Bioorganic & Medicinal Chemistry 20 (2012) 4872–4880

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and anticonvulsant activity of new N-Mannich bases derived from 3-(2-fluorophenyl)- and 3-(2-bromophenyl)-pyrrolidine-2,5-diones. Part II

Jolanta Obniska *, Sabina Rzepka, Krzysztof Kamiński

Department of Medicinal Chemistry, Jagiellonian University, Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

ARTICLE INFO

Article history:
Received 3 January 2012
Revised 13 May 2012
Accepted 14 May 2012
Available online 23 May 2012

Keywords:
N-Mannich bases
3-Phenyl-pyrrolidine-2,5-diones
Succinimides
Anticonvulsant activity

ABSTRACT

Synthesis and anticonvulsant activity of new N-Mannich bases of 3-(2-fluorophenyl)- and 3-(2-bromophenyl)-pyrrolidine-2,5-diones have been described. Initial anticonvulsant screening was performed in mice after intraperitoneal administration in the maximal electroshock seizure test (MES) and subcutaneous pentylenetetrazole seizures test (*sc*PTZ). The neurotoxicity was determined applying the rotarod test. The in vivo results in mice showed that majority of compounds were effective in the MES test. Only seven molecules showed protection in the *sc*PTZ test. The quantitative evaluation in the MES seizures after oral administration into rats showed that the most active were 1-[{4-(4-fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (**14**) with ED_{50} of 7.4 mg/kg and 1-[{4-(3-bromophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (**16**) with ED_{50} of 26.4 mg/kg. These molecules were more potent and also less neurotoxic than phenytoin which was used as reference antiepileptic drug.

1. Introduction

Epilepsy is the most prevalent neurological disorder affecting approximately 50 million people worldwide. Despite of over 40 antiepileptic drugs (AEDs) currently in clinical use, convulsions in 30% of epileptics are inadequately controlled by standard drug therapy.[1,2] Furthermore, compliance is often limited by adverse side effects most notably related to CNS exposure like diminished attention, executive function, intelligence, language skills, memory and processing speed.[3]

For these reasons, constant attempts are made to investigate new chemical agents and mechanisms through which epilepsy can be effectively controlled. The currently used AEDs can be classified into several categories on the basis of the main molecular mechanisms of action, as follow: modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, enhancement of GABA-mediated inhibition or other effect on the GABA system, and inhibition of synaptic excitation mediated by ionotropic glutamate receptors.[4]

The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority AEDs make it difficult to use rational methodologies of discovery new antiepileptic drugs. Therefore the most important method to obtain new anticonvulsants is the ligand-based approach that utilizes existing biological data from old and new drugs, other historical compounds or different pharmacophore models that were established through the analysis of structural characteristics of clinically effective AEDs, as well as other anticonvulsant active compounds. This method is applied mainly for chemical (structural) modifications of currently available AEDs in aim to obtain more efficacious drugs that will suppress seizures and/or drugs with minimal or no adverse effects compared to maternal compound.[5]

The past decades have demonstrated many attempts to identify the structural features of compounds crucial for anticonvulsant activity. As a result it was proved that one of the important core fragments is defined by nitrogen heteroatomic system (imide or lactam), at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system.[6,7] Taking into consideration the above our investigations have been focused on different structural modifications of pyrrolidine-2,5-dione ring.[8–12] It should be stressed here that this scaffold appears in structures of known AEDs such as ethosuximide, mesuximiede or phensuximide (Fig. 1).

The SAR studies showed that the most active were the N-Mannich bases of 3-phenyl-pyrrolidine-2,5-diones with differently substituted phenylpiperazines as amine function.[14–16] Their activities were also closely connected with the presence of electron withdrawing trifluoromethyl group or chlorine atom in the phenyl ring at position-3 of pyrrolidine-2,5-dione moiety. The structures of chosen, active compounds obtained in previous studies are shown in Figure 2.

The high anticonvulsant activity of N-Mannich bases was observed also among structurally connected imidazoldine-2,4-diones.[13] Therefore all the above supports an efforts on the search

* Corresponding author. Tel.: +48 12 620 54 53; fax: +48 12 657 02 62.
E-mail address: mfobnisk@cyf-kr.edu.pl (J. Obniska).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.032

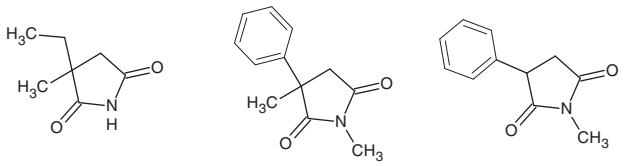


Figure 1. Structures of known AEDs bearing pyrrolidine-2,5-dione scaffold.

of new anticonvulsant agents among N-Mannich bases derived from 3-substituted pyrrolidine-2,5-diones.

Thus as a continuation of systematic chemical and pharmacological studies in the present work we have synthesized a new series of N-Mannich bases of 3-(2-fluorophenyl)- (**5**–**11**, **19**–**23**) and 3-(2-bromophenyl)-pyrrolidine-2,5-diones. These compounds have been designed as analogs of molecules described previously in which chlorine atom or trifluoromethyl group at the 3-phenyl ring have been changed into other electron withdrawing bromine or fluorine atoms. Based on the previous results, at the imide nitrogen atom we have introduced secondary amines favorable for anticonvulsant activity, for example, 4-phenyl-piperazines (unsubstituted, with electron-withdrawing Cl, F atoms or with CF_3 group), benzyl-piperazine, 2-hydroxyethyl-piperazine or morpholine. Such structural modifications enable to assess the influence of both the 2-fluorophenyl- or 2-bromophenyl-substituents and also the kind of amine function on anticonvulsant activity. All the compounds synthesized have been evaluated for anticonvulsant and neurotoxic properties within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA.

2. Results and discussion

2.1. Chemistry

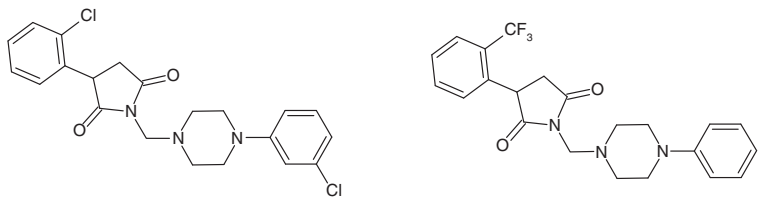
Compounds **5**–**28** were synthesized according to Scheme 1. The starting 2-(2-fluorophenyl)- (**1**) and 2-(2-bromophenyl)-succinic (**2**) acids were prepared according to the method described by Miller and Long.[17] The cyclocondensation reaction of **1** or **2** with the 25% ammonia at 180 °C for 1 h yielded in 3-(2-fluorophenyl)- (**3**) or 3-(2-bromophenyl)- (**4**) pyrrolidine-2,5-diones. The final compounds **5**–**28** were synthesized in the aminoalkylation (Mannich-type) reaction from the appropriately substituted 3-phenyl-pyrrolidine-2,5-diones (**3**, **4**), formaldehyde and corresponding 4-substituted piperazines or morpholine. The reaction was carried out in 96% ethanol at room temperature for ca. 12 h or the mixture was refluxed for 0.5 h, additionally. The crude products were crystallized from 96% ethanol giving the final compounds in yields ranging from 55% to 80%. Except of **22**, **24** and **27**, which were isolated as hydrochloride salts, the other derivatives were obtained as a free bases. The purities were assessed by thin-layer chromatography. The structures of compounds were confirmed by both spectral (^{1}H NMR) and elemental (C, H, N) analyses.

The ^{1}H NMR spectra revealed characteristic chemical shifts agreed with proposed chemical structures. In all derivatives (**5**–**28**) the signals of pyrrolidine-2,5-dione ring protons (H_a, H_b, H_c) appeared at about 2.76 ppm (H_b), 3.50 ppm (H_a) as a doublet of doublets and 4.30 ppm (H_c) as quartets. Appearance of sharp singlets at about 4.55–4.80 ppm correspond to the two protons of methylene linker and confirm the formation of N-Mannich bases **5**–**28**. The piperazine protons were observed as triplets, broad singlets or multiplets at about 2.70–2.80 ppm and 2.90–3.40 ppm. The aromatic protons were observed at expected chemical shifts. The detailed physical and spectral data are presented in experimental section.

2.2. Anticonvulsant activity

The profile of anticonvulsant activity of all compounds was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, after intraperitoneal (ip) injection into mice at doses of 30, 100 and 300 mg/kg. Except of compounds **22**, **24** and **27** tested as hydrochloride salts, all other molecules were evaluated as free bases. An observation was carried out at two different time intervals, namely 0.5 and 4 h. It should be noticed that despite significant advances that have been made in epilepsy research during last years, the MES and *sc*PTZ models are still recognized as the 'gold standards' in the early stages of testing of new anticonvulsants. These tests are capable to detect compounds affording protection against generalized tonic–clonic seizures and generalized absence seizures, respectively.[18–20] The acute neurological toxicity (NT) was determined in the minimal motor impairment—rotarod test (NT). The in vivo results for **5**–**28** are shown in Tables 1 and 2.

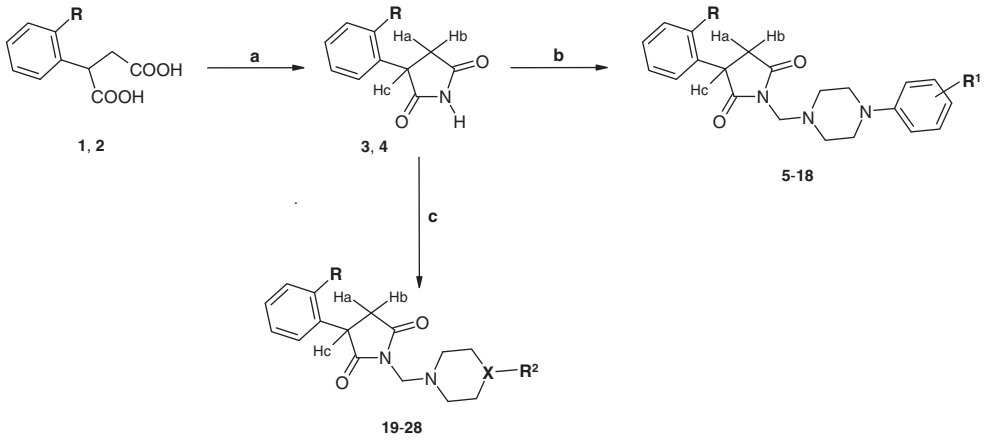
The compounds **5**–**28** showed diversified anticonvulsant properties including the strength, spectrum and time of protection that depended mainly on the kind of the amine function at position-1 of pyrrolidne-2,5-dione. The 4-phenylpiperazin-1-yl-methyl derivatives **5**–**18** showed protection only in the MES test (or were devoid of activity), whereas their benzyl, 2-pyrimidinyl or 2-hydroxyethyl analogues, as well as morpholine derivatives (**19**–**28**) were effective in both MES and *sc*PTZ seizures, in general. Furthermore, except of **11** all other 4-phenylpiperazine derivatives revealed protection only at 4 h after ip administration that indicates delayed onset however long duration of anticonvulsant protection. The exchange of the phenyl substituent into benzyl, 2-pyrimidinyl or 2-hydroxyethyl moieties or introduction of morpholine ring yielded in molecules active in both time intervals (0.5 and 4 h). The activity at 0.5 h is equivalent to quick onset of protection in contrast to 4-phenylpiperazines described above. In the whole series only six compounds (**6**, **8**, **9**, **13**, **21**, **24**), were devoid of activity among



ED_{50} = 14.18 mg/kg (MES, *p.o.*, rats)
TD_{50} < 125 mg/kg (rotarod test, *p.o.*, rats)

ED_{50} = 20.78 mg/kg (MES, *p.o.*, rats)
TD_{50} > 500 mg/kg (rotarod test, *p.o.*, rats)

Figure 2. Structures of model anticonvulsant active compounds.



R = F, Br

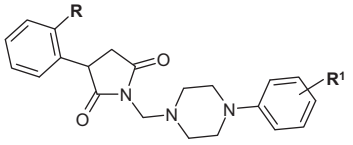
R^1 = H, 2-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 3-CF_3

X = N, O

R^2 = Benzyl, 2-pyrimidinyl, cyclohexyl, 2-hydroxyethyl

Scheme 1. Synthetic routes to intermediates **3**, **4** and final compounds **5–28**. Reagents and conditions: (a) 25% NH_4OH, 180 °C, 1 h, (b) 4-phenylpiperazine derivatives, formaldehyde, 96% ethyl alcohol, reflux - 0.5 h or room temperature ca 12 h, (c) 4-substituted piperazines or morpholine, formaldehyde, 96% ethyl alcohol, reflux - 0.5 h or room temperature ca 12 h.

Table 1
The results after intraperitoneal injection of compounds **5–18** in mice



Compound	R	R^1	MES[a]		NT[b]	
			0.5 h	4 h	0.5 h	4 h
5	F	H	—	300	300	—
6	F	2-F	—	—	—	—
7	F	4-F	—	30	—	300
8	F	2-Cl	—	—	—	—
9	F	3-Cl	—	—	—	—
10	F	4-Cl	—	100	—	—
11[*]	F	3-CF_3	300	100	—	—
12	Br	H	—	100	—	—
13	Br	2-F	—	—	—	—
14	Br	4-F	—	100	—	—
15	Br	2-Cl	—	300	—	—
16	Br	3-Cl	—	100	—	—
17	Br	4-Cl	—	300	—	—
18	Br	3-CF_3	—	300	—	
PTH[c]	—	—	30	30	100	100

Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby anticonvulsant activity or neurotoxicity was demonstrated in half or more animals. The animals were examined at 0.5 and 4 h. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg).

[a] Maximal electroshock test.

[b] Neurotoxicity—rotarod test.

[c] Phenytoin (PHT) reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 27.

[*] Compound **11** revealed also anti-MES activity at a dose of 100 mg/kg at 0.25 and 1 h.

which five were also non-neurotoxic. The other showed protection in at least one animal epilepsy seizure model. The comparison of the results obtained for 3-(2-fluorophenyl)-pyrrolidine-2,5-diones (**5–11**, **19–23**) versus 3-(2-bromophenyl)-pyrrolidine-2,5-diones (**12–18**, **24–28**) showed a little higher activity of the latter.

The most active in the mice ip MES test was **7** active at a dose of 30 mg/kg and compounds **10–12**, **14**, **16**, **20**, **22**, **23**, **25–28** that showed protection at 100 mg/kg. The activity of **7** was comparable with phenytoin used as reference drug preventing electrically induced seizures. The lower anti-MES activity was observed for **5**, **15** and **17–19—**300 mg/kg. The inhibition of MES seizures indicative of the ability of substance to prevent seizure spread which is characteristic especially for phenytoin and phenytoin-like drugs and may indicate the influence of compounds synthesized on voltage-depended sodium channels as the most plausible mechanism of anticonvulsant action. This hypothesis has been confirmed in our previous studies.[13]

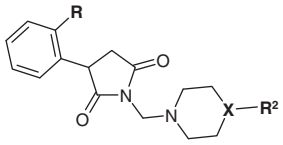
From the whole series only seven molecules **19**, **20**, **22**, **23**, **25**, **27** and **28** showed protection at doses of 100 mg/kg or 300 mg/kg in the *sc*PTZ test which identifies substances elevating seizure threshold.

In the rotarod test for acute neurological toxicity (NT), compounds **5**, **7**, **19**, **20**, **23–25**, **27** and **28** showed neurotoxicity in the maximum dose administered—300 mg/kg. The other molecules did not exhibit motor impairment.

Ten compounds representing 4-phenylpiperazin-1-yl-methyl derivatives (**7**, **10**, **11**, **14**, **16**), their 2-hydroxyethyl (**22**, **27**) and cyclohexyl (**26**) analogues, as well as, morpholine derivatives (**23**, **28**), were examined for their anticonvulsant activity in the MES test and neurotoxicity (rotarod test) after p.o. administration into rats at a dose of 30 mg/kg. According to the ADD Program dispositions the selection was based on mice ip data and the results are shown in Table 3.

The most active were **11**, **16** and **26** that protected all animals at time points 0.5 h, 1 h, 2 h (**11**), 2 h, 4 h (**16**) and 1 h (**26**). These compounds protected also 50% of rats at 4 h (**11**), 1 h (**16**), 0.25 h and 0.5 h (**26**). The other derivatives (**7**, **10**, **14**, **22**, **23**, **27** and **28**) were less active and protected from 25% to 75% of rats in different time intervals. When given orally none of the compounds tested caused motor impairment in the rotarod test. The in vivo data in rats confirmed their absorption from gastrointestinal tract and also penetration to central nervous system.

Table 2
The results after intraperitoneal injection of compounds **19–28** in mice



Compound	R	X	R^2	MES[a]		*sc*PTZ[b]		NT[c]	
				0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
19	F	N	Benzyl	300	300	300[25]	–	300[14]	–
20	F	N	2-Pyrimidinyl	–	100	300[25]	–	300	–
21	F	N	Cyclohexyl	–	–	–	–	–	–
22	F	N	2-Hydroxyethyl	300	100	300[25]	–	–	–
23	F	O	–	100	100	300	300[25]	300[13,14]	–
24	Br	N	Benzyl	–	–	–	–	300[21]	–
25[e]	Br	N	2-Pyrimidinyl	–	100	100[25]	300[25]	300	300
26	Br	N	Cyclohexyl	–	100	–	–	–	–
27	Br	N	2-Hydroxyethyl	100	100	100[25]	300[25]	300	–
28	Br	O	–	100	100	300	–	300	–
PHT[d]	–	–	–	30	30	–	–	100	100
ETX[d]	–	–	–	–	–	100	300	–	–

Response comments:[13] loss righting reflex,[14] unable to grasp rotarod,[21] tonic extension,[25] myoclonic jerks.
Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby anticonvulsant activity or neurotoxicity was demonstrated in half or more animals. The animals were examined at 0.5 and 4 h. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg).
[a] Maximal electroshock test.
[b] Subcutaneous pentylenetetrazole test.
[c] Neurotoxicity—rotarod test.
[d] Phenytoin (PHT), ethosuximide (ETX), reference drugs, tested by use of ADD Program procedures in NIH/NINDS, data for Ref. 27.
[e] Compound **25** revealed anti-MES activity at a dose of 100 mg/kg at 6 h.

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

Compound	MES[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
7	0	0	0	1	2
10	0	0	0	1	2
11	1	4	4	4	2
14	1	1	1	2	3
16	0	1	2	4	4
22	1	1	2	1	1
23	1	1	2	2	1
26	2	2	4	1	1
27	1	1	0	0	1
28	0	0	0	0	1
PHT[b]	1	4	3	3	3

The data indicate the number of rats of four that were protected.
[a] Maximal electroshock test.
[b] Phenytoin (PHT) reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 28.

Four compounds active in rats—**14**, **16**, **22** and **26** have been chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}) after p.o. application (Table 4).

The median effective doses (ED_{50}) in the MES test, as well as neurotoxic doses (TD_{50}) were determined at previously estimated time of peak effect (TPE). The quantitative p.o. data revealed that compounds **14** and **16** showed higher activity than phenytoin in the electrically induced seizures. At the same time they revealed lower neurotoxicity that resulted in higher protection index (PI). Additionally, it should be stressed that in case of both molecules **14** and **16** the time of peak effect (TPE) was twice longer than for phenytoin. This fact may indicate an elongated time of protection in comparison with reference drug.

During further studies, **7**, **11** and **14** have been chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}) after ip administration in mice. As a result all compounds tested showed longer TPE, weaker anticonvulsant activity but also lower neurotoxicity than standard drug—phenytoin (Table 5).

According to the Antiepileptic Drug Development (ADD) Program disposition eleven compounds **10**–**14**, **16**–**19**, **22** and **23** compounds were randomly selected for the evaluation of anticonvulsant activity in the 6-Hz test. Eight of them were active and the results are shown in Table 6. The 6-Hz test has been validated recently as a model of therapy-resistant epilepsy. It was not used widely because of its lack clinical validity since the hydantoins such as phenytoin failed to show protective activity. Nevertheless, the clinically effective antiepileptic drug levetiracetam, which is not active in the conventional MES and *sc*PTZ tests, does exhibit protective activity in the 6-Hz model. This suggested that the 6-Hz model might be capable for identifying anti-seizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action.[21]

As can be seen from mice ip data, the most active was compounds **23** that showed maximal 100% protection at time points 0.25 h, 0.5 h, 1 h. The other molecules were less active and protected up to 75% of mice. The quantitative studies carried out for **23** (Table 7) revealed unfortunately lower activity and higher neurotoxicity than levetiracetam that was used as model antiepileptic drug for this type of seizures.

In summary, all compounds were prepared as racemic mixtures and no attempt has been made to resolve the enantiomers. Due to interesting profile of anticonvulsant activity further investigations that enable exhaustive SAR studies in this group of derivatives are urgently necessary.

3. Conclusion

The library of 24 new N-Mannich bases derived from 3-(2-fluorophenyl)- and 3-(2-bromophenyl)-pyrrolidine-2,5-diones has been synthesized and tested for anticonvulsant and neurotoxic properties. The results obtained revealed that majority of

Table 4
Quantification studies of chosen compounds in the MES and rotarod test (NT) in rats after p.o. administration

Compound	TPE[a] (h)	ED_{50}[b] MES (mg/kg)	TD_{50}[b] NT (mg/kg)	PI[c] MES (TD_{50}/ED_{50})
14	4	7.4 (4.80–10.4)	62.4 (44.6–80.6)	8.43
16	4	26.4 (19.9–32.2)	126 (107.1–144.2)	4.77
22	0.5	>500	>500	ND
26	2	<240	ND	ND
PHT[d]	2	28.1 (20.7–35.2)	>100	>3.60

ND—no data.
[a] Time to peak effect.
[b] Results are represented as mean ± SEM at 95% confidence limit (MES-maximal electroshock test; NT-neurotoxicity, rotarod test).
[c] Protection index (TD_{50}/ED_{50}).
[d] Phenytoin (PHT) reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 29.

Table 5
Quantification studies of **7**, **11** and **14** in the MES and rotarod test (NT) in mice after ip administration

Compound	TPE[a] (h)	ED_{50}[b] MES (mg/kg)	TD_{50}[b] NT (mg/kg)	PI[c] MES (TD_{50}/ED_{50})
7	4	100.7 (52.8–208.2)	166.1 (129.4–208.3)	1.65
11	4	41.35 (28.44–62.04)	>500	>12.09
14	4	79.9 (58.49–112.75)	465.84 (401.01–551.93)	5.83
PHT[d]	1	5.32 (5.44–7.23)	41.2 (36.9–46.1)	7.74

[a] Time to peak effect.
[b] Results are represented as mean ± SEM at 95% confidence limit (MES-maximal electroshock test; NT-neurotoxicity, rotarod test).
[c] Protection index (TD_{50}/ED_{50}).
[d] Phenytoin (PHT) reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 29.

compounds exhibited anticonvulsant activity, especially in the maximal electroshock (MES) test, which is the animal model of human generalized tonic–clonic seizures. The anticonvulsant properties including the strength, spectrum and time of protection, depend in general on the kind of the amine function at position-1 of pyrrolidne-2,5-dione. The quantitative studies in rats after oral administration showed that two compounds were more potent than phenytoin in the MES test. The highest activity was observed for 1-[{4-(4-fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione that showed ED_{50} value of 7.4 mg/kg.

4. Experimental section

4.1. Chemistry

All the chemicals and solvents were purchased from Sigma–Aldrich (St. Louis, USA) 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} aluminum sheets (Merck; Darmstadt, Germany), using subsequent developing systems: S_1–chloroform:acetone (9:1, *v/v*). S_2–chloroform:2-propanol:25% ammonia (9:11:2, *v/v/v*). Spots were detected by their absorption under UV light (λ = 254 nm). Elemental analysis for C, H, and N were 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, ^{13}C NMR and ^{19}F NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$ or DMSO, operating at 300 MHz, 282 MHz and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to TMS δ = 0 (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). The mass spectra for compounds **3**–**28** were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, USA). The ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm column was used (Waters, Milford, USA).

The starting 2-(-2-fluorophenyl)- (**1**) and 2-(2-bromophenyl)-(**2**) succinic acids were prepared by use of method described by Miller and Long.[17]

Table 6
Anticonvulsant activity—psychomotor seizure test in mice (6-Hz, current 32 mA)

Compound	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
10	1	2	1	0	0
11	1	3	0	1	1
12	1	0	2	3	2
13	0	1	1	1	1
16[b]	1	1	1	1	1
19	0	1	0	0	0
22	2	1	0	0	0
23	4	4	4	3	1

[a] Dose of 100 mg/kg was administrated ip. The data indicate the number of mice of four that were protected.
[b] Dose of 75 mg/kg was administrated ip.

Table 7
Quantification data—psychomotor seizure tests (6-Hz) after ip injection into mice

Compound	TPE[a] (h)	ED_{50}[b] (mg/kg)	TD_{50}[c] (mg/kg)	PI[d] (TD_{50}/ED_{50})
23	4	103 (83.1–123.4)	253.2 (168.4–293.1)	2.46
Levetiracetam[e]	1	19.4 (20.7–35.2)	>500	>26

[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] Reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 29.

4.1.1. General procedure for the synthesis of 3-(2-fluorophenyl)- and 3-(2-bromophenyl)-pyrrolidine-2,5-diones (3, 4)

A total of 0.05 mol of the 2-(2-fluorophenyl)- (**1**) or 2-(2-bromophenyl)- (**2**) 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 term-regulated sand bath (ST 72 Roth, Karlsruhe, Germany) with simultaneous distillation of water. After complete removal of water, the temperature of the reaction mixture raised up to 180 °C and was maintained for 1 h. The crude products were crystallised from methanol.

4.1.1.1. 3-(2-Fluorophenyl)-pyrrolidine-2,5-dione (3). White solid. Yield: 70%; mp 107–109 °C; TLC: R_f = 0.60 (S_1); ^{1}H NMR ($CDCl_3$) δ: 2.90 (dd, 1H, H_b imide, *J* = 5.90 Hz, *J* = 18.72 Hz), 3.30 (dd, 1H, H_a imide, *J* = 9.74 Hz, *J* = 18.46 Hz), 4.49 (dd, 1H, H_c imide, *J* = 5.50 Hz, *J* = 9.74 Hz), 7.10–7.25 (m, 2H, ArH), 7.29–7.38 (m, 1H, ArH), 7.60–7.64 (m, 1H, ArH), 8.40 (br s 1H, NH); ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.04 (s, 1F). ESI-MS: 194.10 ($C_{10}H_8O_2NF$

$[M+H]^+$). Anal. calcd for $C_{10}H_8O_2NF$ (193.18) C: 62.23, H: 4.18, N: 7.26; found C: 62.60, H: 4.27, N: 7.33.

4.1.1.2. 3-(2-Bromophenyl)-pyrrolidine-2,5-dione (4). White solid. Yield: 60%; mp 130–132 °C; TLC: $R_f = 0.53$ (S_1); ^{1}H NMR ($CDCl_3$) δ: 2.81 (dd, 1H, H_b imide, $J = 5.91$ Hz, $J = 18.74$ Hz), 3.31 (dd, 1H, H_a imide, $J = 9.48$ Hz, $J = 18.72$ Hz), 4.47 (dd, 1H, H_c imide, $J = 5.90$ Hz, $J = 9.76$ Hz), 7.15–7.21 (m, 2H, ArH), 7.22–7.28 (m, 1H, ArH) 7.60–7.64 (m, 1H, ArH), 8.32 (br s, 1H, NH). ESI-MS: 254.91 ($C_{10}H_8O_2NBr$ $[M+H]^+$). Anal. calcd for $C_{10}H_8O_2NBr$ (254.09) C: 47.29, H: 3.17, N: 5.51; found C: 47.03, H: 3.00, N: 5.66.

4.1.2. General procedure for the synthesis of compounds 5–28

The mixture of 3-(2-fluorophenyl)-pyrrolidine-2,5-dione (**3**) (0.01 mol) or 3-(2-bromophenyl)-pyrrolidine-2,5-dione (**4**) (0.01 mol), 40% formaldehyde solution (0.01 mol) and corresponding 4-substituted piperazines or morpholine (0.01 mol) in 96% ethanol (40 ml) was left for ca. 12 h at room temperature (or was refluxed for 0.5 h additionally), and then refrigerated at ca. −10 °C for 24 h. The precipitated crude products were separated by filtration, washed with cold ethanol and recrystallized from 96% ethanol. Due to oily form of **22**, **24** and **27**, they were converted into hydrochloride salts in anhydrous ethanol saturated with HCl gas. The obtained precipitates were crystallized from anhydrous ethanol.

4.1.2.1. 1-[(4-Phenylpiperazin-1-yl)-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (5). White solid. Yield: 73%; mp 127–129 °C; TLC: $R_f = 0.82$ (S_1), $R_f = 0.91$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.81 (t, 4H, piperazine, $J = 5.13$ Hz), 2.86 (dd, 1H, H_b imide, $J = 5.64$ Hz, $J = 13.60$ Hz), 3.16–3.21 (m, 4H, piperazine), 3.24 (dd, 1H, H_a, imide, $J = 5.64$ Hz, $J = 13.60$ Hz), 4.12 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.62 (s, 2H, CH_2), 6.83–6.93 (m, 3H, ArH), 7.06–7.24 (m, 1H, ArH), 7.26–7.36 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.61, 46.88, 50.90, 51.24, 60.48, 120.38, 123.79, 123.99, 127.51, 128.20, 128.68, 129.66, 129.91, 130.59, 133.58, 136.71, 149.12, 176.59, 178.08; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.03 (s, 1F). ESI-MS: 368.19 ($C_{21}H_{22}O_2N_3F$ $[M+H]^+$). Anal. calcd for $C_{21}H_{22}O_2N_3F$ (367.43): C: 68.73, H: 6.04, N: 11.45; found C: 68.42, H: 6.01, N: 11.22.

4.1.2.2. 1-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (6). White solid. Yield: 81%; mp 128–130 °C; TLC: $R_f = 0.89$ (S_1), $R_f = 0.90$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.79–2.87 (m, 5H, 4H piperazine, 1H, H_b, imide), 3.07–3.22 (m, 4H, piperazine), 3.25 (dd, 1H, H_a imide, $J = 1.02$ Hz, $J = 7.44$ Hz), 4.14 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.63 (s, 2H, CH_2), 6.89–6.98 (m, 2H, ArH), 7.01–7.24 (m, 3H, ArH), 7.27–7.37 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.51 46.83, 50.94, 51.25, 60.49, 120.40, 123.77, 123.94, 127.53, 128.24, 128.79, 129.66, 129.95, 130.62, 133.68, 136.79, 149.19, 176.65, 178.11; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.04 (s, 1F), −122.78 (s, 1F). ESI-MS: 386.20 ($C_{21}H_{21}O_2N_3F_2$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3F_2$ (385.42): C: 65.52, H: 5.50, N: 10.91; found C: 65.38, H: 5.24, N: 11.13.

4.1.2.3. 1-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (7). White solid. Yield: 65%; mp 145–147 °C; TLC: $R_f = 0.89$ (S_1), $R_f = 0.86$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.81 (m, 4H, piperazine), 2.87 (dd, 1H, H_b, imide, $J = 1.28$ Hz, $J = 7.44$ Hz), 3.08–3.21 (m, 4H, piperazine), 3.25 (dd, 1H, H_a imide, $J = 1.28$ Hz, $J = 7.44$ Hz), 4.10 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.62 (s, 2H, CH_2), 6.83–6.99 (m, 2H, ArH), 7.06–7.24 (m, 5H, ArH), 7.29–7.37 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.52, 41.88, 50.21, 50.57, 60.32, 115.36, 115.65, 116.02, 116.30, 117.89, 117.99, 124.57, 124.75, 124.79, 129.98, 130.09, 130.33, 130.39, 147.90, 155.61, 158.78, 159.11, 162.37, 176.70; 178.20; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -116.04 (s, 1F), -124.48 (s, 1F). ESI-MS: 386.14 ($C_{21}H_{21}O_2N_3F_2$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3F_2$ (385.42): C: 65.52, H: 5.50, N: 10.91; found C: 65.78, H: 5.14, N: 10.73.

4.1.2.4. 1-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (8). White solid. Yield: 55%; mp 112–114 °C; TLC: $R_f = 0.88$ (S_1), $R_f = 0.92$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.80–2.90 (m, 5H, 4H piperazine, 1H, H_b imide), 3.05 (t, 4H, piperazine $J = 4.87$ Hz), 3.22 (dd, 1H. H_a imide, $J = 1.02$ Hz, Hz, $J = 9.74$ Hz), 4.16 (q, 1H, H_c imide, $J = 5.38$ Hz), 4.63 (s, 2H, CH_2), 6.94–7.36 (m, 8H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.54, 46.81, 50.95, 51.23, 60.44, 120.41, 123.73, 123.91, 127.57, 128.20, 128.75, 129.66, 129.91, 130.62, 133.63, 136.74, 149.11, 176.63, 178.08; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.06 (s, 1F). ESI-MS: 402.21 ($C_{21}H_{21}O_2N_3FCl$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3FCl$ (401.87): C: 52.33, H: 5.27, N: 10.45; found C: 52.68, H: 5.14, N: 10.23.

4.1.2.5. 1-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (9). White solid. Yield: 85%; mp 108–110 °C; TLC: $R_f = 0.62$ (S_1), $R_f = 0.88$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.77–2.80 (m, 4H piperazine), 2.86 (dd, 1H, H_b imide, $J = 1.28$ Hz, $J = 9.23$ Hz), 3.16–3.19 (m, 4H, piperazine), 3.24 (dd, 1H, H_a imide, $J = 1.28$ Hz, $J = 9.43$), 4.10 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.61 (s, 2H, CH_2), 6.74–6.88 (m, 3H, ArH), 7.06–7.23 (m, 3H, ArH), 7–26–7.40 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.49, 46.75, 50.91, 51.19, 60.42, 120.40, 123.72, 123.93, 127.53, 128.19, 128.72, 129.64, 129.91, 130.62, 133.66, 136.77, 149.14, 176.68, 178.09; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.04 (s, 1F). ESI-MS: 402.22 ($C_{21}H_{21}O_2N_3FCl$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3FCl$ (401.87): C: 52.33, H: 5.27, N: 10.45; found C: 52.12, H: 5.38, N: 10.59.

4.1.2.6. 1-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (10). White solid. Yield: 85%; mp 98–100 °C; TLC: $R_f = 0.85$ (S_1), $R_f = 0.93$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.79 (t, 4H piperazine, $J = 5.00$ Hz), 2.87 (d, 1H, H_b imide, $J = 5.65$ Hz), 3.12–3.20 (m, 4H, piperazine), 3.25 (dd, 1H, H_a imide, $J = 1.02$ Hz, $J = 9.74$ Hz), 4.11 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.62 (s, 2H, CH_2), 6.79–6.86 (m, 2H, ArH), 7.06–7.24 (m, 5H, ArH), 7–29–7.37 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.53, 46.82, 50.90, 51.24, 60.45, 120.42, 123.78, 123.95, 127.58, 128.22, 128.79, 129.60, 129.95, 130.62, 133.63, 136.74, 149.11, 176.65, 178.11; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.04 (s, 1F). ESI-MS: 402.21 ($C_{21}H_{21}O_2N_3FCl$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3FCl$ (401.87): C: 52.33, H: 5.27, N: 10.45; found C: 52.18, H: 5.10, N: 10.33.

4.1.2.7. 1-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (11). White solid. Yield: 75%; mp 102–104 °C; TLC: $R_f = 0.79$ (S_1), $R_f = 0.87$ (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.80–2.83 (m, 4H piperazine), 2.89 (dd, 1H, H_b imide, $J = 0.52$ Hz, $J = 8.71$ Hz), 3.17–3.27 (m, 5H, 4H piperazine, 1H, H_a imide), 4.10 (q, 1H, H_c imide, $J = 5.64$ Hz), 4.63 (s, 2H, CH_2), 7.02–7.21 (m, 4H, ArH), 7.24–7.37 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.51, 46.80, 50.91, 51.26, 60.47, 120.41, 123.75, 123.93, 127.59, 128.25, 128.80, 129.62, 129.92, 130.61, 133.64, 136.76, 149.10, 176.63, 178.10; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.04 (s, 1F), −62.84 (s, 3F). ESI-MS: 436.10 ($C_{22}H_{21}O_2N_3F_4$ $[M+H]^+$). 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: 4.64, N: 9.43.

4.1.2.8. 1-[(4-Phenylpiperazin-1-yl)-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (12). White solid. Yield: 78%; mp 88–90 °C; TLC: $R_f = 0.43$ (S_1), $R_f = 0.64$ (S_2); ^{1}H NMR

(300 MHz, $CDCl_3$) δ: 2.77 (d, 1H, H_b imide, J = 5.90 Hz), 2.82–2.85 (m, 4H, piperazine), 3.19 (t, 4H, piperazine, J = 5.13 Hz), 3.29 (dd, 1H, H_a imide, J = 9.74 Hz, J = 18.46 Hz), 4.43 (q, 1H, H_c imide J = 5.90 Hz), 4.63 (s, 2H, CH_2), 6.83–6.93 (m, 3H, ArH), 7.16–7.35 (m, 5H, ArH), 7.59–7.62 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.57, 46.81, 50.97, 51.20, 60.48, 120.44, 123.75, 123.98, 127.58, 128.25, 128.79, 129.67, 129.93, 130.64, 133.64, 136.78, 149.17, 176.68, 178.11. ESI-MS: 428.30 ($C_{21}H_{22}O_2N_3Br$ $[M+H]^+$). Anal. calcd for $C_{21}H_{22}O_2N_3Br$ (428.33): C: 58.93, H: 5.18, N: 9.82; found C: 58.82, H: 5.22, N: 9.75.

4.1.2.9. 1-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (13). White solid. Yield: 87%; mp 147–149 °C; TLC: R_f = 0.84 (S_1), R_f = 0.78 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.77 (d, 1H, H_b imide, J = 6.15 Hz), 2.82–2.87 (m, 4H, piperazine), 3.09 (t, 4H, piperazine, J = 4.87 Hz), 3.30 (dd, 1H, H_a imide, J = 10.0 Hz, J = 18.72 Hz), 4.45 (q, 1H, H_c imide, J = 5.90 Hz), 4.63 (s, 2H, CH_2), 6.90–7.09 (m, 5H, ArH), 7.17–7.24 (m, 1H, ArH), 7.31–7.36 (m, 1H, ArH), 7.59–7.63 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.53, 41.89, 50.20, 50.54, 60.30, 115.31, 115.61, 116.04, 116.28, 117.83, 117.91, 124.54, 124.72, 124.76, 129.96, 130.02, 130.31, 130.39, 147.90, 155.58, 158.78, 159.08, 162.35, 176.68; 178.19; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −122.86 (s, 1F). ESI-MS: 446.22 ($C_{21}H_{21}O_2N_3FBr$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3FBr$ (446.32): C: 56.55, H: 4.75, N: 9.42; found C: 56.78, H: 4.84, N: 9.23.

4.1.2.10. 1-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (14). White solid. Yield: 65%; mp 96–98 °C; TLC: R_f = 0.83 (S_1), R_f = 0.87 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.78 (d, 1H, H_b imide, J = 5.90 Hz), 2.83 (t, 4H, piperazine, J = 5.0 Hz), 3.11 (t, 4H piperazine, J = 4.87 Hz), 3.29 (dd, 1H, H_a imide, J = 9.74 Hz, J = 18.46 Hz), 4.43 (q, 1H, H_c imide, J = 5.90 Hz), 4.63 (s, 2H, CH_2), 6.84–6.99 (m, 4H, ArH), 7.17–7.23 (m, 2H, ArH), 7.30–7.36 (m, 1H, ArH), 7.60–7.62 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.52, 46.78, 50.91, 51.19, 60.45, 120.40, 123.75, 123.95, 127.56, 128.22, 128.76, 129.68, 129.93, 130.64, 133.68, 136.75, 149.17, 176.68, 178.10; ^{19}F NMR (282 MHz, $CDCl_3$) δ: -124.29 (s, 1F). ESI-MS: 446.23 ($C_{21}H_{21}O_2N_3FBr$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3FBr$ (446.32): C: 56.55, H: 4.75, N: 9.42; found C: 56.28, H: 4.59, N: 9.63.

4.1.2.11. 1-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (15). White solid. Yield: 55%; mp 113–115 °C; TLC: R_f = 0.67 (S_1), R_f = 0.75 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.80–2.92 (m, 5H, 4H piperazine, 1H, H_b imide), 3.05 (t, 4H, piperazine J = 4.49 Hz), 3.31 (dd, 1H, H_a imide, J = 10.0 Hz, J = 18.46 Hz), 4.44 (q, 1H, H_c, imide, J = 5.90 Hz), 4.64 (s, 2H, CH_2), 6.94–7.05 (m, 2H, ArH), 7.17–7.27 (m, 3H, ArH), 7.31–7.36 (m, 2H, ArH), 7.60–7.64 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.56, 46.80, 50.96, 51.21, 60.47, 120.43, 123.76, 123.97, 127.59, 128.23, 128.78, 129.65, 129.92, 130.63, 133.65, 136.76, 149.15, 176.66, 178.10. ESI-MS: 462.11 ($C_{21}H_{21}O_2N_3ClBr$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3ClBr$ (462.72): C: 54.48, H: 4.57, N: 9.08; found C: 54.18, H: 4.44, N: 8.93.

4.1.2.12. 1-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (16). White solid. Yield: 70%; mp 122–124 °C; TLC: R_f = 0.46 (S_1), R_f = 0.67 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.79–2.86 (m, 5H, 4H piperazine, 1H, H_b imide), 3.19 (t, 4H, piperazine J = 4.91 Hz), 3.29 (dd, 1H, H_a imide, J = 10.0 Hz, J = 18.46 Hz), 4.42 (q, 1H, H_c imide, J = 5.90 Hz), 4.61 (s, 2H, CH_2), 6.74–6.86 (m, 3H, ArH), 7.13–7.24 (m, 3H, ArH), 7.29–7.35 (m, 1H, ArH), 7.59–7.62 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.54, 46.79, 50.95, 51.19, 60.46, 120.42, 123.76, 123.98, 127.60, 128.24, 128.79, 129.64, 129.93, 130.61, 133.64, 136.77, 149.16, 176.67, 178.09. ESI-MS: 462.13 ($C_{21}H_{21}O_2N_3ClBr$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3ClBr$ (462.72): C: 54.48, H: 4.57, N: 9.08; found C: 54.70, H: 4.55, N: 9.09.

4.1.2.13. 1-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (17). White solid. Yield: 62%; mp 126–128 °C; TLC: R_f = 0.32 (S_1), R_f = 0.61 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.77–2.89 (m, 5H, 4H piperazine, 1H, H_b imide), 3.15 (t, 4H, piperazine J = 4.87 Hz), 3.29 (dd, 1H, H_a imide, J = 10.0 Hz, J = 18.46 Hz), 4.42 (q, 1H, H_c imide, J = 5.90 Hz), 4.62 (s, 2H, CH_2), 6.79–6.91 (m, 2H, ArH), 7.15–7.35 (m, 5H, ArH), 7.59–7.62(m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.53, 46.78, 50.93, 51.18, 60.45, 120.41, 123.75, 123.96, 127.57, 128.20, 128.77, 129.61, 129.90, 130.61, 133.64, 136.75, 149.12, 176.65, 178.09. ESI-MS: 462.12 ($C_{21}H_{21}O_2N_3ClBr$ $[M+H]^+$). Anal. calcd for $C_{21}H_{21}O_2N_3ClBr$ (462.72): C: 54.48, H: 4.57, N: 9.08; found C: 54.79, H: 4.52, N: 9.05.

4.1.2.14. 1-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (18). White solid. Yield: 63%; mp 108–110 °C; TLC: R_f = 0.45 (S_1), R_f = 0.69 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.78–2.89 (m, 5H, 4H piperazine, 1H, H_b imide), 3.12–3.58 (m, 5H, 4H piperazine, 1H imide), 4.12 (q, 1H, H_c imide, J = 6.15 Hz), 4.63 (s, 2H, CH_2), 7.03–7.19 (m, 3H, ArH), 7.22–7.37 (m, 4H, ArH), 7.59–7.62 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.55, 46.79, 50.96, 51.21, 60.48, 120.44, 123.76, 123.98, 127.60, 128.24, 128.79, 129.65, 129.93, 130.64, 133.66, 136.76, 149.16, 176.66, 178.11; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −62.82 (s, 3F). ESI-MS: 496.18 ($C_{22}H_{21}O_2N_3F_3Br$ $[M+H]^+$). Anal. calcd for $C_{22}H_{21}O_2N_3F_3Br$ (496.33): C: 53.28, H: 4.27, N: 8.47; found C: 53.58, H: 4.24, N: 8.23.

4.1.2.15. 1-[(4-Benzylpiperazin-1-yl)-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (19). White solid. Yield: 85%; mp 57–59; TLC: R_f = 0.83 (S_1), R_f = 0.87 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (br s, 4H, piperazine), 2.68 (t, 4H, piperazine, J = 4.49 Hz), 2.76 (dd, 1H, H_b imide, J = 1.02 Hz, J = 5.64 Hz), 2.82 (dd, 1H, H_a imide, J = 1.02 Hz, J = 5.77 Hz), 3.49 (d, 2H, CH_2, J = 5.90 Hz), 4.12 (q, 1H, H_c imide, J = 9.74 Hz), 4.56 (s, 2H, CH_2), 7.07–7.36 (m, 9H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.19, 41.66, 50.58, 52.93, 60.34, 62.90, 115.96, 116.24, 124.63, 124.70, 124.75, 124.81, 127.05, 128.20, 129.14, 129.89, 129.99, 130.24, 130.29, 137.96, 159.11, 162.38, 176.67, 178.13; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.18 (s, 1F). ESI-MS: 382.26 ($C_{22}H_{24}FN_3O_2$ $[M+H]^+$). 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.

4.1.2.16. 1-[{4-(Pyrimid-2-yl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (20). White solid. Yield: 72%; mp 123–126 °C; TLC: R_f = 0.83 (S_1), R_f = 0.93 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.70 (t, 4H, piperazine, J = 5.0 Hz), 2.79 (dd, 5H, 1H, H_b imide, J = 0.77 Hz, J = 5.26 Hz), 3.16 (dd, 1H, H_a imide, J = 1.02 Hz, J = 5.77 Hz), 2.81 (t, 4H piperazine, J = 5.13 Hz), 4.08 (q, 1H, H_c imide, J = 5.64 Hz), 4.61 (s, 2H, CH_2), 6.46–6.49 (m, 1H, pyrimidine), 7.02–7.34 (m, 4H, ArH), 8.29 (d, 2H, pyrimidine, J = 4.62 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.45, 36.47, 41.81, 41.83, 43.67, 50.46, 60.49, 109.93, 115.97, 116.25, 124.58, 124.69, 124.74, 124.76, 129.91, 130.02, 130.32, 130.37, 157.68, 159.06, 161.70, 162.33, 176.58, 178.06; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.19 (s, 1F). ESI-MS: 370.26 ($C_{19}H_{20}O_2N_5F$ $[M+H]^+$). Anal. calcd for $C_{19}H_{20}O_2N_5F$ (369.40): C: 61.85, H: 5.56, N: 18.98; found C: 61.58, H: 5.24, N: 19.13.

4.1.2.17. 1-[(4-Cyclohexylpiperazin-1-yl)-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (21). White solid. Yield: 76%; mp 179–181 °C; TLC: R_f = 0.85 (S_1), R_f = 0.81 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06–1.29 (m, 5H, cyclohexane), 1.60–1.76 (m, 2H, cyclohexane), 1.80–1.86 (m, 3H, cyclohexane), 2.17–2.24 (m, 1H, cyclohexane), 2.57 (br s, 4H piperazine), 2.68 (br s, 4H, piperazine), 2.77 (dd, 1H, H_b imide, J = 4.87 Hz, J = 18.34 Hz), 3.20 (dd, 1H, H_a, imide, J = 1.02 Hz, J = 18.46 Hz), 4.12 (q, 1H, H_c imide, J = 5.21 Hz), 4.56 (s, 2H, *CH_2*), 7.06–7.35 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.53, 41.89, 50.22, 50.57, 60.32, 115.36, 115.65, 116.03, 116.31, 117.90, 118.0, 124.75, 129.98, 130.09, 130.33, 176.69, 178.20; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.17 (s, 1F). ESI-MS: 374.36 ($C_{21}H_{28}O_2N_3F$ [M+H]$^+$). Anal. calcd for $C_{21}H_{28}O_2N_3F$ (373.48): C: 67.44, H: 7.55, N: 11.24; found C: 67.58, H: 7.34, N: 11.13.

4.1.2.18. 1-[{4-(2-Hydroxyethyl)-piperazin-1-yl}-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione dihydrochloride (22). White solid. Yield: 63%; mp 144–145 °C; TLC: R_f = 0.75 (S_1), R_f = 0.85 (S_2); ^{1}H NMR (300 MHz, DMSO) δ: 2.07 (br s, 1H, OH), 2.65–2.78 (m, 3H, 1H, H_b imide, 2H, *CH_2*), 2.90–3.09 (m, 4H, piperazine), 3.13–3.45 (m, 5H, 4H piperazine, 1H, H_a imide), 3.73–3.79 (m, 2H, *CH_2*), 4.29 (q, 1H, H_c, imide, J = 5.90 Hz), 4.34 (s, 2H, *CH_2*), 7.19–7.24 (m, 2H, ArH), 7.34–7.47 (m, 2H, ArH), 9.89 (br s, 1H, +NH), 10.29 (br s, 1H, +NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.17, 38.22, 41.50, 50.49, 52.81, 60.22, 62.41, 115.88, 116.12, 124.55, 124.53, 124.64, 124.70, 126.91, 128.11, 128.97, 129.81, 129.82, 130.05, 130.15, 137.86, 159.01, 162.25, 176.44, 178.02; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.19 (s, 1F). ESI-MS: 336.20 ($C_{17}H_{22}FN_3O_3$ [M+H]$^+$). Anal. calcd for $C_{17}H_{24}O_3N_3FCl_2$ (408.31): C: 50.05, H: 5.93, N: 10.30; found C: 49.18, H: 6.04, N: 10.23.

4.1.2.19. 1-[(Morpholin-1-yl)-methyl]-3-(2-fluorophenyl)-pyrrolidine-2,5-dione (23). White solid. Yield: 75%; mp 90–92 °C; TLC: R_f = 0.82 (S_1), R_f = 0.91 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.64 (t, 4H morpholine, J = 4.74 Hz), 2.81 (dd, 1H, H_b imide, J = 5.64 Hz, J = 18.20 Hz), 3.25 (dd, 1H, H_a imide, J = 9.87 Hz, J = 18.46 Hz), 3.67 (t, 4H morpholine, J = 4.61 Hz), 4.10 (q, 1H, H_c, imide, J = 5.64 Hz), 4.53 (s, 2H, *CH_2*), 7.07–7.18 (m, 2H, ArH), 7.21–7.27 (m, 1H, ArH), 7.29–7.37 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.55, 46.81, 50.97, 51.21, 60.45, 120.41, 123.75, 123.95, 127.57, 130.63, 133.62, 136.74, 149.12, 176.63, 178.09; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −116.18 (s, 1F). ESI-MS: 293.18 ($C_{15}H_{17}O_3N_2F$ [M+H]$^+$). Anal. calcd for $C_{15}H_{17}O_3N_2F$ (292.31): C: 61.76, H: 5.87, N: 9.59; found C: 61.58, H: 6.00, N: 9.43.

4.1.2.20. 1-[(4-Benzylpiperazin-1-yl)-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione monohydrochloride (24). White solid. Yield: 85%; mp 168–170 °C TLC: R_f = 0.76 (S_1), R_f = 0.87 (S_2); ^{1}H NMR (300 MHz, DMSO) δ: 2.76 (dd, 1H, H_b imide, J = 0.77 Hz, J = 6.15 Hz), 2.95 (t, 4H, piperazine, J = 5.15 Hz), 3.18–3.24 (m, 7H, 2H, *CH_2*, 4H, piperazine, 1H_a imide), 4.26 (q, 1H, H_c, imide, J = 5.85 Hz), 4.47 (s, 2H, *CH_2*),7.23–7.28 (m, 1H, ArH), 7.39–7.45 (m, 5H, ArH), 7.57–7.65 (m, 3H, ArH), 10.81 (br s, 1H, +NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.18, 41.56, 50.55, 52.90, 60.30, 62.57, 115.94, 116.22, 124.61, 124.66, 124.70, 124.79, 127.03, 128.19, 129.11, 129.86, 129.90, 130.18, 130.26, 137.92, 159.08, 162.36, 176.65, 178.10. ESI-MS: 442.20 ($C_{22}H_{24}BrN_3O_2$ [M+H]$^+$). Anal. calcd for $C_{22}H_{25}O_2N_3BrCl$ (478.82): C: 55.17, H: 5.26, N: 8.77; found C: 55.47, H: 5.15, N: 8.52.

4.1.2.21. 1-[{4-(Pyrimid-2-yl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (25). White solid. Yield: 64%; mp 131–133 °C; TLC: R_f = 0.69 (S_1), R_f = 0.79 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.73 (t, 4H, piperazine, J = 5.13 Hz), 2.81 (d, 1H, H_b imide, J = 5.90 Hz), 33.27 (dd, 1H, H_a, imide, J = 5.13 Hz, J = 18.46 Hz), 3.82 (t, 4H, piperazine, J = 5.13 Hz), 4.40 (q, 1H, H_c imide, J = 6.03 Hz), 4.62 (s, 2H, *CH_2*), 6.49 (t, 1H, pyrimidine, J = 4.10 Hz), 7.13–7.21 (m, 2H, ArH), 7.27–7.32 (m, 1H, ArH), 7.59 (d, 1H, ArH, J = 7.95 Hz), 8.30 (dd, 2H, pyrimidine, J = 0.51 Hz, J = 4.62 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.41, 36.44, 41.78, 41.80, 43.63, 50.43, 60.47, 109.90, 115.94, 116.21, 124.55, 124.66, 124.75, 124.76, 129.90, 130.0, 130.28, 130.36, 157.65, 159.02, 161.68, 162.30, 176.55, 178.01. ESI-MS: 430.11 ($C_{19}H_{20}O_2N_5Br$ [M+H]$^+$). Anal. calcd for $C_{19}H_{20}O_2N_5Br$ (430.31): C: 53.07, H: 4.69, N: 16.29; found C: 52.85, H: 4.56, N: 16.40.

4.1.2.22. 1-[(4-Cyclohexylpiperazin-1-yl)-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (26). White solid. Yield: 82%; mp 152–153 °C TLC: R_f = 0.88 (S_1), R_f = 0.85 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06–1.25 (m, 5H, cyclohexane), 1.55–1.64 (m, 2H, cyclohexane), 1.77–1.87 (m, 3H, cyclohexane), 2.17–2.23 (m, 1H, cyclohexane), 2.58 (br s, 4H, piperazine), 2.69 (br s, 4H, piperazine), 2.73 (d, 1H, H_b imide, J = 5.90 Hz), 3.27 (dd, 1H, H_a imide, J = 5.88 Hz, J = 18.46 Hz), 4.42 (q, 1H, H_c imide, J = 6.15 Hz), 4.58 (s, 2H, *CH_2*), 7.16–7.25 (m, 2H, ArH), 7.29–7.34 (m, 1H, ArH), 7.60 (d, 1H, ArH, J = 7.95 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.50, 41.86, 50.19, 50.54, 60.30, 115.33, 115.64, 116.01, 116.29, 117.87, 118.07, 124.71, 129.92, 130.05, 130.31, 176.66, 178.15. ESI-MS: 434.24 ($C_{21}H_{28}O_2N_3Br$ [M+H]$^+$). Anal. calcd for $C_{21}H_{28}O_2N_3Br$ (434.39): C: 58.12, H: 6.50, N: 9.68; found C: 58.22, H: 6.64, N: 9.59.

4.1.2.23. 1-[{4-(2-Hydroxyethyl)-piperazin-1-yl}-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione dihydrochloride (27). White solid. Yield: 55%; mp 161–163 °C; TLC: R_f = 0.76 (S_1), R_f = 0.90 (S_2); ^{1}H NMR (300 MHz, DMSO) δ: 2.01 (br s, 1H, OH), 2.69–2.2.88 (m, 3H, 1H, H_b imide, 2H, *CH_2*), 2.91–3.02 (m, 4H, piperazine), 3.12–3.25 (m, 5H, 4H piperazine, 1H, H_a imide), 3.40–3.79 (m, 2H, *CH_2*), 4.37 (s, 2H, *CH_2*), 4.48 (q, 1H, H_c imide, J = 6.28 Hz) 7.24–7.47 (m, 3H, ArH), 7.65 (d, 1H, ArH, J = 7.26 Hz), 9.68 (br s, 1H, +NH), 10.20 (br s, 1H, +NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.20, 38.28, 41.53, 50.51, 52.78, 60.22, 62.39, 115.80, 116.14, 124.55, 124.60, 124.68, 124.72, 126.93, 128.11, 128.92, 129.80, 129.88, 130.15, 130.18, 137.87, 159.08, 162.31, 176.48, 178.12. ESI-MS: 396.28 ($C_{17}H_{22}BrN_3O_3$ [M+H]$^+$). Anal. calcd for $C_{17}H_{24}O_3N_3BrCl_2$ (469.22): C: 43.54, H: 5.16, N: 8.95; found C: 43.19, H: 5.35, N: 8.63.

4.1.2.24. 1-[(Morpholin-1yl)-methyl]-3-(2-bromophenyl)-pyrrolidine-2,5-dione (28). White solid. Yield: 63%; mp 107–110 °C; TLC: R_f = 0.80 (S_1), R_f = 0.81 (S_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38 (br s, 2H, morpholine), 1.54 (br s, 2H, morpholine), 2.61 (t, 4H morpholine, J = 5.38 Hz), 2.78 (dd, 1H, H_b imide, J = 5.90 Hz, J = 18.46 Hz), 3.28 (dd, 1H, H_a imide J = 5.92 Hz, J = 18.46 Hz), 4.41 (q, 1H, H_c imide, J = 5.89 Hz), 4.54 (s, 2H, *CH_2*), 7.17–7.22 (m, 2H, ArH), 7.30–7.36 (m, 1H, ArH), 7.61 (d, 1H, ArH, J = 7.95 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.53, 46.80, 50.96, 51.20, 60.44, 120.39, 123.71, 123.92, 127.53, 130.63, 133.61, 136.72, 149.10, 176.64, 178.10. ESI-MS: 353.14 ($C_{15}H_{17}O_3N_2Br$ [M+H]$^+$). Anal. calcd for $C_{15}H_{17}O_3N_2Br$ (353.22): C: 51.01, H: 4.85, N: 7.93; found C: 50.90, H: 4.74, N: 7.63.

4.2. Pharmacology

The initial anticonvulsant evaluation was performed within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere.[22,23]

Male albino mice (CF-1 strain) and male albino rats (Sprague–Dawley) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The compounds were suspended in 0.5% methylcellulose/water mixture.

The ASP initially evaluates anticonvulsant activity for newly submitted compounds following intraperitoneal (ip) administration in mice and oral administration in rats. Groups of eight mice or four rats are employed. Phase I studies in mice involved two convulsant tests: maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole seizure test (*sc*PTZ) and rotarod test for neurological toxicity (NT).

4.2.1. Maximal electroshock test (MES)

In the MES test, an electrical stimulus of 0.2 s in duration (50 mA in mice and 150 mA in rat at 60 Hz) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

4.2.2. Subcutaneous pentylenetetrazole seizure test (*sc*PTZ)

This test utilizes a dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) that 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 convulsant is administered subcutaneously.

All the compounds were injected intraperitoneally into mice at the dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h after administration. The results are presented in Tables 1 and 2.

Selected derivatives were administrated orally into rats using four animals at a fixed dose of 30 mg/kg (MES test) or 50 mg/kg (*sc*PTZ test), that discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity. For both doses the motor impairment was studied in parallel. Rats were tested at five time periods ranging from one quarter to 4 h post substance administration. The results are shown in Table 3.

4.2.3. Neurological toxicity (NT)

Neurological toxicity induced by a compound was detected in mice using standardized rotarod test.[24] Untreated control mice or rats, when placed on the rod, can maintained their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of the animal to maintain equilibrium for a given time (1 min).

4.2.4. Quantification studies

The quantitative determination of ED_{50} and TD_{50} values was performed at previously estimated time of peak effect after oral administration into rats or ip administration into mice. Groups of eight animals received various doses of the compound until at least three points were established in the range of 10–90% seizure protection or minimal motor impairment. From the plot of the data obtained, 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. The results are shown in Tables 4 and 5.

4.2.5. The 6-Hz model

This test was carried out according to the protocol originally described by Brown et al.[25] and more recently by Barton et al.[21] and Kaminski et al.[26] 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.[26] (Table 6)

Acknowledgments

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, Rockville, MD, USA), and Professor Katarzyna Kieć-Kononowicz (Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Kraków, Poland) the coordinator of the ADD Project from Polish site.

References and notes

1. McNamara, J. O. Pharmacotherapy of the Epilepsies. In *Goodman s The Phamacological Basis of Therapeutics*; Brunton, L. L., Chabner, B. A., Knollmann, B. C., Eds., 12th ed.; McGraw-Hill: New York, 2011; pp 583–608.
2. Picot, M. C.; Baldy-Moulinier, M.; Daurs, J. P.; Dujols, P.; Crespel, A. *Epilepsia* **2008**, *49*, 1230.
3. Cramer, J. A.; Mintzer, S.; Wheless, J.; Mattson, R. H. *Expert. Rev. Neurother.* **2010**, *10*, 885.
4. Rogawski, M. A. *Epilepsy Res.* **2006**, *69*, 273.
5. Khan, H. N.; Kulsoom, S.; Rashid, H. *Epilepsy Res.* **2012**, *98*, 62.
6. Wong, M. G.; Defina, J. A.; Andrews, P. R. *J. Med. Chem.* **1986**, *29*, 562.
7. Tasso, S. M.; Bruno-Blanch, L. E.; Moon, S. C.; Estiú, G. L. *J. Mol. Struct.* **2000**, *504*, 229.
8. Kamiński, K.; Obniska, J.; Wiklik, B.; Atamanyuk, D. *Eur. J. Med. Chem.* **2011**, *46*, 4634.
9. Kamiński, K.; Rzepka, S.; Obniska, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5800.
10. Kamiński, K.; Obniska, J. *Bioorg. Med. Chem.* **2008**, *16*, 4921–4931.
11. Kamiński, K.; Obniska, J.; Dybała, M. *Eur. J. Med. Chem.* **2008**, *43*, 53–61.
12. Obniska, J.; Kamiński, K.; Dzierżawska-Majewska, A.; Zagórska, A.; Karolak-Wojciechowska, J. *J. Fluorine Chem.* **2006**, *127*, 417.
13. Obniska, J.; Byrtus, H.; Kamiński, K.; Pawłowski, M.; Szczesio, M.; Karolak-Wojciechowska, J. *Bioorg. Med. Chem.* **2010**, *18*, 6134.
14. Obniska, J.; Kamiński, K.; Skrzyńska, D.; Pichór, J. *Eur. J. Med. Chem.* **2009**, *44*, 2224.
15. Obniska, J.; Kopytko, M.; Zagórska, A.; Chlebek, I.; Kamiński, K. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 333.
16. Obniska, J.; Zagórska, A. *Il Farmaco* **2003**, *58*, 1227.
17. Miller, C. A.; Long, L. M. *J. Am. Chem. Soc.* **1951**, *73*, 4895.
18. Löscher, W. *Seizure* **2011**, *20*, 359.
19. Rogawski, M. A. *Epilepsy Res.* **2006**, *68*, 22.
20. Stables, J. P.; Kupferberg, H. J. The NIH Anticonvulsant Drug Development (Add) Program: Preclinical Anticonvulsant Screening Project. In *Molecular and Cellular Targets for Antiepileptic Drugs*; Avanzini, G., Regesta, G., Tanganelli, O., Avoli, M., Eds.; John Libby & Company Ltd: London, 1997; pp 191–198.
21. Barton, M. E.; Klein, B. D.; Wolf, H. H.; White, H. S. *Epilepsy Res.* **2001**, *47*, 217.
22. Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409.
23. Kupferberg, H. J. *Epilepsia* **1989**, *30*, 51.
24. Dunham, N. W.; Miya, T. A. *J. Am. Pharm. Assoc. Sci.* **1957**, *46*, 208.
25. Brown, W. C.; Schiffman, D. O.; Swinyard, E. A.; Goodman, L. S. *J. Pharmacol. Exp. Ther.* **1953**, *107*, 273.
26. Kaminski, R. F.; Livingood, M. R.; Rogawski, M. A. *Epilepsia* **2004**, *45*, 864.
27. Thirumurugan, R.; Sriram, D.; Saxena, A.; Stables, J.; Yogeeswari, P. *Bioorg. Med. Chem.* **2006**, *14*, 3106.
28. Yogeeswari, P.; Sriram, D.; Thirumurugan, R.; Raghavendran, J. V.; Sudhan, K.; Pavana, P. K.; Stables, J. P. *J. Med. Chem.* **2005**, *48*, 6202.
29. White, H. S.; Woodhead, J. H.; Wilcox, K. S.; Stables, J. P.; Kupferberg, H. J.; Wolf, H. H. In *Antiepileptic Drugs*; Levy, R. H., Mattson, R. H., Meldrum, B. S., Perucca, E., Eds.; Lippincott: Philadelphia, PA, 2002; pp 43–44.