04$ Hz), 3.17–3.24 (m, 4H, H-f,g), 3.29–3.34 (d, 1H, H-a, $J = 9.82$), 4.32–4.40 (q, 1H, H-c, $J = 6.02$ Hz), 4.64 (s, 2H, CH_2), 6.87–7.40 (m, 9H, arom.)
3b	2.73–2.86 (m, 4H, H-d,e), 2.89–2.91 (d, 1H, H-b, $J = 5.78$ Hz), 3.21–3.25 (m, 4H, H-f,g), 3.27–3.30 (d, 1H, H-a, $J = 9.90$), 4.10–4.12 (d, 2H, CH_2-benzilic, $J = 5.22$) 4.37–4.43 (q, 1H, H-c, $J = 6.05$ Hz), 4.49–4.59 (q, 2H, CH_2, $J = 13$,4 Hz), 7.23–7.62 (m, 8H, arom.)
3c	2.74–2.83 (m, 4H, H-d,e), 2.88–2.90 (d, 1H, H-b, $J = 6.05$ Hz), 3.16–3.24 (m, 4H, H-f,g), 3.26–3.30 (d, 1H, H-a, $J = 9.90$ Hz), 4.31–4.36 (q, 1H, H-c, $J = 6.05$ Hz), 4.56–4.66 (q, 2H, CH_2, $J = 13.2$ Hz), 6.74–7.43 (m, 8H, arom.)
3d	2.75–2.83 (m, 4H, H-d,e), 2.96–2.98 (d, 1H, H-b, $J = 5.22$ Hz), 3.18–3.28 (m, 4H, H-f,g), 3.25–3.28 (d, 1H, H-a, $J = 9.35$ Hz), 3.90 (s, 3H, OCH_3), 4.36–4.38 (q, 1H, H-c, $J = 6.60$ Hz), 4.58–4.62 (q, 2H, CH_2, $J = 13.4$ Hz), 6.91–7.39 (m, 8H, arom.)
3e	2.76–2.83 (m, 4H, H-d,e), 2.86–2.88 (2d, 1H, H-b, $J = 5.22$ Hz), 3.15–3.21 (m, 4H, H-f,g), 3.25–3.28 (2d, 1H, H-a, $J = 9.62$ Hz), 4.00–4.04 (q, 1H, H-c, $J = 5.22$ Hz), 4.60 (s, 2H, CH_2), 6.83–7.47 (m, 9H, arom.)
3f	2.80–2.85 (m, 4H, H-d,e), 2.86–2.87 (2d, 1H, H-b, $J = 5.22$ Hz), 3.03–3.18 (m, 4H, H-f,g), 3.24–3.28 (2d, 1H, H-a, $J = 9.90$ Hz), 3.84 (s, 2H, CH_2 benzylic), 4.01–4.06 (q, 1H, H-c, $J = 5.50$ Hz), 4.56–4.66 (q, 2H, CH_2, $J = 13.20$ Hz), 6.83–7.47 (m, 9H, arom.)
3g	2.81–2.83 (m, 4H, H-d,e), 2.87–2.89 (d, 1H, H-b, $J = 5.22$ Hz), 3.20–3.23 (m, 4H, H-f,g), 3.26–3.29 (d, 1H, H-a, $J = 9.62$ Hz), 4.03–4.08 (q, 1H, H-c, $J = 5.22$ Hz), 4.56–4.66 (q, 2H, CH_2, $J = 13.20$ Hz), 6.89–7.48 (m, 8H, arom.)
3h	2.80–2.85 (m, 4H, H-d,e), 2.86–2.88 (d, 1H, H-b, $J = 5.50$ Hz), 3.05–3.23 (m, 4H, H-f,g), 3.25–3.28 (d, 1H, H-a, $J = 9.62$ Hz), 3.84 (s, 3H, OCH_3), 4.01–4.06 (q, 1H, H-c, $J = 5.50$ Hz), 4.56–4.66 (q, 2H, CH_2, $J = 13.20$ Hz), 6.83–7.47 (m, 8H, arom.)

Table 2
^{1}H NMR data of synthesized compounds **6a**–**f**



Comp	6a	6b	6c	6d	6e	6f
n	0	1	0	0	0	0
R	H	H	3-Cl	2-OCH_3	2-F	4-F

Comp.	^{1}H NMR (ppm)
6a	1.63–2.17 (m, 8H, cyclopentane), 2.62 (s, 2H, Ha, Hb), 2.70–2.79 (m, 4H, Hc, Hd), 3.13–3.18 (m, 4H, He, Hf), 4.53 (s, 2H, CH_2), 6.86–7.24 (m, 5H, arom.)
6b	1.65–2.15 (m, 8H, cyclopentane), 2.43–2.45 (m, 4H, Hc, Hd), 2.59 (s, 2H, Ha, Hb), 2.62–2.64 (m, 4H, He, Hf), 3.49 (s, 2H, CH_2 benzylic), 4.46 (s, 2H, CH_2), 7.25–7.29 (m, 5H, arom.)
6c	1.65–2.16 (m, 8H, cyclopentane), 2.61 (s, 2H, Ha, Hb), 2.70–2.75 (m, 4H, Hc, Hd), 3.02–3.06 (m, 4H, He, Hf), 4.52 (s, 2H, CH_2), 6.72–7.26 (m, 4H, arom.)
6d	1.65–2.16 (m, 8H, cyclopentane), 2.61 (s ,2H, Ha, Hb), 2.79–2.83 (m, 4H, Hc, Hd), 3.11–3.16 (m, 4H, He, Hf), 3.83 (s, 3H, OCH_3), 4.54 (s, 2H, CH_2), 6.82–6.99 (m, 4H, arom.)
6e	1.65–2.13 (m, 8H, cyclopentane), 2.59 (s, 2H, Ha, Hb), 2.61–2.82 (m, 4H, Hc, Hd), 3.10–3.14 (m, 4H, He, Hf), 4.52 (s, 2H, CH_2), 6.92–7.26 (m, 4H, arom.)
6f	1.63–2.11(m, 8H, cyclopentane), 2.48 (s, 2H, Ha, Hb), 2.67–2.96 (m, 4H, Hc, Hd), 3.00–3.06 (m, 4H, He, Hf), 4.31 (s, 2H, CH_2), 6.87–7.04 (m, 4H, arom.)

(0.02 mol), followed by the appropriately substituted 4-phenylpiperazine (0.02 mol). The mixture was refluxed for 30 min, kept at a room temperature for 3–6 h, and eventually refrigerated. The precipitated crude product was washed with cold ethanol and recrystallized from a 96% ethanol.

Hydrochlorides **3d** and **3g** were prepared by introducing dry hydrogen chloride into an alcoholic solution of the corresponding compounds. The crude products were recrystallized from ethanol. ^{1}H NMR spectral data, yields, m.p. and R_f values are presented in Tables 1–3.

3.2. Pharmacology

Compounds **3a**–**h** and **6a**–**f** were pharmacologically pre-evaluated using the testing procedures described elsewhere [16,17] as part of the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program, National Institutes of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda). Phase I studies of the investigated compounds involved three testes: maximal electroshock (MES), subcutaneous metrazole (sc.MET) and a rotarod test for neurological toxicity (TOX). Phase I involved i.p. administration of the compound as a suspension in a 0.5% methylcellulose. Phase I was a qualitative assay involving a small number of mice (1–4) at doses of 30, 100, and 300 mg/kg. The compounds were classified into the following categories: active at $<$ 100 mg/kg (class 1), active at $>$100 mg/kg (class 2), inactive (class 3), and toxic or active but toxic (class 4). These data are presented in Tables 4 and 5. Compounds **3b**, **3c**, **3d**, **3e**, **3h** were advanced to phase VIa and were administered orally into rats at a dose of 30 mg/kg. The rat classifications are as follows: 4/4 of animals protected (class 4), 3/4 of animals protected (class 3), 2/4 of animals active (class 2), 1/4 of animals protected (class 1). The results of the above classification are shown in Table 6. Compounds **3c** and **3h**, which were active in mice and rats were evaluated quantitatively (phase II). In phase II, the median effective dose (ED_{50}) and the median toxic dose (TD_{50}) were determined. ED_{50} and TD_{50} values and their confidence limits were determined at the time of peak effect for each compound by the method previously described [18]. The results of quantitative studies are shown in Table 7.

4. Results and discussion

The synthesized derivatives of 1-[(4-arylpiperazin-1-yl-)methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione (**3a**–**d**) showed anticonvulsant properties in the phase I screening project. In that series of compounds, 1-[(4-

benzylpiperazin-1-yl)-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione **3b** was highly active at dose of 100 mg/kg (3/3 animals protected at 0.5 h) and 300 mg/kg (1/1 animals protected at 0.5 and 4 h) in the MES test. Compound **3b** was also active at dose of 100 mg/kg (5/5 animals protected at 0.5 h) and 300 mg/kg (1/1 animals protected at 0.5 and 4 h) in the sc.MET test. Conversion of 4-benzylpiperazine (**3b**) to 4-phenylpiperazine (**3a**) led to a less active and more toxic compound. In that group of compounds, the most active was 1-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione (**3c**) in the MES test. Compound **3c** was active at dose of 30 mg/kg (1/3 animals protected at 4 h) and at dose 100 mg/kg (1/3 animals protected at 4 h). The replacement of 3-chloro substituent (**3c**) with 2-methoxy group (**3d**) decreased activity and increased the neurotoxicity. In a series of the derivatives with the 3-bromophenyl substituent in the 3-position of the pyrrolidine-2,5-dione ring (**3e**–**h**), only compound **3f** was inactive. 1-[(4-arylpiperazin-1-yl)-methyl]-3-(3-bromophenyl)-pyrrolidine-2,5-dione (**3e**) showed a protective effect towards seizures at doses of 100 mg/kg (1/3 animals protected at 4 h) and 300 mg/kg (1/1 animals protected at 4 h) in the MES test. The methoxy analogue **3h** exhibited anticonvulsant activity at dose of 100 mg/kg (3/7 animals protected at 4 h) and 300 mg/kg (1/5 animals protected at 4 h) in the MES screening. In the neurological toxicity screening, all the compounds tested were neurotoxic at a dose of 100 mg/kg. Mice were unable to grasp rotorod after administration of the following compounds: **3b** (300 mg/kg, 0.5 h), **3e** (100 mg/kg, 0.5 h and 300 mg/kg, 0.5 and 4 h), **3h** (100 mg/kg, 0.5 h and 300 mg/kg, 0.5 and 4 h) (Table 4).

Compounds **3b**–**e**, and **3h** were selected for oral evaluation of the anti-MES and neurotoxic activity in rats (phase IVa). These compounds were administered per os in a dose of 30 mg/kg and their effects were studied after 0.25, 0.5, 1, 2, and 4 h. When given per orally none of the compounds were neurotoxic. The most active compounds **3b**, **3c**, **3e**, **3f** protected 100% of the animals (4/4) at **1**, **2** and **4h** (Table 6).

Quantitative evaluation was performed in rats for compounds **3c** and **3h** (after oral administration) in the MES and sc.MET tests (Table 7). Compound **3c** was active at dose of 250 mg/kg (100% protection at 4 h) in the sc.MET test. In the MES test, the best $ED_{50} = 14.18$ mg/kg and PI = 8.8 were recorded for compound **3c**. Compound **3h** was less active $ED_{50} = 33.64$ mg/kg and PI = 2.47. Time to peak effects (TPE) were 4 h for compound **3c** and 2 h for compound **3h**.

In the series of 1-[(4-arylpiperazin-1-yl)-methyl]-2-azaspiro[4.4]nonane-1,3-dione derivatives, compounds **6a**–**f** were devoid of anticonvulsant activity and were toxic at doses of 100 and 30 mg/kg **6a**, **6c**. The data obtained in the present study, showed that introduction of the spirocyclopentyl ring into the 3-position of pyrrolidine-2,5-dione did not enhance anticonvulsant activity. Moreover, some of them (**6a**–**b**) exhibited convulsant activity in a pharmacological study.

Table 3
Physical data of compounds **3a**–**h** and **6a**–**f**

Comp.	Yield (%)	M.p. (°C)	Molecular formula	R_f [a]
3a	52	104–106	$C_{21}H_{22}N_3O_2Cl$	0.56 A 0.81 B
3b	58	138–140	$C_{22}H_{24}N_3O_2Cl$	0.48 A 0.76 B
3c	54	136–138	$C_{21}H_{21}N_3FCl_2$	0.61 A 0.89 B
3d	63	186–188	$C_{22}H_{25}N_3O_3Cl_2$	0.13 A 0.73 B
3e	62	94–96	$C_{21}H_{22}N_3O_2Br$	0.64 A 0.84 B
3f	75	123–125	$C_{22}H_{24}N_3O_2Br$	0.52 A 0.67 B
3g	60	178–180	$C_{21}H_{22}N_3O_2BrCl_2$	0.50 A 0.61 B
3h	56	128–130	$C_{22}H_{24}N_3O_3Br$	0.64 A 0.86 B
6a	71	127–128	$C_{19}H_{25}N_3O_2$	0.54 A 0.65 B
6b	73	88–90	$C_{20}H_{27}N_3O_2$	0.31 A 0.88 B
6c	71	96–98	$C_{19}H_{24}N_3O_2Cl$	0.60 A 0.74 B
6d	64	102–104	$C_{20}H_{27}N_3O_3$	0.50 A 0.61 B
6e	60	100–102	$C_{19}H_{24}N_3O_2F$	0.59 A 0.71 B
6f	68	99–101	$C_{19}H_{24}N_3O_2F$	0.57 A 0.73 B

[a] Solvent: (A) benzene:ethyl acetate:acetone (10:5:1); (B) acetone:isopropanol:ammonia (9:11:2).

In conclusion, our investigations permit an assumption that the following structural elements are required for anticonvulsant activity: aromatic ring at the 3-position of pyrrolidine-2,5-dione moiety and a 4-arylpiperazine fragment with selected substituents at the phenyl ring.

Acknowledgements

The authors wish to thank Dr James Stables for providing them with pharmacological data through the Antiepileptic Drug Development Program. (Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, MD, USA).

Table 4
Anticonvulsant screening project (ASP) phase I test in mice (**3a**–**h**)

Comp.	Dose (mg/kg)	MES [a]		sc.MET [b]		Tox [c]		ASP [d]
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
3a	30	0/1	0/1	0/1	0/1	2/4	0/2	4
	100	0/3	1/3	0/1	0/1[5]	7/8	3/4	
	300	1/1	1/1	0/1	0/1	4/4	2/2	
3b	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	3/3	0/3	5/5	0/1	1/8	0/4	
	300	1/1	1/1	1/1	1/1	4/4[14]	2/2	
3c	10	0/1	0/1	0/1	0/1	0/4	0/2	4
	30	0/3	1/3	0/1	0/1	2/8	1/4	
	100	0/1	1/1	0/1	0/1	3/4	1/2	
3d	30	0/1	0/1	0/1[4]	0/1	0/4	1/2	1
	100	2/3	0/3	0/1[5]	0/1[4]	6/8	4/4	
	300					4/4[1]		
3e	30	0/1	0/1	0/1[4]	0/1	0/4	0/2	1
	100	0/3	1/3	0/1	0/1[5]	3/8[14]	1/4	
	300	0/1	1/1	0/1	0/1	4/4[14]	1/2[14]	
3f	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1[4]	0/1	0/8	0/4	
	300	0/1	0/1	0/1[4]	0/1	3/4	0/2	
3g	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	3/3	0/3	0/1[4]	0/1	0/8	0/4	
	300	1/1	1/1	0/1[4]	0/1	3/4	0/2	
3h	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	0/3	3/7	0/1	0/1	3/8[14]	0/4	
	300	0/1	1/5	0/1	0/1	4/4[14]	1/2[14]	

Response comments: [1]death; [4]death following tonic extension; [5]death following clonic seizure; [14]unable to grasp rotorod.
[a] MES test (number of animals protected/number of animals tested).
[b] Subcutaneous pentylenetetrazole test.
[c] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested).
[d] The classification are as follows: 1-anticonvulsant activity at doses 100 mg/kg or less; 2-anticonvulsant activity at doses greater than 100 mg/kg; 3-compound inactive at 300 mg/kg; 4-compound inactive or active and toxic at doses 100 mg/kg.

Table 5
Anticonvulsant screening project (ASP) phase I test in mice (**6a**–**f**)

Comp.	Dose (mg/kg)	MES [a]		sc.MET [b]		Tox [c]		ASP [d] classification
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
6a	30	0/1	0/1	0/1	0/1	1/4	0/2	4
	100	0/3	0/3	0/1	0/1[4]	5/8	0/4	
	300	0/1		0/1[5]	0/1[4]	4/4[1,23]	0/1	
6b	30	0/1	0/1	0/1[4]	0/1	0/4	0/2	3
	100	0/1	0/1	0/1[4,22]	0/1	7/8[1]	2/3[1]	
	300					4/4[1]		
6c	30	0/1	0/1	0/1	0/1	1/4	0/2	4
	100	0/3	0/3	0/1	0/1	5/8	0/2	
	300	0/1		0/1[5]		4/4[14]	2/2[1]	
6d	30	0/1	0/1	0/1[5]	0/1	0/4	0/2	3
	100	0/3	0/3	0/1[4]	0/1	5/8	1/4	
	300					4/4[1,14]1/1[1]	1/1[1]	
6e	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/2	0/2			4/8[1]	0/2	
	300					4/4[1]		
6f	30	0/1	0/1	0/1[5]	0/1	0/4	0/2	3
	100	0/3	0/3	0/1[5]	0/1	8/8[33,14]	0/4	
	300					4/4[1]		

Response comments: [1]death; [4]death following tonic extension; [5]death following clonic seizure; [14]unable to grasp rotorod; [22]continuous seizure activity; [23]clonic seizure; [33]tremors.

[a] MES test (number of animals protected/number of animals tested).

[b] Subcutaneous pentylenetetrazole test.

[c] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested).

[d] The classification are as follows: 1-anticonvulsant activity at doses 100 mg/kg or less; 2-anticonvulsant activity at doses greater than 100 mg/kg; 3-compound inactive at 300 mg/kg; 4-compound inactive or active and toxic at doses 100 mg/kg.

Table 6
Anticonvulsant screening project (ASP) phase VIa

Comp.	MES					TOX				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
3b	2/4	3/4	3/4	4/4	1/4	0/4	0/4	0/4	0/4	0/4
3c	0/4	2/4	0/4	3/4	4/4	0/4	0/4	0/4	0/4	0/4
3d	1/4	1/4	1/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4
3e	2/4	2/4	3/4	3/4	4/4	0/4	0/4	0/4	0/4	0/4
3f	1/4	2/4	4/4	4/4	3/4	0/4	0/4	0/4	0/4	0/4

Test results in rats (dose at 30 mg/kg).

Table 7
Qanticonvulsant data in rats dosed orally (phase II ASP)

Comp.	ED_{50} MES (mg/kg)	ED_{50} sc.MET (mg/kg)	TD_{50} (mg/kg)	PI MES (TD_{50}/ED_{50})	TPE MES (h)
3c	14.18 (8.43–22.71)	< 250	< 125	8.8	4
3h	33.64 (22.92–48.76)	> 84	83.33 (58.83–111.93)	2.47	2
Phenytoin [a]	29.8 (21.9–38.9)	> 800	> 3000	> 100	4

Pharmacological values given in this table are follows: the ED_{50} dose of drug required to assure anticonvulsant protection in 50% animals. The TD_{50} dose eliciting minimal neurological toxicity in 50% animals. The PI, protection index; the TPE, time to peak effect.

[a] Data on phenytoin are from ref. H.S. White, J.H. Woodhead, M.R. Franklin, General Principles: Experimental Selection, Quantification and Evaluation of Anticonvulsant Drugs, in: R. Levy, Mattson, B.S. Meldrum (eds.), Antiepileptic drugs, 4th ed., Raven Press, New York, 1995, pp. 99–110.

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