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Short communication

Synthesis and anticonvulsant activity of new *N*-[(4-arylpiperazin-1-yl)-alkyl] derivatives of 3-phenyl-pyrrolidine-2,5-dione

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Abstract

In the present study, on the development of new anticonvulsants, the series of *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl)- (**8a–e**, **10a–h**) and 3-(2-trifluoromethyl-phenyl)-pyrrolidine-2,5-diones (**9a–e**, **11a–i**) were synthesized and tested for anticonvulsant activity using the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) screens. Their neurotoxicity were determined applying the rotorod test. In this series, the most active were *N*-[(4-phenylpiperazin-1-yl)-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9a**) with the $ED_{50} = 20.78$ mg/kg, when given orally to rats and *N*-[3-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11i**) with the $ED_{50} = 132.13$ mg/kg after intraperitoneally injection to mice.

Keywords: 3-(2-Methylphenyl)-pyrrolidine-2,5-dione; 3-(2-Trifluoromethylphenyl)-pyrrolidine-2,5-dione; 4-Arylpiperazine derivatives; Anticonvulsant activity

1. Introduction

Epilepsy is one of the most common neurological disorders, affecting about 1% of the world's population. The currently available anticonvulsants (AEDs) are effective in reducing the severity and number of seizures in less than 70% of patients. Moreover, their usage is associated with undesirable side effects ranging from cosmetic (gingival hyperplasia) to life threatening (hepatotoxicity, megaloblastic anemia) [1–3]. Therefore, the continued search for the safer and more effective AEDs is urgently necessary.

The SAR studies of clinically available AEDs and other anticonvulsant active compounds indicated, that the number of such molecules contain 5- or 6-member heterocyclic rings, one or two carbonyl groups as well as an aromatic system [4–6]. Following these findings, in the course of developing new anticonvulsants our attention has been focused on a group of 3-substituted pyrrolidine-2,5-diones with the piperazin-1-yl-alkyl fragment at the imide nitrogen atom. Among these derivatives, the anticonvulsant activity was observed especially for compounds with an aromatic area at position-3 of the pyrrolidine-2,5-dione and 4-aryl or 4-methyl-piperazin-1-yl alkyl moiety at the imide nitrogen atom. Several of these molecules (**1**, **2** and **3**) exhibited potent anticonvulsant activity, which was comparable with standard AEDs [7–9] (Fig. 1).

In line with the above findings, in the present study we have synthesized a new series of *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-phenylpyrrolidine-2,5-diones with different length of alkyl spacer between imide nitrogen atom and 4-arylpiperazine moiety. On the other hand, we have introduced the electron-donating CH_3 (**8a–8e**, **10a–10h**) or electron-attracting CF_3 (**9a–9e**, **11a–11i**) substituents at position-2 of the phenyl ring. It is noteworthy that, CF_3 group is recognized as bioactive one and plays a significant role in development of new drugs, including anticonvulsant active molecules [10,11].

2. Chemistry

The synthesis of compounds **8a–e**, **9a–e**, **10a–h** and **11a–i** is shown in Scheme 1. The starting materials, 2-(2-methylphenyl)- and 2-(2-trifluoromethylphenyl)-succinic acids (**4**, **5**) were prepared by using methods reported elsewhere [12]. The 3-(2-methylphenyl)- and 3-(2-trifluoromethylphenyl)-

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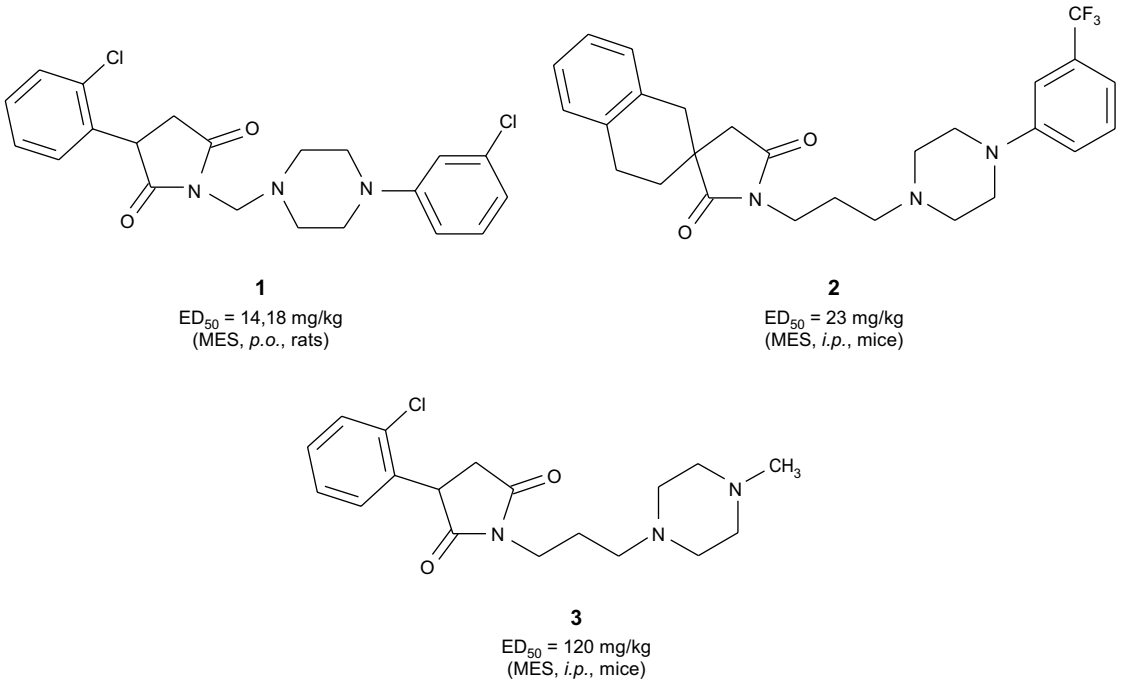
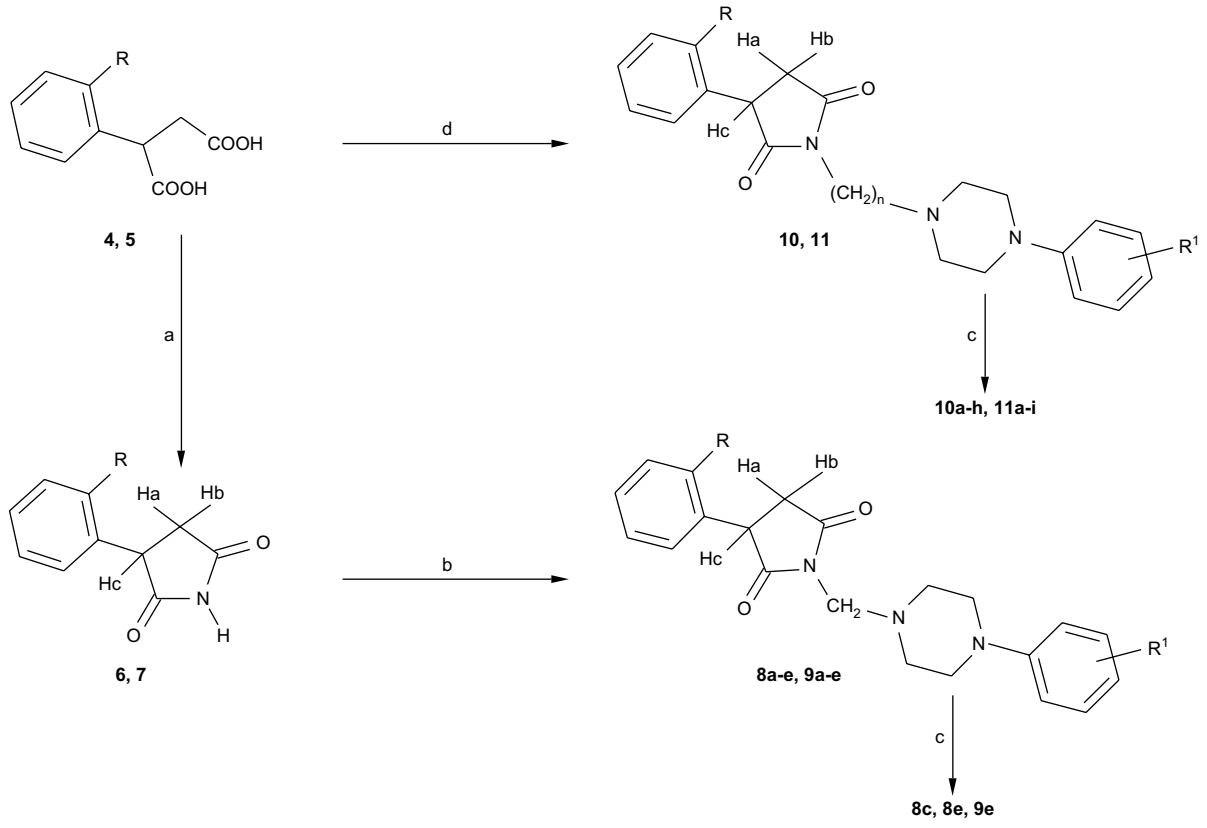


Fig. 1. Chemical structures of compounds **1**–**3**.

pyrrolidine-2,5-diones (**6**, **7**) were obtained in the cyclization reaction of the dicarboxylic acids **4** or **5** with the 25% ammonia by heating them at ca. 190 °C for 2 h. Compounds **8a**–**e** and **9a**–**e** were synthesized in a Mannich-type reaction from the appropriately substituted 3-phenylpyrrolidine-2,5-diones (**6**, **7**), formaldehyde and corresponding 4-arylpiperazine. The reaction was carried out in ethanol at a room temperature for ca. 6–12 h and was eventually refluxed for 30 min.



Scheme 1. Synthetic procedures of compounds **8a**–**e**, **9a**–**e**, **10a**–**h** and **11a**–**i**. Reagents and conditions: (a) 25% NH_4OH, 190 °C, 2 h, (b) 4-arylpiperazine derivatives, formaldehyde, 96% ethyl alcohol, reflux for 0.5 h or ca. 6–12 h room temperature, (c) anhydrous ethanol HCl solution and (d) 1-aminoalkyl-4-arylpiperazine, cyclocondensation, 190 °C, 2 h.

Compounds **10a–h** and **11a–i** were prepared using a one-pot cyclization reaction of corresponding succinic acid **4** or **5** with appropriately substituted 1-amino-alkyl-4-arylpiperazines, by heating them at ca. 190 °C for 2 h.

Because of oil form of compounds **10a–h**, **11a–i**, **8c**, **8e** and **9e** they were isolated as hydrochloride salts and were recrystallized from anhydrous ethanol. The other products were crystallized from 96% ethanol.

The final compounds were obtained in yields ranging from 48% to 78%. Their purity was assessed by TLC chromatography. The structures were confirmed by both spectral (^{1}H NMR) and elemental analysis.

The physical and analytical data are listed in the experimental section.

3. Pharmacology

3.1. Anticonvulsant screening

The pre-clinical discovery and development of new chemical agents for the treatment of epilepsy are based mainly on the use of predictable animal models, from which the MES and scPTZ screens are recognized as the ''gold standards'' in the early stages of testing [13].

The anticonvulsant activity for all synthesized compounds was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) tests after intraperitoneal injection to mice at doses of 30, 100 and 300 mg/kg. The neurotoxic properties were determined in the minimal motor impairment-rotorod screen (NT).

The obtained compounds revealed diversified anticonvulsant properties. Excluding **9c**, which was active in the scPTZ and MES test, the other molecules (**8a**, **8c**, **8e**, **9a–e**, **10b**, **10d–e**, **10g–h**, **11d**, **11f** and **11h–i**) showed protection against electrically induced seizures (MES-test) or were inactive (**8b**, **8d**, **10a**, **10c**, **10f**, **11a–c**, **11e** and **11g**). Compounds **8e**, **9a**, **9b** and **11i** exhibited activity at a dose of 30 mg/kg, whereas **9d**, **9e**, **10d**, **10g–h** and **11h** at a dose of 100 mg/kg. The other molecules (**8a**, **8c**, **9c**, **10b**, **10e**, **11d** and **11f**) were effective at a dose of 300 mg/kg. The obtained results are presented in Tables 1 and 2.

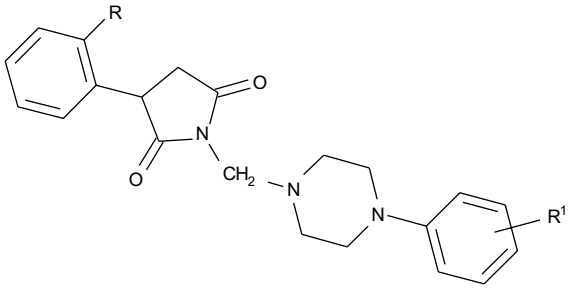
On the basis of obtained data in *i.p.* screen in mice and according to Anticonvulsant Screening Project (ASP) procedures, selected compounds (**8e**, **9a**, **9b**, **9d**, **10d**, **10g–h**, **11d** and **11i**) were evaluated orally to rats at a dose of 30 mg/kg for both anticonvulsant and neurotoxic properties (Table 3). Furthermore, compounds **8e** and **10d** were tested after intraperitoneal injection to rats (Table 4).

Compounds **9a** and **11i** were chosen for phase II evaluation for quantification of ED_{50} and TD_{50}. These parameters were determined after *i.p.* administration to mice (**11i**) and *p.o.* administration to rats (**9a**). The quantitative data are presented in Tables 5 and 6.

4. Results and discussion

In the present study, a library of twenty seven *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl)- (**8a–e**, **10a–h**) and 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-diones (**9a–e**, **11a–i**) was synthesized. In this series of compounds the anticonvulsant activity depended mainly on the length of alkyl spacer between imide nitrogen atom and 4-arylpiperazine moiety, as well as, substitution mode of the latter and the kind of substituents at position-2 of the phenyl ring.

Table 1
Anticonvulsant screening project (ASP) phase I results for compounds **8a–e**, **9a–e**



Compound	R	R_1	Intraperitoneal injection in mice			
			MES[a] (h)		NT[b] (h)	
			0.5	4	0.5	4
8a	CH_3	H	–	300	100	300
8b	CH_3	2-F	–	–	300[14]	–
8c	CH_3	2-OCH_3	300	–	100	300[33]
8d	CH_3	3-Cl	–	–	–	–
8e	CH_3	3-CF_3	30	30	300[1,14,33]	–
9a	CF_3	H	–	30	300[14]	–
9b	CF_3	2-F	–	30	100	300
9c[c]	CF_3	2-OCH_3	300	300	100	100
9d	CF_3	3-Cl	–	100	100	100
9e	CF_3	3-CF_3	100	100	300[1]	–
Phenytoin[d]			30	30	100	100

The figures in the table indicate the minimum dose whereby bioactivity or neurotoxicity was demonstrated in half or more animals. A dash indicates the absence of activity or neurotoxicity at the maximum dose administrated (300 mg/kg).

Response comments: [1]death, [14]unable to grasp rotorod, [33]tremors, [34]muscule spasm.

[a] Maximal electroshock test.

[b] Rotorod toxicity.

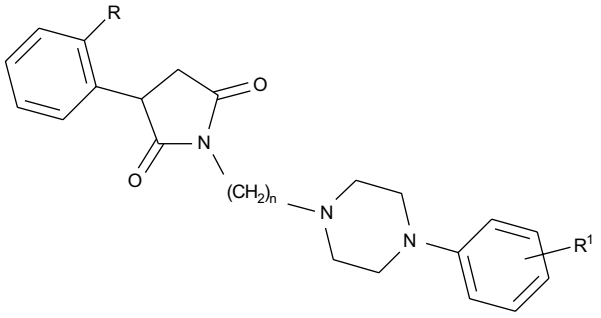
[c] Compound 9c revealed anti-scPTZ activity at a dose of 100 mg/kg and 300 mg/kg at 4 h, however, at the same time caused myoclonic jerks.

[d] Reference drug, data for phenytoin from Ref. [18].

The most active were 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-diones with methylene bridge between two nitrogen atoms (**9a–e**). In this series, the highest activity was observed for **9a** and **9b** that showed anti-MES protection at a dose of 30 mg/kg at time periods of 4 h. The other compounds were less active and revealed protection at doses of 100 mg/kg (**9d** and **9e**) or 300 mg/kg (**9c**). As mentioned above **9c** was also active in the scPTZ test at a dose of 100 mg/kg and 300 mg/kg at 4 h.

The replacement of trifluoromethyl group at position-2 of the phenyl ring (**9a–e**) with methyl substituent (**8a–e**) decreased activity. Among these compounds **8a** and **8c** showed protection at a dose of 300 mg/kg, whereas compound **8e** with trifluoromethyl group at position-3 of 4-arylpiperazine fragment, was active at a dose of 30 mg/kg but at a dose of 300 mg/kg mice were unable to grasp rotorod, had a tremors and caused death of animals.

Table 2
Anticonvulsant screening project (ASP) phase I results for compounds **10a–h** and **11a–i**



Compound	R	R_1	*n*	Intraperitoneal injection in mice			
				MES[a] (h)		NT[b] (h)	
				0.5	4	0.5	4
10a	CH_3	H	2	–	–	300	–
10b	CH_3	2-F	2	–	300	300	–
10c	CH_3	2-OCH_3	2	–	–	300[1]	–
10d	CH_3	3-Cl	2	–	100	–	–
10e	CH_3	3-CF_3	2	300	300	–	–
10f	CH_3	2-OCH_3	3	–	–	100	–
10g	CH_3	3-Cl	3	300	100	300	–
10h	CH_3	3-CF_3	3	100	100	–	–
11a	CF_3	H	2	–	–	–	–
11b	CF_3	2-F	2	–	–	–	–
11c	CF_3	2-OCH_3	2	–	–	300[14,34]	–
11d	CF_3	3-Cl	2	–	100	–	–
11e	CF_3	3-CF_3	2	–	–	–	–
11f	CF_3	H	3	300	–	300[14]	–
11g	CF_3	2-OCH_3	3	–	–	100	300
11h	CF_3	3-Cl	3	100	100	100	300
11i	CF_3	3-CF_3	3	30	100	300	300
Phenytoin[c]				30	30	100	100

Response comments: [1]death, [14]unable to grasp rotorod, [34]muscule spasm.
[a] Maximal electroshock test.
[b] Rotorod toxicity.
[c] Reference drug, data for phenytoin from Ref. [18].

Table 3
Test results in rats after oral administration at a dose of 30 mg/kg

Compound	MES[a] (h)					NT[b] (h)				
	0.25	0.5	1	2	4	0.25	0.5	1	2	4
8e	0	0	2	1	2	2	1	0	0	0
9a	1	0	1	2	3	0	0	0	0	0
9b	1	0	1	0	1	0	0	0	0	0
9d	0	1	2	4	4	0	0	0	0	0
10d	0	0	1	0	2	0	0	0	0	0
10g	0	0	0	1	0	0	0	0	0	0
10h	0	0	0	0	0	0	0	0	1	0
11d	0	0	0	0	0	0	0	0	0	0
11i	0	1	1	0	0	0	0	0	0	0
Phenytoin[c]	1	4	3	3	3	ND	ND	ND	ND	ND

The data indicate the number of rats of four that were protected or in which the motor impairment was observed.
ND, no data.
[a] Maximal electroshock test.
[b] Rotorod test for neurological toxicity.
[c] Data for phenytoin from Ref. [19].

Table 4
Test results in rats after intraperitoneal administration at a dose of 30 mg/kg

Compound	MES[a] (h)					NT[b] (h)				
	0.25	0.5	1	2	4	0.25	0.5	1	2	4
8e	2	2	4	2	2	4	4	2	2	0
10d	0	0	0	0	0	0	0	0	0	0

The data indicate the number of rats of four that were protected or in which the motor impairment was observed.
[a] Maximal electroshock test.
[b] Rotorod toxicity.

The change of the length of alkylene spacer between two nitrogen atoms from methylene (**8a–e**, **9a–e**) to ethylene (**10a–e**, **11a–e**) decreased anticonvulsant properties, especially in a series of 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione derivatives (**11a–e**). In this series only **11d** exhibited activity at a dose of 100 mg/kg at 4 h, all other derivatives (**11a–c** and **11e**) were inactive. The better results were obtained for ethylene analogues with methyl group at position-2 of the phenyl ring (**10a–e**). Among these derivatives, compounds with electron-attracting substituents (**10b**, **10d–e**) exhibited activity at a dose of 100 mg/kg (**10d**) and 300 mg/kg (**10b**, **10e**).

Introduction of propylene spacer between imide and 4-arylpiperazine nitrogen atoms increased activity in both series (**10f**,**h** and **11f–i**). In this group the most active was **11i**, which showed the anti-seizure protection at a dose of 30 mg/kg at 0.5 h and 100 mg/kg at 4 h. Compounds **10g**,**h** and **11h** were effective at doses of 100 mg/kg and/or 300 mg/kg (**10g**, **11f**). It is noteworthy that, except of compound **11f** which was active only at 0.5 h, all other derivatives (**10g**,**h**, **11h–i**) showed long duration of anticonvulsant action and were active within the time period 0.5–4 h. Compounds **10f** and **11g** with methoxy group at the position-2 of 4-arylpiperazine moiety, comparable to their ethylene analogues (**10c** and **11c**) were inactive in both tests used.

Results from the rotorod toxicity evaluations demonstrate that compounds **8d**, **10d–e**, **10h**, **11a–b** and **11d–e** did not show neurotoxicity at the maximum dose administered (300 mg/kg). Additionally, among these **8d**, **11a–b** and **11e** were inactive. The other derivatives revealed neurotoxicity at a dose of 100 mg/kg (**8a**, **8c**, **9b–d**, **10f** and **11g–h**) and/or 300 mg/kg (**8a–c**, **8e**, **9a–b**, **9e**, **10a–c**, **10g**, **11c** and **11f–i**). Active compounds **10d–e**, **10h** and **11d** emerged as anticonvulsants without neurotoxic properties.

A valuable feature of a candidate anticonvulsant is its ability to inhibit convulsions, when given by the oral route. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the total duration of anticonvulsant activity or neurotoxicity. Therefore, on the basis of data obtained in mice and according to the Anticonvulsant Screening Project (ASP) disposition, nine compounds **8e**, **9a–b**, **9d**, **10d**, **10g–h**, **11d** and **11i** were selected and examined for their anticonvulsant activity in the MES screen as well as neurotoxicity after *p.o.* administration to rats at a dose of 30 mg/kg. The results are presented in Table 3.

Table 5
Quantitative anticonvulsant data in mice dosed intraperitoneally for compound **11i**

Compound	TPE (h)[a]	ED_{50}[b] MES (mg/kg)	ED_{50}[c] scPTZ (mg/kg)	TD_{50}[d] (mg/kg)	PI[e] MES (TD_{50}/ED_{50})
11i	2	132.13 (82.55–202.8)[f]	>350	359.59 (254.73–502.16)	2.72
Phenytoin[g]	1	5.32 (5.44–7.23)	>500	41.2 (36.9–46.1)	6.52

[a] Time to peak effect.
[b] Maximal electroshock test. The ED_{50} – median effective dose required assure anticonvulsant protection in 50% animals.
[c] Subcutaneous pentylenetetrazole seizure threshold. The ED_{50} – median effective dose required assure anticonvulsant protection in 50% animals.
[d] TD_{50} – median toxic dose eliciting minimal neurological toxicity in 50% animals.
[e] PI protective index (TD_{50}/ED_{50}).
[f] 95% Confidence limits given in parentheses.
[g] Data for phenytoin from Ref. [18].

As can be seen from these data, the most active was compound **9d** that protected 100% of animals at 2 h and 4 h as well as 50% and 25% at time periods 1 h or 0.5 h, respectively. The satisfactory anticonvulsant action of this molecule was quite long and comparable to phenytoin, used as standard anticonvulsant drug. The potent activity revealed also compounds **8e** and **9a**. These substances protected 50% of animals at 1 h and 4 h (**8e**), or 2 h (**9a**). Moreover, **9a** showed one peak of 75% protection at time point 4 h. They protected also 25% of rats at 0.25 h, 1 h (**9a**) or 2 h (**8e**). Compounds **9b**, **10d**, **10g** and **11i** showed only a marginal 25% protection at 0.25 h (**9b**), 0.5 h (**11i**), 1 h (**9b**, **10d** and **11i**), 2 h (**10g**) and 4 h (**9b**). Derivative **10d** protected also 50% of rats at time point 4 h. Among molecules studied **10h** and **11d** were inactive. In the neurotoxicity screen, compound **8e** caused motor impairment in 50% (0.25 h) and 25% (0.5 h) of animals, whereas **10h** was toxic in 25% of rodents at time point 2 h. The other derivatives did not show neurotoxicity.

Additionally, according to the ASP rule, compounds **8e** and **10d** were examined for their anti-MES activity as well as neurotoxicity after intraperitoneally administration to rats at a dose of 30 mg/kg (Table 4). The results indicated that only compound **8e** was active and showed a peak of 100% protection at time point 1 h. This molecule protected also 50% of animals in other time intervals. Despite potent anticonvulsant activity, **8e** revealed neurotoxicity at the same time and has been excluded from the further studies. Comparison of activity after oral and intraperitoneal administration for compound **8e** may indicate that this molecule is weakly absorbed from the gastrointestinal tract.

From the whole series, two compounds (**9a** and **11i**), which were active at a dose of 30 mg/kg in mice, have been chosen for phase II evaluation for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative evaluation of the MES median effective dose (ED_{50}) and median toxic dose (TD_{50}) were performed after *i.p.* administration to mice (**11i**) and after *p.o.* administration to rats (**9a**). Results of the quantitative tests along with the data for phenytoin are shown in Tables 5 and 6.

As can be seen from the above data **11i** gave the ED_{50} of 132.13 mg/kg and TD_{50} value of 359.59 mg/kg, resulting in protection index (PI, TD_{50}/ED_{50}) of 2.72. This molecule was less active and showed lower PI when compared with phenytoin ($ED_{50} = 6.32$ mg/kg, PI = 6.52). Much better results were obtained for **9a** after *p.o.* administration to rats. This compound was more active ($ED_{50} = 20.78$ mg/kg), safer (PI = 24.1) and possessed longer period of activity (TPE = 8 h) than reference substance.

The preliminary anticonvulsant screening revealed, that majority of compounds exhibited anti-MES activity. The results of pharmacological studies enabled to establish several structures–activity relationships (SAR). At first, the anticonvulsant properties depended on the length of alkyl spacer between the nitrogen atoms of imide and 4-arylpiperazine moiety, as well as the kind of substituents connected to both phenyl rings. In general, the most potent were molecules with methylene linker and highly electron-attracting trifluoromethyl group at the position-2 of the 3-phenylpyrrolidine-2,5-dione moiety (**9a–e**), as well as compounds with trifluoromethyl or/and chloro substituents at the position-3 of the 4-arylpiperazine fragment (**8e**, **10d–e**, **10g–h**, **11d** and **11h–i**). The most noticeable exceptions can be observed for molecules **11e** and **11i** with two trifluoromethyl substituents in their structures. The compound with ethylene spacer (**11e**) was inactive, whereas its propylene analogue (**11i**) displayed potent activity. The elongation of the alkyl chain from methylene to ethylene

Table 6
Quantitative anticonvulsant data in rats dosed orally for compound **9a**

Compound	TPE (h)[a]	ED_{50}[b] MES (mg/kg)	ED_{50}[b] scPTZ (mg/kg)	TD_{50}[c] (mg/kg)	PI[d] MES (TD_{50}/ED_{50})
9a	8	20.78 (9.05–39.7)[e]	>250	>500	>24.1
Phenytoin[f]	2	23.2 (21.4–25.4)	>500	>500	>21.6

[a] Time to peak effect.
[b] ED_{50} – median effective dose required to assure anticonvulsant protection in 50% animals.
[c] TD_{50} – median toxic dose eliciting minimal neurological toxicity in 50% animals.
[d] PI protective index (TD_{50}/ED_{50}).
[e] 95% Confidence limits given in parentheses.
[f] Data for phenytoin from Ref. [18].

decreased activity, in contrast to derivatives with propylene spacer, which exhibited activity comparable with methylene analogues. It is also noteworthy, that in general, 3-(2-methylphenyl)-pyrrolidine-2,5-diones were less active than respective 3-(2-trifluoromethylphenyl) derivatives. It proves an essential role of the bioactive trifluoromethyl groups in respect to the anticonvulsant properties such type of compounds.

5. Conclusions

In summary, the series of new *N*-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl)- and 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-diones were synthesized and tested for their anticonvulsant activity. The most potent was *N*-[(4-phenylpiperazin-1-yl)-methyl]-3-(2-trifluoromethyl-phenyl)-pyrrolidine-2,5-dione (**9a**) with the ED_{50} value of 20.78 mg/kg. This compound showed greater ED_{50} and comparable TD_{50} to phenytoin used as reference anticonvulsant.

6. Experimental protocols

6.1. Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany), using subsequent developing systems: S_1 – chloroform: acetone (9:1, v/v). S_2 – chloroform: 2-izopropanol: 25% ammonia (9:11:2, v/v). Spots were detected by their absorption under UV light ($\lambda = 254$ nm) and by visualization with 0.05 mol I_2 in 10% HCl. The elemental analysis for C, H, and N was carried out by a micro method using the elemental Vario EI III Elemental analyser (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values.

^{1}H NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, operating at 300 MHz. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet).

The starting 2-(2-methylphenyl)- (**4**) and 2-(2-trifluoromethylphenyl) (**5**) succinic acids were prepared by the method described by Miller and Long [12].

The synthesis of 1-(2-aminoethyl)- and 1-(3-aminopropyl)-4-arylpiperazines was reported by Glennon et al. [14].

6.1.1. General procedure for the synthesis of 3-(2-methylphenyl)- and 3-(2-trifluoro-methylphenyl)-pyrrolidine-2,5-diones (6, 7)

A total of 0.05 mol of the 2-(2-methylphenyl)- (**4**) or 2-(2-trifluoromethylphenyl) (**5**) succinic acid were dissolved in 50 ml of water and 0.05 mol of the 25% ammonia was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After the water was completely removed, the temperature of reaction rose up to 190 °C and was maintained for 1.5 h. The solid products were separated by filtration and crystallized from methanol (Scheme 1).

*6.1.1.1. 3-(2-Methylphenyl)-pyrrolidine-2,5-dione (**6**).* White powdery crystals. Yield: 88%; mp 126–128 °C; TLC: $R_f = 0.42$ (S_1); ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 2.76 (dd, 1H, H_b imide, $J = 5.13$ Hz, $J = 18.46$ Hz), 3.26 (dd, 1H, H_a imide, $J = 9.75$ Hz, $J = 18.46$ Hz), 4.32 (q, 1H, H_c imide, $J = 5.13$ Hz), 7.21–7.23 (m, 4H, ArH), 8.30 (brs, 1H, NH). Anal. Calcd for $C_{11}H_{11}O_2N$ (189.22), C: 69.91, H: 5.86, N: 7.40; Found C: 70.28, H: 5.74, N: 7.63.

*6.1.1.2. 3-(2-Trifluoromethylphenyl)-pyrrolidine-2,5-dione (**7**).* White powdery crystals. Yield: 67%; mp 132–134 °C; TLC: $R_f = 0.53$ (S_1); ^{1}H NMR ($CDCl_3$): δ 2.71 (dd, 1H, H_b imide, $J = 5.89$ Hz, $J = 18.72$ Hz), 3.27–3.36 (m, 1H, H_a imide), 4.54 (q, 1H, H_c imide, $J = 5.21$ Hz), 7.29 (d, 1H, ArH, $J = 7.95$ Hz), 7.45 (t, 1H, ArH, $J = 7.55$ Hz), 7.60 (t, 1H, ArH, $J = 7.29$ Hz), 7.72 (d, 1H, ArH, $J = 8.46$ Hz), 8.30 (brs, 1H, NH). Anal. Calcd for $C_{11}H_8O_2N_1F_3$ (243.19), C: 54.37, H: 3.32, N: 5.76; Found C: 54.58, H: 3.44, N: 5.66.

6.1.2. General procedure for the synthesis of N-[(4-arylpiperazinyl-1-yl)-methyl]-3-(2-methylphenyl)- (**8a–e**) and 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9a–e**)

The mixture of 3-(2-methylphenyl)- (0.01 mol) (**6**) or 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (0.01 mol) (**7**), 40% formaldehyde solution (0.01 mol) and corresponding 4-arylpiperazine (0.01 mol) in 96% ethanol (40 ml) was left for ca. 6–12 h at room temperature or additionally refluxed for 0.5 h, then refrigerated ca. −10 °C for 24 h. The products were washed with cold ethanol and the solid products (**8a–b**, **8d** and **9a–d**) were separated by filtration and recrystallized from 96% ethanol. Compounds **8c**, **8e** and **9e** were obtained as light oils. These molecules were converted to hydrochloride salts in anhydrous ethanol saturated with HCl gas. They were crystallized from anhydrous ethanol (Scheme 1).

*6.1.2.1. N-[(4-phenylpiperazin-1-yl)-methyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**8a**).* White powdery crystals. Yield: 72%; mp 90–92 °C; TLC: $R_f = 0.54$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 3H, CH_3), 2.71 (dd, 1H, H_b imide, $J = 5.13$ Hz, $J = 18.46$ Hz), 2.77–2.83 (m, 4H, piperazine), 3.17–3.22 (m, 4H, piperazine), 3.27 (dd, 1H, H_a imide, $J = 9.24$ Hz, $J = 18.46$ Hz), 4.29 (q, 1H, H_c imide, $J = 5.13$ Hz), 4.62 (s, 2H, CH_2), 6.84–6.93 (m, 3H, ArH), 7.02–7.06 (m, 1H, ArH), 7.16–7.30 (m, 5H, ArH). Anal. Calcd for $C_{22}H_{25}O_2N_3$ (363.46), C: 72.79, H: 6.94, N: 11.58; Found C: 72.50, H: 6.80, N: 11.30.

*6.1.2.2. N-[{4-(2-fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**8b**).* White powdery crystals. Yield: 77%; mp 128–130 °C; TLC: $R_f = 0.63$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 3H, CH_3), 2.76 (dd, 1H, H_b imide,

$J = 5.13$ Hz, $J = 18.46$ Hz), 2.85 (brs, 4H, piperazine), 3.08 (brs, 4H, piperazine), 3.25 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.46$ Hz), 4.31 (q, 1H, H_c imide, $J = 5.13$ Hz), 4.62 (s, 2H, CH_2), 6.90–7.09 (m, 5H, ArH), 7.19–7.23 (m, 3H, ArH). Anal. Calcd for $C_{22}H_{24}O_2N_3F$ (381.45), C: 69.36, H: 6.35, N: 11.03; Found C: 69.58, H: 6.24, N: 11.23.

*6.1.2.3. Monohydrochloride N-[{4-(2–methoxyphenyl)-piperazin-1-yl}-methyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**8c**).* White powdery crystals. Yield: 70%; mp 195–197 °C; TLC: $R_f = 0.55$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 3H, CH_3), 2.73 (dd, 1H, H_b imide, $J = 5.39$ Hz, $J = 18.46$ Hz), 2.84–2.87 (m, 4H, piperazine), 3.05–3.07 (m, 4H, piperazine), 3.24 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.46$ Hz), 3.85 (s, 3H, OCH_3), 4.30 (q, 1H, H_c imide, $J = 5.38$ Hz), 4.63 (s, 2H, CH_2), 6.83–7.08 (m, 5H, ArH), 7.18–7.23 (m, 3H, ArH), 11.42 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{28}O_3N_3Cl$ (429.93), C: 64.25, H: 6.56, N: 9.77; Found C: 64.58, H: 6.34, N: 9.83.

*6.1.2.4. N-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**8d**).* White powdery crystals. Yield: 65%; mp 100–102 °C; TLC: $R_f = 0.69$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 3H, CH_3), 2.76 (dd, 1H, H_b imide, $J = 4.99$ Hz, $J = 18.21$ Hz), 2.81–2.87 (m, 4H, piperazine), 3.19 (brs, 4H, piperazine), 3.25 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.46$ Hz), 4.30 (q, 1H, H_c imide, $J = 5.13$ Hz), 4.62 (s, 2H, CH_2), 6.75–6.87 (m, 2H, ArH), 7.01–7.04 (m, 1H, ArH), 7.13–7.23 (m, 5H, ArH). Anal. Calcd for $C_{22}H_{24}O_2N_3Cl$ (397.89), C: 66.39, H: 6.08, N: 10.56; Found C: 66.58, H: 5.90, N: 10.45.

*6.1.2.5. Monohydrochloride N-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**8e**).* White powdery crystals. Yield: 62%; mp 160–163 °C; TLC: $R_f = 0.71$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.38 (s, 3H, CH_3), 2.76 (dd, 1H, H_b imide, $J = 5.38$ Hz, $J = 18.46$ Hz), 2.85 (brs, 4H, piperazine), 3.08 (brs, 4H, piperazine), 3.26 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.46$ Hz), 4.32 (q, 1H, H_c imide, $J = 5.39$ Hz), 4.78 (s, 2H, CH_2), 7.11–7.29 (m, 7H, ArH), 7.42–748 (m, 1H, ArH), 11.39 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{25}O_2N_3F_3Cl$ (467.92), C: 59.03, H: 5.38, N: 8.98; Found C: 58.90, H: 5.44, N: 8.83.

*6.1.2.6. N-[(4-phenylpiperazin-1-yl)-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9a**).* White powdery crystals. Yield: 60%; mp 98–100 °C; TLC: $R_f = 0.58$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.70 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 18.72$ Hz), 2.85 (brs, 4H, piperazine), 3.20 (brs, 4H, piperazine), 3.29 (dd, 1H, H_a imide, $J = 9.24$ Hz, $J = 18.46$ Hz), 4.49 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.66 (s, 2H, CH_2), 6.89–6.94 (m, 3H, ArH), 7.19–7.30 (m, 3H, ArH), 7.43 (t, 1H, ArH, $J = 7.69$ Hz), 7.57 (t, 1H, ArH, $J = 7.69$ Hz), 7.71 (d, 1H, ArH, $J = 7.95$ Hz). Anal. Calcd for $C_{22}H_{22}O_2N_3F_3$ (417.44), C: 63.37, H: 5.32, N: 10.08; Found C: 63.58, H: 5.54, N: 10.03.

*6.1.2.7. N-[{4-(2-fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9b**).* White powdery crystals. Yield 69%; mp 70–72 °C; TLC: $R_f = 0.63$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.71 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 18.72$ Hz), 2.87 (brs, 4H, piperazine), 3.09–3.12 (m, 4H, piperazine), 3.31 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.72$ Hz), 4.51 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.66 (s, 2H, CH_2), 6.92–7.10 (m, 4H, ArH), 7.22–7.26 (m, 1H, ArH), 7.41–7.47 (m, 1H, ArH), 7.58 (t, 1H, ArH, $J = 7.69$ Hz), 7.71 (d, 1H, ArH, $J = 7.95$ Hz). Anal. Calcd for $C_{22}H_{21}O_2N_3F_4$ (435.42), C: 60.75, H: 4.87, N: 9.66; Found C: 60.58, H: 4.74, N: 9.83.

*6.1.2.8. N-[{4-(2-methoxyphenyl)-piperazin-1-yl}-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9c**).* White powdery crystals. Yield 64%; mp 86–88 °C; TLC: $R_f = 0.50$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.95 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 18.97$ Hz), 3.37 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.90$ Hz), 3.40–3.64 (m, 4H, piperazine), 3.73–3.94 (m, 4H, piperazine), 4.00 (s, 3H, OCH_3), 4.56 (q, 1H, H_c imide, $J = 6.15$ Hz), 4.76 (s, 2H, CH_2), 6.90–7.07 (m, 2H, ArH), 7.26–7.37 (m, 3H, ArH), 7.43 (dd, 1H, ArH, $J = 6.58$ Hz), 7.68–7.83 (m, 2H, ArH). Anal. Calcd for $C_{23}H_{24}O_3N_3F_3$ (447.46), C: 61.80, H: 5.41, N: 9.40; Found C: 61.58, H: 5.54, N: 9.63.

*6.1.2.9. N-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9d**).* White powdery crystals. Yield: 78%; mp 83–85 °C; TLC: $R_f = 0.73$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.69 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 18.46$ Hz), 2.80–2.84 (m, 4H, piperazine), 3.19–3.20 (m, 4H, piperazine), 3.30 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.72$ Hz), 4.49 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.65 (s, 2H, CH_2), 6.77–6.87 (m, 4H, ArH), 7.14–7.20 (m, 1H, ArH), 7.41–7.46 (m, 1H, ArH), 7.54–7.59 (m, 1H, ArH), 7.71 (d, 1H, ArH, $J = 7.97$ Hz). Anal. Calcd for $C_{22}H_{21}O_2N_3F_3Cl$ (451.88), C: 58.46, H: 4.68, N: 9.30; Found C: 58.62, H: 4.74, N: 9.53.

*6.1.2.10. Monohydrochloride N-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**9e**).* White powdery crystals. Yield: 69%; mp 229–231 °C; TLC: $R_f = 0.77$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.68 (dd, 1H, H_b imide, $J = 5.68$ Hz, $J = 18.46$ Hz), 2.97 (brs, 4H, piperazine), 3.04 (brs, 4H, piperazine), 3.44 (dd, 1H, H_a imide, $J = 9.23$ Hz, $J = 18.21$ Hz), 4.65 (q, 1H, H_c imide, $J = 6.15$ Hz), 4.90 (s, 2H, CH_2), 7.27–7.38 (m, 3H, ArH), 7.44–7.59 (m, 2H, ArH), 7.60–7.73 (m, 3H, ArH), 10.27 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{22}O_2N_3F_6Cl$ (521.89), C: 52.92, H: 4.25, N: 8.05; Found C: 53.12 H: 4.08, N: 8.17.

*6.1.3. General procedure for the synthesis of N-[(4-arylpiperazin-1-yl)-alkyl]-3-(2-methylphenyl)- (**10a–h**) and 3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-diones (**11a–i**)*

A total of 0.01 mol of the appropriately substituted 1-(2-aminoethyl)- or 1-(3-aminopropyl)-4-arylpiperazine were mixed with 20 ml of water, and 0.01 mol of the

2-(2-methylphenyl)- (**4**) or 2-(2-trifluoromethylphenyl)- (**5**) succinic acid was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After the water was completely removed, the temperature of reaction was rose up to 190 °C and was maintained for 2 h. Free bases, obtained as light oil, were converted to hydrochloride salts in anhydrous ethanol saturated with HCl gas. The obtained precipitates salts were crystallized from anhydrous ethanol (Scheme 1).

*6.1.3.1. Monohydrochloride N-[2-(4-phenylpiperazin-1-yl)-ethyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10a**).* White powdery crystals. Yield: 64%; mp 206–208 °C; TLC: $R_f = 0.73$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 2.67 (dd, 1H, H_b imide, $J = 4.87$ Hz, $J = 18.20$ Hz), 2.99 (brs, 2H, CH_2–CH_2) 3.36 (d 2H, CH_2–CH_2, $J = 5.39$ Hz), 3.60–3.74 (m, 5H, 4H piperazine, 1H, H_a imide), 3.95–4.06 (m, 4H, piperazine), 4.82 (q, 1H, H_c imide, $J = 4.62$ Hz), 6.93–7.32 (m, 9H, ArH), 12.72 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{28}O_2N_3Cl$ (413.95), C: 66.73, H: 6.82, N: 10.15; Found C: 66.58, H: 6.74, N: 10.03.

*6.1.3.2. Monohydrochloride N-[2-{4-(2-fluorophenyl)-piperazin-1-yl}-ethyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10b**).* White powdery crystals. Yield: 68%; mp 194–196 °C; TLC: $R_f = 0.79$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 2.68 (dd, 1H, H_b imide, $J = 4.87$ Hz, $J = 18.20$ Hz), 3.00–3.07 (m, 2H, CH_2–CH_2), 3.34–3.47 (m, 4H, piperazine), 3.61–3.74 (m, 3H, 1H, H_a imide, 2H, CH_2–CH_2), 3.96–4.07 (m, 4H, piperazine), 4.83 (q, 1H, H_c imide, $J = 4.87$ Hz), 6.96–7.20 (m, 5H, ArH), 7.23–7.28 (m, 3H, ArH), 12.69 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{27}O_2N_3F_1Cl$ (431.94), C: 63.95, H: 6.30, N: 9.73; Found C: 63.83, H: 6.58, N: 9.85.

*6.1.3.3. Monohydrochloride N-[2-{4-(2-methoxyphenyl)-piperazin-1-yl}-ethyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10c**).* White powdery crystals. Yield: 70%, mp 223–225 °C; TLC: $R_f = 0.72$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 2.71 (dd, 1H, H_b imide, $J = 4.87$ Hz, $J = 18.20$ Hz), 3.51–3.67 (m, 5H, 4H, piperazine, 1H, H_a imide), 3.97–4.02 (m, 4H, piperazine), 4.05 (s, 3H, OCH_3), 4.39 (brs, 2H, CH_2–CH_2), 4.80 (q, 1H, H_c imide, $J = 4.87$ Hz), 5.02 (d, 2H, CH_2–CH_2, $J = 12.30$ Hz), 7.02–7.08 (m, 3H, ArH), 7.14–7.22 (m, 3H, ArH), 7.46 (t, 1H, ArH, $J = 7.95$ Hz), 8.16 (d, 1H, ArH, $J = 7.44$ Hz), 13.50 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{30}O_3N_3Cl$ (443.98), C: 64.93, H: 6.81, N: 9.46; Found C: 64.72, H: 7.02, N: 9.55.

*6.1.3.4. Monohydrochloride N-[2-{4-(3-chlorophenyl)-piperazin-1-yl}-ethyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10d**).* White powdery crystals. Yield: 65%; mp 230–232 °C; TLC: $R_f = 0.80$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 2.68 (dd, 1H, H_b imide, $J = 4.62$ Hz, $J = 18.20$ Hz), 2.92 (d, 2H, CH_2–CH_2, $J = 8.72$ Hz), 3.36 (d, 2H, CH_2–CH_2, $J = 4.87$ Hz), 3.60–3.80 (m, 5H, 1H_a imide, 4H, piperazine), 3.95–4.06 (m, 4H, piperazine), 4.82 (q, 1H, H_c imide, $J = 4.62$ Hz), 6.77–6.80 (m, 1H, ArH), 6.90–6.94 (m, 2H, ArH), 7.04–7.06 (m, 1H, ArH), 7.13–7.23 (m, 4H, ArH), 12.84 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{27}O_2N_3Cl_2$ (448.40), C: 61.66, H: 6.07, N: 9.38; Found C: 61.40, H: 6.15, N: 9.58.

*6.1.3.5. Monohydrochloride N-[2-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-ethyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10e**).* White powdery crystals. Yield: 62%; mp 254–256 °C; TLC: $R_f = 0.85$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH_3), 2.68 (dd, 1H, H_b imide, $J = 4.62$ Hz, $J = 18.20$ Hz), 2.93 (d, 2H, CH_2–CH_2, $J = 8.72$ Hz), 3.35–3.40 (m, 2H, CH_2–CH_2), 3.60–3.95 (m, 5H, 1H, H_a imide, 4H, piperazine), 3.98–4.07 (m, 4H, piperazine), 4.82 (q, 1H, H_c imide, $J = 4.87$ Hz), 7.04–7.23 (m, 7H, ArH), 7.39 (t, 1H, ArH, $J = 7.95$ Hz), 12.88 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{27}O_2N_3F_3Cl$ (481.93), C: 59.81, H: 5.65, N: 8.72; Found C: 59.92, H: 5.58, N: 8.63.

*6.1.3.6. Monohydrochloride N-[3-{4-(2-methoxyphenyl)-piperazin-1-yl}-propyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10f**).* White powdery crystals. Yield: 68%; mp 167–169 °C; TLC: $R_f = 0.82$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.29 (brs, 2H, CH_2–CH_2–CH_2), 2.39 (s, 3H, CH_3), 2,74 (dd, 1H, H_b imide, $J = 4.61$ Hz, $J = 18.46$ Hz), 3.21 (brs, 2H, CH_2–CH_2–CH_2), 3.32 (dd, 1H, H_a imide, $J = 9.74$ Hz, $J = 18.46$ Hz), 3.58–3.63 (m, 4H, piperazine), 3.68–3.83 (m, 2H, CH_2–CH_2–CH_2), 4.06 (s, 3H, OCH_3), 4.28 (brs, 2H, piperazine), 4.40 (q, 1H, H_c imide, $J = 4.87$ Hz), 4.97 (brs, 2H, piperazine), 6.99–7.08 (m, 3H, ArH), 7.22–7.24 (m, 3H, ArH), 7.44 (t, 1H, ArH, $J = 7.99$ Hz), 8.12 (d, 1H, ArH, $J = 7.69$ Hz), 13.80 (brs, 1H, HCl). Anal. Calcd for $C_{25}H_{32}O_3N_3Cl$ (458.00), C: 65.56, H: 7.04, N: 9.17; Found C: 65.72, H: 7.17, N: 9.35.

*6.1.3.7. Monohydrochloride N-[3-{4-(3-chlorophenyl)-piperazin-1-yl}-propyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10g**).* White powdery crystals. Yield: 65%; mp 159–161 °C; TLC: $R_f = 0.90$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.33 (brs, 2H, CH_2–CH_2–CH_2), 2.38 (s, 3H, CH_3), 2.72 (dd, 1H, H_b imide, $J = 4.67$ Hz, $J = 18.43$ Hz), 2.95 (brs, 2H, CH_2–CH_2–CH_2), 3.08 (brs, 2H, CH_2–CH_2–CH_2), 3.30 (dd, 1H, H_a imide $J = 9.35$ Hz, $J = 18.43$ Hz), 3.59–3.73 (m, 8H, piperazine), 4.38 (q, 1H, H_c imide, $J = 4.95$ Hz), 6.77–6.81 (m, 1H, ArH), 6.89–6.99 (m, 3H, ArH), 7.18–7.23 (m, 4H, ArH), 13.11 (brs 1H, HCl). Anal. Calcd for $C_{24}H_{29}O_2N_3Cl_2$ (462.43), C: 62.40, H: 6.33, N: 9.10; Found C: 62.72, H: 6.18, N: 9.15.

*6.1.3.8. Monohydrochloride N-[3-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-3-(2-methylphenyl)-pyrrolidine-2,5-dione (**10h**).* White powdery crystals. Yield: 55%; mp 195–197 °C; TLC: $R_f = 0.96$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.33 (brs, 2H, CH_2–CH_2–CH_2), 2.38 (s, 3H, CH_3), 2.71 (dd, 1H, H_b imide, $J = 4.67$ Hz, $J = 18.46$ Hz), 2.97 (brs, 2H, CH_2–CH_2–CH_2), 3.11 (brs, 2H, CH_2–CH_2–CH_2), 3.31 (dd, 1H, H_a imide $J = 8.98$ Hz, $J = 18.72$ Hz), 3.63–3.74 (m, 8H, piperazine), 4.39 (q, 1H, H_c imide, $J = 4.95$ Hz), 7.01–7.24 (m, 7H, ArH),

7.40 (t, 1H, ArH, $J = 7.94$ Hz), 13.16 (brs 1H, HCl). Anal. Calcd for $C_{25}H_{29}O_2N_3F_3Cl$ (453.95), C: 66.14, H: 6.44, N: 9.26; Found C: 66.40, H: 6.62, N: 9.13.

*6.1.3.9. Monohydrochloride N-[2-(4-phenylpiperazin-1-yl)-ethyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11a**).* White powdery crystals. Yield: 68%; mp 247–249 °C; TLC: $R_f = 0.81$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.89 (dd, 1H, H_b imide, $J = 5.90$ Hz, $J = 18.20$ Hz), 2.98 (brs 2H, CH_2–CH_2), 3.37 (brs, 2H, CH_2–CH_2), 3.50 (dd, 1H, H_a imide, $J = 9.36$ Hz, $J = 17.95$ Hz), 3.60–3.71 (m, 4H, piperazine), 3.92–4.02 (m, 4H, piperazine), 4.83 (q, 1H, H_c imide, $J = 5.80$ Hz), 6.91–7.00 (m, 2H, ArH), 7.26–7.39 (m, 3H, ArH), 7.41–7.43 (m, 1H, ArH), 7.53–7.57 (m, 2H, ArH), 7.68 (d, 1H, ArH, $J = 7.69$ Hz), 12.92 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{25}O_2N_3F_3Cl$ (467.92), C: 59.03, H: 5.38, N: 8.98; Found C: 59.19, H: 5.23, N: 9.15.

*6.1.3.10. Monohydrochloride N-[2-{4-(2-fluorophenyl)-piperazin-1-yl}-ethyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11b**).* White powdery crystals. Yield: 60%; mp 228–230 °C; TLC: $R_f = 0.84$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.89 (dd, 1H, H_b imide, $J = 5.90$ Hz, $J = 18.20$ Hz), 3.25 (brs 2H, CH_2–CH_2), 3.41–3.57 (m, 5H, 4H piperazine, 1H, H_a imide), 3.82–4.05 (m, 6H, 4H piperazine, 2H, CH_2–CH_2), 4.84 (q, 1H, H_c imide, $J = 5.90$ Hz), 7.04–7.24 (m, 4H, ArH), 7.38–7.44 (m, 1H, ArH), 7.55–7.57 (m, 2H, ArH), 7.68 (d, 1H, ArH, $J = 7.69$ Hz), 13.02 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{24}O_2N_3F_4Cl$ (485.91), C: 56.84, H: 4.98, N: 8.65; Found C: 56.68, H: 5.09, N: 8.45.

*6.1.3.11. Monohydrochloride N-[2-{4-(2-methoxyphenyl)-piperazin-1-yl}-ethyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11c**).* White powdery crystals. Yield: 57%; mp 200–202 °C; TLC: $R_f = 0.69$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.90 (dd, 1H, H_b imide, $J = 5.76$ Hz, $J = 18.20$ Hz), 3.07 (brs. 2H, CH_2–CH_2), 3.37 (brs, 2H, CH_2–CH_2), 3.48–3.57 (m, 5H, 4H piperazine, 1H, H_a imide), 3.86 (s, 3H, OCH_3), 3.90–4.05 (m, 4H piperazine), 4.83 (q, 1H, H_c imide, $J = 5.90$ Hz), 6.87–6.96 (m, 3H, ArH), 7.07 (t, 1H, ArH, $J = 7.43$ Hz), 7.38–7.53 (m, 1H, ArH), 7.56–7.59 (m, 2H, ArH), 7.68 (d, 1H, ArH, $J = 7.95$ Hz), 12.78 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{27}O_3N_3F_3Cl$ (497.95), C: 57.89, H: 5.46, N: 8.44; Found C: 57.62, H: 5.40, N: 8.55.

*6.1.3.12. Monohydrochloride N-[2-{4-(3-chlorophenyl)-piperazin-1-yl}-ethyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11d**).* White powdery crystals. Yield: 77%, mp 233–235 °C; TLC: $R_f = 0.89$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.91 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 17.69$ Hz), 3.10 (brs 2H, CH_2–CH_2), 3.42 (dd, 1H, H_a imide, $J = 9.10$ Hz, $J = 17.95$ Hz), 3.60–3.76 (m, 6H, 4H piperazine, 2H, CH_2–CH_2), 3.97–4.05 (m, 4H piperazine), 4.84 (q, 1H, H_c imide, $J = 5.76$ Hz), 6.78–6.94 (m, 3H, ArH), 7.18–7.23 (t, 1H, ArH, $J = 8.20$ Hz), 7.41–7.60 (m, 3H, ArH), 7.69 (d, 1H, ArH, $J = 7.95$ Hz), 13.15 (brs, 1H, HCl). Anal. Calcd for $C_{23}H_{24}O_2N_3F_3Cl_2$ (502.38), C: 55.03, H: 4.82, N: 8.37; Found C: 55.33, H: 4.65, N: 8.52.

*6.1.3.13. Monohydrochloride N-[2-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-ethyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11e**).* White powdery crystals. Yield: 55%, mp 238–240 °C; TLC: $R_f = 0.93$ (S_2). ^{1}H NMR ($CDCl_3$) δ: 2.90 (dd, 1H, H_b imide, $J = 6.04$ Hz, $J = 17.88$ Hz), 3.06 (brs 2H, CH_2–CH_2), 3.40 (brs, 2H, CH_2–CH_2) 3.53 (dd, 1H, H_a imide, $J = 9.49$ Hz, $J = 17.60$ Hz), 3.65–3.86 (m, 4H piperazine), 4.04 (brs, 4H piperazine), 4.86 (q, 1H, H_c imide, $J = 6.05$ Hz), 7.13–7.23 (m, 3H, ArH), 7.42 (t, 2H, ArH, $J = 7.42$ Hz), 7.56–7.60 (m, 2H, ArH), 7.69 (d, 1H, ArH, $J = 7.98$ Hz), 13.17 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{24}O_2N_3F_6Cl$ (500.47), C: 57.65, H: 4.84, N: 8.40; Found C: 57.82, H: 4.76, N: 8.59.

*6.1.3.14. Monohydrochloride N-[3-(4-phenylpiperazin-1-yl)-propyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11f**).* White powdery crystals. Yield: 65%; mp 203–205 °C; TLC: $R_f = 0.88$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.30–2.40 (m, 2H, CH_2–CH_2–CH_2), 2.71 (dd, 1H, H_b imide, $J = 5.40$ Hz, $J = 18.47$ Hz), 2.99 (brs, 2H, piperazine), 3.12 (t, 2H, CH_2–CH_2–CH_2, $J = 4.69$ Hz), 3.32 (dd, 1H, H_a imide, $J = 9.66$ Hz, $J = 18.47$ Hz), 3.60–3.70 (m, 6H, piperazine), 3.77 (t, 2H, CH_2–CH_2–CH_2, $J = 6.40$ Hz), 4.52 (q, 1H, H_c imide, $J = 5.54$ Hz), 6.90–6.99 (m, 3H, ArH), 7.27–7.32 (m, 3H, ArH), 7.43 (t, 1H, ArH, $J = 7.67$ Hz), 7.62 (t, 1H, ArH, $J = 7.39$ Hz), 7.70 (d, 1H, ArH, $J = 7.95$ Hz), 13.05 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{27}O_2N_3F_3Cl$ (481.95), C: 59.81, H: 5.65, N: 8.72; Found C: 59.68, H: 5.43, N: 8.58.

*6.1.3.15. Monohydrochloride N-[3-{4-(2-methoxyphenyl)-piperazin-1-yl}-propyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11g**).* White powdery crystals. Yield 51%; mp 161–163 °C; TLC: $R_f = 0.76$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.36 (brs, 2H, CH_2–CH_2–CH_2), 2.72 (dd, 1H, H_b imide, $J = 5.50$ Hz, $J = 18.70$ Hz), 3.12 (brs, 4H, piperazine), 3.32 (dd, 1H, H_a imide, $J = 9.63$ Hz, $J = 18.43$ Hz), 3.49–3.62 (m, 6H, 2H, CH_2–CH_2–CH_2, 4H, piperazine), 3.71–3.79 (m, 2H, CH_2–CH_2–CH_2), 3.89 (s, 3H, OCH_3), 4.52 (q, 1H, H_c imide, $J = 5.64$ Hz), 6.88–7.12 (m, 4H, ArH), 7.31 (d, 1H, ArH, $J = 7.70$ Hz), 7.44 (t, 1H, ArH, $J = 7.70$ Hz), 7.63 (t, 1H, ArH, $J = 7.43$ Hz), 7.70 (d, 1H, ArH, $J = 7.98$ Hz), 12.94 (brs, 1H, HCl). Anal. Calcd for $C_{25}H_{29}O_3N_3F_3Cl$ (511.98), C: 58.65, H: 5.71, N: 8.21; Found C: 58.38, H: 5.53, N: 8.38.

*6.1.3.16. Monohydrochloride N-[3-{4-(3-chlorophenyl)-piperazin-1-yl}-propyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11h**).* White powdery crystals. Yield: 53%; mp 224–227 °C; TLC: $R_f = 0.92$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.06 (t, 2H, CH_2–CH_2–CH_2, $J = 8.11$ Hz), 2.72 (dd, 1H, H_b imide, $J = 5.50$ Hz, $J = 18.70$ Hz), 2.93–3.09 (m, 4 H, 2H piperazine, 2H CH_2–CH_2–CH_2), 3.31 (dd, 1H, H_a imide, $J = 9.63$ Hz), 3.58–3.77 (m, 8H, 6H piperazine, 2H CH_2–CH_2–CH_2), 4.50 (q, 1H, H_c imide, $J = 5.50$ Hz), 6.76–6.97

(m, 3H, ArH), 7.18–7.24 (m, 2H, ArH), 7.44 (t, 1H, ArH, $J = 7.70$ Hz), 7.60–7.72 (m, 2H, ArH), 13.10 (brs, 1H, HCl). Anal. Calcd for $C_{24}H_{26}O_2N_3F_3Cl_2$ (516.40), C: 55.87, H: 5.08, N: 8.14; Found C: 55.58, H: 5.22, N: 8.18.

*6.1.3.17. Monohydrochloride N-[3-{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-propyl]-3-(2-trifluoromethylphenyl)-pyrrolidine-2,5-dione (**11i**).* White powdery crystals. Yield: 48%; mp 222–224 °C; TLC: $R_f = 0.95$ (S_2); ^{1}H NMR ($CDCl_3$) δ: 2.40 (brs, 2H, CH_2–*CH_2*–CH_2), 2.74 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 18.46$ Hz), 3.04–3.15 (m, 4H, piperazine), 3.34 (dd, 1H, H_a imide, $J = 8.20$ Hz, $J = 18.21$ Hz), 3.68–3.85 (m, 8H, 4H piperazine, 4H *CH_2*–CH_2–*CH_2*), 4.54 (q, 1H, H_c imide, $J = 5.51$ Hz), 6.91–7.21 (m, 2H, ArH), 7.32–7.41 (m, 4H, ArH), 7.61–7.66 (m, 1H, ArH), 7.71 (d, 1H, ArH, $J = 7.43$ Hz), 12.35 (brs, 1H, HCl). Anal. Calcd for $C_{25}H_{26}O_2N_3F_6Cl$ (549.95), C: 54.60, H: 4.76, N: 7.64; Found C: 54.42, H: 4.74, N: 7.71.

6.2. Anticonvulsant screening

All the compounds **8a–e**, **9a–e**, **10a–h** and **11a–i** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, by using the testing procedures described elsewhere [15,16]. Phase I studies of the investigated compounds involved three testes: maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and rotorod test for neurological toxicity (NT). Male albino mice (CF#1 strain, weighing 18–25 g) were used as experimental animals.

Compounds were injected intraperitoneally to mice as a suspension in 0.5% methylcellulose at a dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h intervals after administration.

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA) was delivered *via* corneal electrodes primed with an electrolyte solution containing an anaesthetic agent. Abolition of the hindlimb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread.

The scPTZ test utilizes of pentylenetetrazole (85 mg/kg). This produces clonic seizures lasting for a period of at least 5 s in 97% (CD_{97}) of animals tested. At the anticipated time of testing the pentylenetetrazole was administrated subcutaneously. Absence of clonic seizures in the observed time period indicated an ability of compounds to abolish the effect of pentylenetetrazole on seizure threshold.

A neurological toxicity test (NT) induced by a compound was detected in mice using standardized rotorod test [17]. Untreated control mice, when placed on the 6 rpm rotation rod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of mice to maintain equilibrium for 1 min in each of three successive trials.

Promising compounds (**8e**, **9a–b**, **9d**, **10d**, **10g–h**, **11d** and **11i**) from phase I underwent phase VIa in which were administrated orally to rats using four animals at a fixed dose of 30 mg/kg for both MES and the rotorod toxicity tests. Rats were tested at five times period ranging from one quarter to 4 h post drug administration. Additionally, compounds **8e** and **10d** were tested in the MES test after intraperitoneally injection to rats at a dose of 30 mg/kg.

The quantitative determination of the median effective dose (ED_{50}) and toxic dose (TD_{50}) were performed after *i.p.* administration to mice (**11i**) and *p.o.* administration to rats (**9a**). Groups of eight mice or rats received various doses of the compound until at least two points were established in the range of 10–90% seizure protection or minimal neurotoxicity. From the plot of obtained data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line and standard error of the slope were calculated by means of a computer program written at NINDS/NIH.

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