Full Paper

Synthesis and Anticonvulsant Properties of New Mannich Bases Derived from 3-Aryl-pyrrolidine-2,5-diones. Part 1

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A series of new Mannich bases of *N*-[(4-arylpiperazin-1-yl)-methyl]-3-(chlorophenyl)-pyrrolidine-2,5-diones **10 – 23** have been synthesized and evaluated for their anticonvulsant activity in maximum electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure threshold tests. Their neurotoxicity was determined using a rotorod screen. Several molecules showed a promising anticonvulsant profile especially in the MES-test. In this model of seizures, the most active were *N*-[{4-(4-chlorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione **16** and *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione **17** with ED_{50} values of 21.4 mg/kg and 28.83 mg/kg, respectively. Selected derivatives **10**, **14**, and **16** were tested in the psychomotor seizure 6-Hz test from which *N*-[{4-(2-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione **10** revealed the highest protection with an ED_{50} of 78 mg/kg. Compounds **10**, **12**, and **17** were also tested in the pilocarpine-induced status PIPS test. Furthermore, **17** was examined in the hippocampal kindling screen after *i.p.* administration to rats.

Keywords: Anticonvulsant activity / *N*-(4-Arylpiperazin-1-yl)-methyl-pyrrolidine-2,5-dione / Mannich bases / 3-Phenyl-pyrrolidine-2,5-dione

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Introduction

Epilepsy is not a disease but a syndrome of different cerebral disorders of the central nervous system (CNS), which is characterized by recurrent spontaneous seizures arising from excessive electrical activity in some portion of the brain [1]. Despite of the introduction of many new antiepileptic drugs (AEDs) and remarkable strides in this research field, more than 30% of the patients with epilepsy remain refractory to available treatments [2]. Moreover, a large number of old and new AEDs cause serious side effects, which include ataxia, nausea, mental dulling, and hepatotoxicity [3, 4]. Therefore, with all of above-mentioned disadvantages in mind, the continued search for safer and more effective AEDs is urgently needed.

Continued efforts are being made in the development of antiepileptic drugs employing a range of strategies, including modification of the structure of existing drugs, targeting novel molecular substrates, as well as nonmechanism-based drug screening of compounds in animal models [5]. Among the experimental models developed to evaluate the anticonvulsant activity, conventional acute seizure models are the most widely used, among which the maximal electroshock (MES) and the subcutaneous pentylenetetrazole (*sc*PTZ) tests are the most popular. Additionally, the use of alternative antiepileptic models *e.g.*, 6-Hz, genetic or kindled models enabled the discovery of new, very efficient and safe drugs like levetiracetam or brivaracetam, which were inactive in the traditional animals seizure models (MES and *sc*PTZ) [6, 7].

One of the important core fragments of anticonvulsants is defined by a nitrogen heteroatomic system usually a cyclic imide, consisting of at least one or two carbonyl groups and phenyl or alkyl substituents attached

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Abbreviations: antiepileptic drugs (AEDs); anticonvulsant screening project (ASP); pilocarpine-induced status prevention (PISP); time-of-peak-effect (TPE)

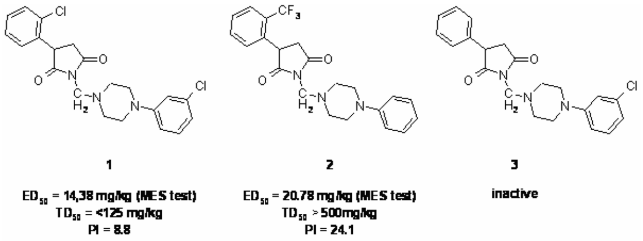


Figure 1. The structures of compounds **1**, **2**, and **3**.

to the heterocyclic system [8–10]. This common template is present in the structures of many older AEDs *e.g.*, phenobarbital, phenytoin, etosuximide, as well as in the newest generation of anticonvulsants *e.g.*, levetiracetam, brivaracetam, or seletracetam.

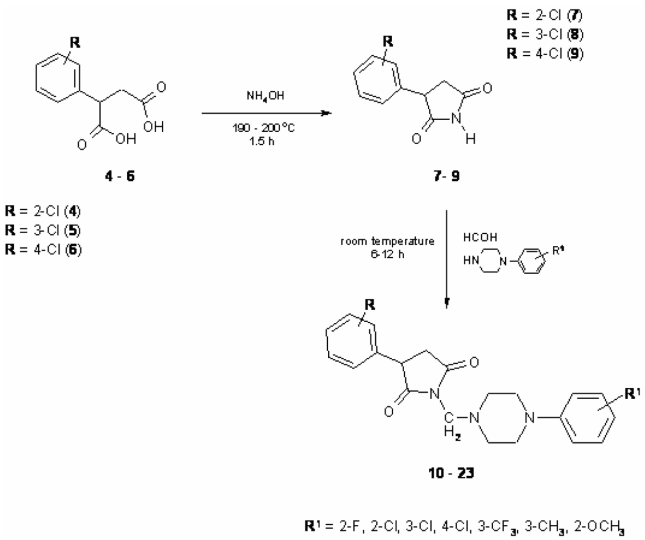
The previous studies from our laboratory have demonstrated the potent anticonvulsant activity in a group of *N*-[4-(phenyl-piperazin-1-yl)-methyl]-3-aryl-pyrrolidine-2,5-diones. These molecules were effective especially in electrically induced seizures (MES). The most active among these compounds were *N*-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione **1** with an ED_{50} = 14.18 mg/kg, protective index PI (TD_{50}/ED_{50}) of 8.8 and *N*-[4-(phenyl-piperazin-1-yl)-methyl]-3-(2-trifluoromethyl-phenyl)-pyrrolidine-2,5-dione **2** that showed an ED_{50} value of 20.78 mg/kg and PI of 24.1 (Fig. 1; [11, 12]). In continuation of our systematic SAR studies of these series of derivatives, recently, we have obtained several analogues of the active compounds **1** and **2**, which were devoid of electron-withdrawing substituents at the 3-phenyl ring. Surprisingly enough, all of these molecules were inactive (compound **3**, Fig. 1; [13]). Taking into consideration the above, it is justified to claim that the presence of electron-withdrawing substituents at the 3-phenyl ring of pyrrolidine-2,5-dione seems to be crucial for the anticonvulsant activity of such type compounds.

Following these results and as part of our efforts to design new anticonvulsant agents, in the present study, a small library of *N*-Mannich bases with a 3-phenyl-pyrrolidine-2,5-dione system as a core fragment and a chloro atom at positions 2, 3, or 4 of the phenyl ring has been synthesized. At the imide nitrogen atom through the methylene bridge, we have introduced 4-arylpiperazines with different, electron-withdrawing (F, Cl, CF_3) or electron-donating (CH_3, OCH_3) groups.

Results and discussion

Chemistry

The synthesis of compounds **10–23** was accomplished as shown in Scheme 1. The starting 3-(2-chlorophenyl)- **4**, 3-(3-chlorophenyl)- **5**, 3-(4-chlorophenyl)- **6** succinic acids



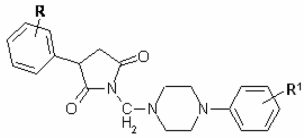
Scheme 1. General method for the synthesis of compounds **10–23**.

were prepared as previously reported [14]. The synthetic procedures of the corresponding imides (**7–9**) have been described in a separate report [15]. The target compounds **10–23** were prepared by a Mannich-type reaction from the appropriately substituted imides **7–9**, formaldehyde, and the corresponding 4-arylpiperazines. The reaction was carried out in ethanol at room temperature for approx. 6–12 h. The crude products were crystallized from 96% ethanol. Because of the oily form of compounds **13** and **17**, they were isolated as hydrochloride salts and were recrystallized from anhydrous ethanol. The final compounds were obtained in yields ranging from 65% to 75%. Their purity was assessed by TLC chromatography. The structures of the compounds synthesized were confirmed by spectral and elemental analyses. The detailed physical and analytical data are listed in the experimental section.

Anticonvulsant activity

The initial anticonvulsant evaluation was performed 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. 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. At the present time, there are three *in-vivo* screens used routinely that include the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole (*sc*PTZ), and the kindling model. From these tests, the MES and *sc*PTZ screens are recognized as the "gold standards" in the early stages of testing [16, 17].

Table 1. Anticonvulsant screening project (ASP) phase I in mice (compounds tested **10–23**).



Compound	R	R^1	Intraperitoneal injection into mice			
			MES[a)]		TOX[b)]	
			0.5 h	4 h	0.5 h	4 h
10	2-Cl	2-Cl	–	–	–	–
11	2-Cl	4-Cl	–	300	–	–
12	2-Cl	3-CF_3	–	100	–	–
13	3-Cl	2-F	–	100	300[1]	–
14	3-Cl	2-Cl	–	–	–	–
15	3-Cl	3-Cl	–	–	–	–
16	3-Cl	4-Cl	300	100	–	–
17	3-Cl	3-CF_3	100	100	300	300
18	3-Cl	3-CH_3	–	30	100	–
19	3-Cl	2-OCH_3	–	–	300	300
20	4-Cl	2-Cl	–	–	300	300
21	4-Cl	3-Cl	–	100	300	300
22	4-Cl	4-Cl	–	–	–	–
23	4-Cl	3-CF_3	–	100	300	–
Phenytoin[c)]	–	–	30	30	100	100

The figures in the table indicate the minimum dose whereby bioactivity or neurotoxicity was demonstrated in half or more of the animals. A dash indicates the absence of activity or neurotoxicity at the maximum dose administrated (300 mg/kg).
a) Maximal electroshock test; b) rotorod toxicity; c) reference drug, data for phenytoin, Ref. [27]; Response comments: [1]death.

The anticonvulsant activity profile of all the compounds was established with maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, after intraperitoneal injection of the test compounds into mice at doses of 30, 100, and 300 mg/kg. An observation was carried out at two different time intervals, namely 0.5 and 4 h. The acute neurological toxicity (NT) was determined in the minimal motor impairment-rotorod screen. The results are shown in Table 1.

The compounds studied revealed diversified anticonvulsant properties, nevertheless, they were effective mainly in the MES test. The initial evaluation in mice (phase I) showed that excluding molecules **10**, **14**, **15**, **19**, **20**, and **22** which were devoid of activity, all other derivatives were effective in the *i. p.* MES screen.

The MES test is claimed to detect compounds affording protection against generalized tonic-clonic seizures. Compounds that revealed protection in the MES test indicating the ability of a substance to prevent seizure spread at a dose of 100 mg/kg include **12**, **13**, **16**, **17**, **21**, and **23**, whereas **11** provided anti-MES protection at a dose of 300 mg/kg. In this series, **16** and **17** were active in both time intervals – 0.5 and 4 h – however, the majority **11–13**, **18**, **21**, and **23** showed activity at 4 h. This indicates the slow onset and long duration of the anticonvulsant action. It is worth to note that only **18** revealed the anti-MES activity at a dose of 30 mg/kg, on the other hand, however, it was neurotoxic at a dose of 100 mg/kg. In the neurotoxicity screen (NT) compounds **10–12**, **14–16**, and **22** did not show neurotoxicity in the maximum dose administered (300 mg/kg). Among these, **10**, **14**, **15**, and **22** were inactive. Compounds **13**, **17**, **19–21**, and **23** exhibited motor impairment at the dose of 300 mg/kg at 0.5 and 4 h. The results of the neurotoxicity screening showed that **12** and **16** emerged as anticonvulsants without neurotoxic properties.

A valuable property of a candidate anticonvulsant is its ability to inhibit convulsions when given orally. This screen discloses the time of onset, the approximate time-of-peak-effect (TPE) and the duration of anticonvulsant activity or neurotoxicity. On the basis of the *i. p.*-screening data in mice, and according to the Anticonvulsant Screening Project (ASP) disposition, six compounds **13**, **16–18**, **21**, and **23** were selected and examined in the MES test as well as neurotoxicity screen after *p. o.* administration into rats at a dose of 30 mg/kg. The results obtained are presented in Table 2. The most active were compounds **16**, **17**, and **18**; they protected 100% of the animals at 0.5 h (**17**) or 4 h (**16** and **18**). The following molecules provided 75% protection at 2 h: **16**, 50% protection at 1 h and 2 h: **17** and **18** or at 4 h: **17**. 25% protection was given at 0.25 h by: **17**, at 0.5 h **18**, and 1 h **16**. The other compounds were less active, namely **21** showed a peak of 75% protection at 1 and 4 h; compound **23** was effective in 25, 75, and 50% of the animals at 1 h, 2 h, and 4 h, respectively. Finally, compound **13** protected 25% of the tested animals at the time of 0.5 h and 1 h as well as 50% of rats at 2 and 4 h. Except for **18**, all other derivatives were non neurotoxic when given orally.

On the basis of oral data from rats, compound **16** and **17** were chosen for phase-II evaluation for the quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative evaluations of the MES median effective dose (ED_{50}) and toxic dose (TD_{50}) were performed after oral administration to rats at the previously estimated time-of-peak-effect (TPE) – 4 h for **16** and 0.5 h for **17**. Results of the quantitative tests along with the data for the standard drug phenytoin are shown in Table 3. Also shown there, compound **16** revealed an ED_{50} of 21.40 mg/kg and TD_{50} > 500 mg/kg, resulting in a protection index (TD_{50}/ED_{50}) of 23.37, whereas **17** was less active and more

Table 2. Anticonvulsant activity of selected compounds administrated *p. o.* to rats at a dose of 30 mg/kg.

Compound	MES[a]					NT[b]				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
13	1	1	0	2	2	0	0	0	0	0
16	0	0	1	3	4	0	0	0	0	0
17	1	4	2	2	2	0	0	0	0	0
18	0	1	2	2	4	0	3	0	0	0
21	0	0	3	0	3	0	0	0	0	0
23	0	0	1	3	2	0	0	0	0	0
Phenytoin[c]	1	4	3	3	3	ND	ND	ND	ND	ND

a) Maximal electroshock test, number of animals protected/number of animals tested; b) rotorod test for neurological toxicity, number of animals exhibiting toxicity/number of animals tested; c) reference drug, data for phenytoin, Ref. [28]; ND: no data.

Table 3. Quantification studies of compounds **16** and **17** in the MES and neurotoxicity test (NT) in rats after *p. o.* administration.

Compound	TPE (h)[a]	ED_{50}[b] (MES) (mg/kg)	TD_{50}[c] (mg/kg)	PI[d] (MES) (TD_{50}/ED_{50})
16	4	21.4 (12.93–40.05)[e]	>500	23.37
17	0.5	28.83 (16.28–46.75)	152.8 (121.45–193.98)	5.30
Phenytoin[f]	2	23.20 (21.4–25.4)	>500 (36.9–46.1)	>21.60

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) reference drug, data for phenytoin, Ref. [29].

Table 4. Quantification studies of compound **16** in the MES, *sc*PTZ, and neurotoxicity test (NT) in mice after *i. p.* administration.

Compound	TPE (h)[a]	ED_{50}[b] (MES) (mg/kg)	ED_{50}[b] (*sc*PTZ) (mg/kg)	TD_{50}[c] (mg/kg)	PI[d] (MES) (TD_{50}/ED_{50})
16	6	71.84 (53.14–96.89)[e]	376.32 (260.62–539.15)	519.16 (477.40–593.75)	7.23 (MES) 1.38 (*sc*PTZ)
Phenytoin[f]	1	5.32 (5.44–7.23)	>500	41.2 (36.9–46.1)	6.52

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) reference drug, data for phenytoin, Ref. [29].

toxic and revealed an ED_{50} value of 28.83 mg/kg, a TD_{50} value of 152.8 mg/kg, and a protective index (PI) of 5.30. The quantitative data showed that compound **16** was more potent and safer (higher PI index) than phenytoin which was used as reference drug for anticonvulsants active in the MES test.

Continuing the pharmacological studies of compound **16**, the quantitative evaluation of the MES and *sc*PTZ, ED_{50} and TD_{50} values were determined after intraperitoneal administration into mice (TPE = 6 h) (Table 4). Compound **16** showed ED_{50} values of 71.84 mg/kg (MES, PI = 7.23), 376.32 mg/kg (*sc*PTZ, PI = 1.38), and TD_{50} of 519.16 mg/kg. It is worth to note that despite of the lower activity of **16** in the MES screen comparing to phenytoin, this molecule was also active in the pentylenetetrazole seizures, which may suggest its potential effectiveness in different types of human epilepsies. Compound **17** underwent further studies in the hippocampal-kindled rats test after *i. p.* administration. Kindling is a phenomenon whereby repeated, low level, initially subconvulsive, electrical brain stimulation results in a progressive intensification of seizure activity, eventually leading to fully generalized electrographic and motor seizures [18]. The hippocampal-kindled rat test is an excellent animal

Table 5. Anticonvulsant evaluation – psychomotor seizure tests (6-Hz) after *i.p.* injection into mice.

Compound	Intraperitoneal injection into mice[a)]				
	0.25 h	0.5 h	1 h	2 h	4 h
10	3	3	4	2	0
14	1	3	1	0	0
16	0	0	0	2	2

a) Dose of 100 mg/kg was administrated. The data indicate the number of mice out of four that were protected.

Table 6. Quantification data – psychomotor seizure tests (6-Hz) after *i.p.* injection into mice.

Compound	TPE (h)[a)]	ED_{50}[b)] (6-Hz) (mg/kg)	TD_{50}[c)] (mg/kg)	PI[d)] (TD_{50}/ED_{50})
10	1	78.00 (54.20–109.30)[e)]	>600 (477.40–593.75)	>7
Levetiracetam[f)]	1	19.40 (9.90–36.00)	>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) 95%-confidence limits given in parentheses; f) data for levetiracetam from ref. [17].

model for the study of seizure activity and is an acceptable model for the study of human partial epilepsy, which is still one of the most important therapeutic challenges. Compound **17** has demonstrated high activity – ED_{50} of 28.5 mg/kg (95% confidence interval: 14.9–44.1 mg/kg), whereas motor impairment was observed at a dose of 300 mg/kg.

According to the ASP dispositions, compounds **10** and **14** inactive both in MES and *sc*PTZ test as well as **16** (active in the MES screen), were chosen for the evaluation of anticonvulsant properties in the 6-Hz test. The selection was made randomly as part of the search for molecules providing anti 6-Hz protection among chemically diversified compounds pursued in the NIH/NINDS. The 6-Hz screen has been validated as a model of therapy-resistant epilepsy recently. It was not used widely because of its lack of 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 test, does exhibit protective activity in the 6-Hz model. This suggested that the 6-Hz model might be capable for identifying antiseizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action [19]. The results obtained for compounds **10**, **14**, and **16** are shown in Table 5. The results obtained revealed that **10** was the most active compound, which, administrated intraperitoneally into mice at a dose of 100 mg/kg, protected 75% of animals at 0.25 h and 0.5 h, 100% at 1 h, and 50% at 2 h. Its 3-chloro analogue **14** protected 25% of mice at 0.25 h and 1 h as well as 75% at 0.5 h. It is worth noticing that **10** and **14** were inactive in mice MES and *sc*PTZ *i. p.* screens. Finally, **16** protected 50% of the animals in two time intervals – 2 h and 4 h. Compound **10**, as the most active, was selected for quantification of *i.p.* ED_{50} and TD_{50} values at TPE = 1 h in mice. This molecule showed an ED_{50} = 78 mg/kg, TD_{50} > 600 mg/kg and a protective index (PI) > 7 (Table 6).

According to the ASP dispositions, compounds **10**, **12**, **16**, and **17** were assessed for potential activity against nerve agents using the pilocarpine-induced status prevention (PISP) screen, which is one of the animal models of *status epilepticus* (SE). The outcome measures are the determination of "protection" or "no-protection". The results are shown in Table 7. In the PISP screen, only **12** was found to be active and protected seven animals (rats) at the time-zero-dose of 200 mg/kg (*i.p.*) . The other compounds **10**, **16**, and **17** were inactive. To determine the acute motor impairment doses of 100 mg/kg, 300 mg/kg, and 600 mg/kg the compounds were administrated *via* the *i.p.* route. Two rats per each dose were used. The animals were observed during the following time periods: 0.25, 0.5, 1, 2, and 4 h. The result of the neurotoxicity screens revealed that compound **12** caused motor impairment at a dose of 100 and 300 mg/kg at the time of 2 h, whereas **17** caused the effect at a dose of 600 mg/kg at a time ranging from 1 to 4 h. Compounds **10** and **16** were non-neurotoxic at all doses applied.

In conclusion, the results of the preliminary screening revealed that anticonvulsant activity depended on the

Table 7. Pilocarpine-induced status prevention (PISP) model. The results for compound **12** after *i.p.* administration into mice.

Compound	Dose (mg/kg)	Time (h)[a)]	Protected rats	Nonprotected rats	Average Weight Change ± SEM (g)[b)]	
					Protected rats	Nonprotected rats
12	200	0	7	8	15.7 ± 2.1	20.0 ± 0.0

a) Post first stage III seizure; b) weight change 24 h post first stage III seizure.

position of the chloro atom at the succinimide 3-phenyl ring and also on the kind and position of the substituents at the 4-phenyl-piperazine moiety. In general, the most active compounds were those with a chloro atom located at the *meta*-position of the 3-phenyl ring. The respective *ortho*- and *para*-analogs were less active. Moreover, taking into consideration the results of our previous studies which showed the lack of activity among unsubstituted 3-phenyl-pyrrolidine-2,5-diones [13] or low activity for the respective 3-(methylphenyl)-pyrrolidine-2,5-diones, it seems that the presence of an electron-withdrawing chloro atom in this fragment is decisive for anticonvulsant activity. Considering the 4-phenyl-piperazine moiety, the most potent compounds were those with a *para*-chloro or *meta*-trifluoromethyl substitution. The introduction of a chloro atom or a methoxy group at the *ortho*-position yielded inactive molecules (one exception was observed for the 2-fluoro derivative **13** that was active in the MES test).

Conclusion

The results obtained revealed that number of *N*-[(4-arylpiperazin-1-yl)-methyl]-3-(chlorophenyl)-pyrrolidine-2,5-diones were effective in the MES screen. The most active were *N*-[{4-(4-chlorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione with ED_{50} of 21.4 mg/kg, TD_{50} > 500 mg/kg and a protection index (TD_{50}/ED_{50}) of 23.37 mg/kg. This molecule was more potent and safer than phenytoin, which was used as reference drug. Several compounds revealed high activity in the 6-Hz seizure test and pilocarpine-induced status prevention (PISP) screen, which may indicate their potential efficacy in the refractory epilepsy as well as *status epilepticus*.

Experimental

Chemistry

All chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (m.p.) 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 thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminum sheets (Merck), using chloroform / acetone (9:1, v/v) as developing system. Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol I_2 in 10% HCl. Elemental analyses for C, H, and N were carried out by a micro method using the elemental Vario EI III Elemental analyser (Heraeus, 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 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), brs (broad singlet), d (doublet), dd (double doublets), t (triplet), q (quartet), m (multiplet).

*General procedure for preparation of compounds **10–23***

To a mixture of 3-(2-chlorophenyl)- (0.01 mol; **7**), 3-(3-chlorophenyl)- (0.01 mol; **8**), or 3-(4-chlorophenyl)-pyrrolidine-2,5-dione (0.01 mol; **9**) in 96% ethanol (20 mL) and 40% solution of formaldehyde (0.01 mol), corresponding 4-arylpiperazines (0.01 mol) dissolved in 96% ethanol, were added. The mixture was left for ca. 6–12 h at room temperature and then refrigerated at approximately −10°C for 24 h. The precipitated crude products were washed with cold ethanol, separated by filtration, and recrystallized from 96% ethanol (Scheme 1). Compounds **13** and **17** 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.

*N-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione **10***

White powdery crystals. Yield: 70%; m.p.: 107–109°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.85 (t, 4H, piperazine, *J* = 5.38 Hz), 2.92 (dd, 1H, imide, *J* = 4.36 Hz, *J* = 18.21 Hz), 3.06 (t, 4H, piperazine, *J* = 4.49 Hz), 3.24 (dd, 1H, imide, *J* = 5.39 Hz, *J* = 10.00 Hz), 4.38 (q, 1H, imide, *J* = 5.90 Hz), 4.64 (t, 2H, -CH_2-, *J* = 13.08 Hz), 6.94–7.05 (m, 2H, ArH), 7.21–7.25 (m, 6H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

*N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione **11***

White powdery crystals. Yield: 75%; m.p.: 106–108°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.82–2.85 (m, 4H, piperazine), 2.95 (dd, 1H, imide, *J* = 5.90 Hz, *J* = 17.65 Hz), 3.16 (brs, 4H, piperazine), 3.26 (dd, 1H, imide, *J* = 9.75 Hz), 4.35 (q, 1H, imide, *J* = 6.16 Hz), 4.61 (t, 2H, -CH_2-, *J* = 13.33 Hz), 6.83 (d, 2H, ArH, *J* = 8.98 Hz), 7.17–7.39 (m, 5H, ArH), 7.40–7.44 (m, 1H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione ***12***

White powdery crystals. Yield: 65%; m.p. 118–120°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.85–2.93 (m, 5H, 4H piperazine; 1H imide,), 3.23–3.32 (m, 5H, 4H piperazine; 1H imide,), 4.36 (q, 1H, imide, *J* = 5.90 Hz), 4.65 (t, 2H, -CH_2-, *J* = 13.33 Hz), 7.05–7.11 (m, 3H, ArH), 7.21–7.43 (m, 5H, ArH). $C_{22}H_{21}N_3O_2Cl_1F_3$ (451.88).

Monohydrochloride N-[{4-(2-fluorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***13***

White powdery crystals. Yield: 72%; m.p.: 157–159°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.92 (dd, 1H, imide, *J* = 6.41 Hz, *J* = 17.95 Hz), 3.06 (brs, 4H, piperazine), 3.21–3.48 (m, 5H, 4H piperazine; 1H imide), 4.37 (q, 1H, imide, *J* = 6.15 Hz), 4.70 (s, 2H, -CH_2-), 6.97–7.19 (m, 3H, ArH), 7.34–7.40 (m, 4H, ArH), 7.56 (s, 1H, ArH), 11.36 (s, 1H, HCl). $C_{21}H_{22}N_3O_2Cl_2F_1$ (438.34).

N-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***14***

White powdery crystals. Yield: 78%; m.p. 95–97°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.85 (brs, 4H, piperazine), 2.91 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.35 Hz), 3.06 (brs, 4H, piperazine), 3.28 (dd, 1H, imide, *J* = 9.75 Hz, *J* = 18.46 Hz), 4.08 (q, 1H, imide, *J* = 5.13 Hz), 4.62 (t, 2H, -CH_2-, *J* = 13.34 Hz), 6.94–7.04 (m, 2H, ArH), 7.14–7.36 (m, 6H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***15***

White powdery crystals Yield: 67%; m.p.: 81–83°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.78 (brs, 4H, piperazine), 2.86 (dd, 1H, imide, *J* = 5.38 Hz, *J* = 18.72 Hz), 3.18 (t, 4H, piperazine, *J* = 4.87 Hz), 3.24 (dd, 1H, imide, *J* = 9.74 Hz, *J* =18.72 Hz), 4.05 (q, 1H, imide, *J* = 5.13 Hz), 4.60 (t, 2H, -CH_2-, *J* = 13.59 Hz), 6.76–6.85 (m, 4H, ArH), 7.10–7.18 (m, 2H, ArH), 7.30–7.35 (m, 2H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***16***

White powdery crystals. Yield: 70%; m.p.: 113–115°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.74–2.78 (m, 4H, piperazine), 2.85 (dd, 1H, imide, *J* = 5.38 Hz, *J* = 18.72 Hz), 3.13 (t, 4H piperazine, *J* = 4.87 Hz), 3.24 (dd, 1H, imide, *J* = 9.49 Hz, *J* = 18.46 Hz), 4.03 (q, 1H, imide, *J* = 5.13 Hz), 4.59 (t, 2H, -CH_2-, *J* = 13.59 Hz), 6.78–6.86 (m, 2H, ArH), 7.10–7.13 (m, 1H, ArH), 7.16–7.21 (m, 2H, ArH), 7.30–7.34 (m, 3H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

Monohydrochloride N-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***17***

White powdery crystals. Yield: 63%; m.p.: 168–170°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.92 (dd, 1H, imide, *J* = 6.41 Hz, *J* = 17.95 Hz), 3.22–3.45 (m, 5H, 1H imide; 4H piperazine), 3.57 (brs, 4H, piperazine), 4.37 (q, 1H, imide, *J* = 6.41 Hz), 4.75 (s, 2H, -CH_2-), 7.09–7.15 (m, 1H, ArH), 7.21–7.32 (m, 2H, ArH), 7.34–7.45 (m, 5H, ArH), 11.35 (brs, 1H, HCl). $C_{22}H_{22}N_3O_2Cl_2F_3$ (488.35).

N-[{4-(3-Methylphenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***18***

White powdery crystals. Yield: 76%; m.p.: 81–83°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.31 (s, 3H, -CH_3), 2.78 (brs, 4H, piperazine), 2.84 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.72 Hz), 3.16 (t, 4H, piperazine, *J* = 4.99 Hz), 3.23 (dd, 1H, imide, *J* = 9.49 Hz, *J* = 18,46 Hz), 4.03 (q, 1H, imide, *J* = 5.13 Hz), 4.60 (t, 2H, -CH_2-, *J* = 13.59 Hz), 6.68–6.73 (m, 3H, ArH), 7.09–7.18 (m, 2H, ArH), 7.29–7.34 (m, 3H, ArH). $C_{22}H_{24}N_3O_2Cl_1$ (397.90).

N-[{4-(2-Methoxyphenyl)-piperazin-1-yl}-methyl]-3-(3-chlorophenyl)-pyrrolidine-2,5-dione ***19***

White powdery crystals. Yield: 67%; m.p.: 99–101°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.85–2.89 (m, 5H, 4H piperazine; 1H imide), 3.09 (brs, 4H, piperazine), 3.25 (dd, 1H, imide, *J* = 9.49 Hz, *J* = 18.46 Hz), 3.85 (s, 3H, -OCH_3), 4.07 (q, 1H, imide, *J* = 5.39 Hz), 4.62 (t, 2H, -CH_2-, *J* = 13.20 Hz), 6.85–7.03 (m, 4H, ArH), 7.16 (d, 1H, ArH, *J* = 6.15 Hz), 7.29–7.36 (m, 3H, ArH). $C_{22}H_{24}N_3O_3Cl_1$ (413.90).

N-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(4-chlorophenyl)-pyrrolidine-2,5-dione ***20***

White powdery crystals. Yield: 72%; m.p.: 114–116°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.82 (brs, 4H, piperazine), 2.89 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.05 (brs, 4H, piperazine), 3.27 (dd, 1H, imide, *J* = 9.74 Hz, *J* =18.46 Hz), 4.07 (q, 1H, imide, *J* = 5.13 Hz), 4.61 (t, 2H, -CH_2-, *J* = 13.34 Hz), 6.94–7.04 (m, 2H, ArH), 7.18–7.24 (m, 3H, ArH), 7.33–7.39 (m, 3H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(4-chlorophenyl)-pyrrolidine-2,5-dione ***21***

White powdery crystals. Yield: 70%; m.p.: 120–122°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.79 (brs, 4H, piperazine), 2.85 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.19 (brs, 4H, piperazine), 3.25 (dd, 1H, imide, *J* = 9.49 Hz, *J* = 18.46 Hz), 4.05 (q, 1H, imide, *J* = 5.13 Hz), 4.60 (s, 2H, -CH_2-), 6.76–6.97 (m, 3H, ArH), 7.14–7.23 (m, 3H, ArH), 7.33–7.37 (m, 2H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-(4-chlorophenyl)-pyrrolidine-2,5-dione ***22***

White powdery crystals. Yield: 75%; m.p.: 115–117°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.78 (brs, 4H, piperazine), 2.84 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.14 (t, 4H, piperazine, *J* = 5.00 Hz), 3.24 (dd, 1H, imide, *J* = 9.74 Hz, *J* = 18.72 Hz), 4.04 (q, 1H, imide, *J* = 5.13 Hz), 4.59 (t, 2H, -CH_2-, *J* = 13.37 Hz), 6.83 (d, 2H, ArH, *J* = 8.72 Hz), 7.16–7.22 (m, 4H, ArH), 7.33–7.37 (m, 2H, ArH). $C_{21}H_{21}N_3O_2Cl_2$ (418.33).

N-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-(4-chlorophenyl)-pyrrolidine-2,5-dione ***23***

White powdery crystals. Yield: 68%; m.p.: 87–89°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.80 (brs, 4H, piperazine), 2.85 (dd, 1H, imide, *J* = 5.13 Hz, *J* = 18.46 Hz), 3.20 (brs, 4H, piperazine), 3.25 (dd, 1H, imide, *J* = 9.74 Hz, *J* =18.46 Hz), 4.05 (q, 1H, imide, *J* = 5.13 Hz), 4.60 (t, 2H, -CH_2-, *J* = 13.33 Hz), 7.03–7.19 (m, 3H, ArH), 7.20–7.24 (m, 2H, ArH), 7.31–7.37 (m, 3H, ArH). $C_{22}H_{21}N_3O_2Cl_1F_3$ (451.88).

Pharmacology

Compounds **10–23** 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), Rockville, MD, USA using procedures described elsewhere [20, 21].

Male albino mice (CF-1 strain) and male albino rats (Sprague-Dawley) were used as experimental animals; they were obtained from Charles River, Portage, MI, USA. 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. 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 Table 1. Selected derivatives (**13**, **16–18**, **21**, and **23**) were administrated orally to the rats using four animals at a fixed dose of 30 mg/kg (MES test; Phase VIa). The motor impairment was studied in parallel. Rats were tested at five time periods ranging from 15 min to 4 h post-substance administration. The results are shown in Table 2.

Maximal electroshock test (MES)

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

Subcutaneous pentylenetetrazole seizure test (scPTZ)

This screen 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 five seconds in 97% (CD_{97}) of the animals tested. At the anticipated time of testing, the convulsant is administered subcutaneously.

Neurological toxicity (NT)

Neurological toxicity induced by the compounds was detected in mice or rats using the standardized rotorod test [22]. Untreated control mice or rats, when placed on the rod, can maintain their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of animal to maintain equilibrium for a given time.

Quantification studies

The quantitative determination of ED_{50} and TD_{50} values for **16** and **17** was performed at the previously estimated time-of-peak-effect after oral administration to rats. Groups of eight rats received various doses of the compound to be tested until at least three points were established in the range of 10–90% seizure protection or minimal neurotoxicity. 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. For compound **16**, the ED_{50} and TD_{50} were also determined after intraperitoneal administration into mice. The results are shown in Tables 3 and 4.

Hippocampal-kindled rats test

The hippocampal kindling paradigm as described by Lothman *et al*. [23] offers a distinct advantage over other kindling models. This procedure requires the surgical placement of bipolar electrodes in the ventral hippocampus of adult male Sprague-Dawley rats (Charles River, USA). Stage-five behavioral seizures (Racine scale [24]) are produced by using a stimulus consisting of a 50 Hz, 10 s train of 1 ms biphasic 200 μA pulses delivered every 30 min for 6 h (12 stimuli per day) on alternating days for a total of 60 stimulations (five stimulus days). Prior to evaluating a candidate's anticonvulsant activity, a drug-free control period consisting of supramaximal stimulations is recorded to verify the stability of a stage-five generalized seizure. A single dose of the candidate compound is then administered intraperitoneally (*i. p.*), 15 min following the last control stimulation. The anticonvulsant activity of the drug is assessed every 30 min for three to four hours starting 15 min after administering the test material. After each stimulation, individual Racine-seizure scores and after-discharge durations are recorded. Rats are used again in drug trials after four to five drug-and stimulus-free days.

The 6-Hz model

This screen was carried out according to the protocol originally described by Brown *et al*. [25] and, more recently, by Barton *et al*. [19] 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].

The pilocarpine-induced status prevention (PISP) model

Male albino rats (Sprague-Dawley, 150–180 g; Charles River, USA) were used as experimental animals. The compounds were administrated via the *i. p.* route of administration. Then, a challenge dose of pilocarpine is given observing for treatment-effects of the substance tested. The seizure severity is determined using the well known Racine scale [24] as follows: (I) immobility, eye closure, twitching of vibrissae, sniffing, and facial clonus; (II) head nodding associated with more severe facial clonus; (III) clonus of one of the fore limbs; (IV) rearing often accomplished by bilateral forelimb clonus, and (V) all of the above plus loss of balance and falling, accomplished by generalized clonic seizures. The anticonvulsant activity of compounds **10**, **12**, **16**, and **17** was assessed at time zero, namely the time from the first-stage III seizures (Test 71). The outcome measures are the determination of "protection" or "no protection" (Table 7).

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