Full Paper

Synthesis and Anticonvulsant Properties of New *N*-Mannich Bases Derived from 3,3-Diphenyl- and 3-Ethyl-3-methyl-pyrrolidine-2,5-diones. Part III

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Twenty-four new *N*-[(4-phenylpiperazin-1-yl)-methyl] derivatives of 3,3-diphenyl- (**7**–**18**) and 3-ethyl-3-methyl-pyrrolidine-2,5-dione (**19**–**30**) were synthesized and evaluated for their anticonvulsant activity in the maximum electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure tests. The acute neurological toxicity was determined using the rotorod screen. Eleven compounds were active and revealed protection only in electrically induced seizures (MES). In the whole series the most effective compound was *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**14**) with an ED_{50} value of 30.3 mg/kg (*p.o.* rats) in the MES test. To explain the possible mechanism of action, for chosen active derivatives **7**, **8**, **9**, **11**, **14**, **23**, and **26**, their influence on $Na_V1.2$ sodium channel currents was evaluated *in vitro*. The crystallographic structures for several molecules (**8**, **10**, and **11**) were solved.

Keywords: 3,3-Disubstituted-pyrrolidine-2,5-diones / Anticonvulsant activity / *In vitro* studies / *In vivo* studies / Mannich bases / Sodium channels

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Introduction

Epilepsy is the most prevalent neurological disorder, affecting approximately 50 million people worldwide [1]. Even though significant advances have been made in epilepsy research, convulsions in about 30% of epileptics are still inadequately controlled by standard drug therapy [2, 3]. 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 [4]. For these reasons, constant attempts are made to investigate new chemical agents and mechanisms through which epilepsy can be effectively controlled. The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of the majority the antiepileptic drugs makes it difficult to use rational methodologies of discovery that are based on the three-dimensional structure of a biological target. Thus the most useful for the design of new anticonvulsants are ligand-based approaches that rely on the use of different pharmacophores established through the analysis of structural characteristics of clinically effective antiepileptic drugs (AEDs) as well as other anticonvulsant active compounds [5]. The two 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 a nitrogen heteroatomic system, usually imide or lactam, with at least one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system [6, 7]. This common template is present in the structures of both first generation AEDs such as ethosuximide and phenytoin as well as among the newest drugs, e.g., levetiracetam, brivaracetam, or seletracetam (Fig. 1).

The currently used AEDs can be classified into four categories on the basis of the main molecular mechanisms of action, as follow: (i) modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, (ii) enhancement of GABA-mediated inhibition or other effect on the GABA system, (iii) inhibition

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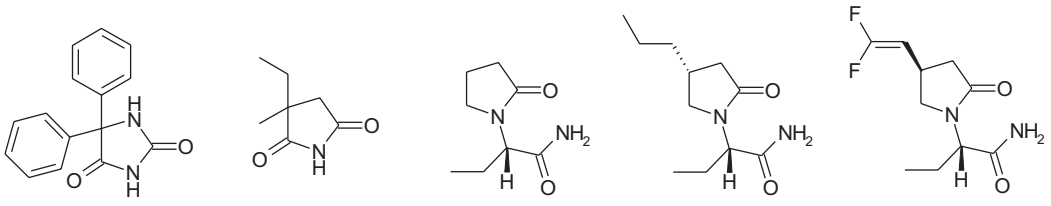


Figure 1. Structures of known AEDs bearing the imide or lactam scaffold.

of synaptic excitation mediated by ionotropic glutamate receptors, and (iv) modulation of synaptic release [8]. Regardless of the complex modes of action, the discovery of new anticonvulsants is still particularly focused on targeting voltage-gated sodium channels (VGSCs), since they play a fundamental role in establishing and regulating the excitability of neurons within the central nervous system (CNS). Furthermore the changes in the expression pattern and behavior of different sodium channel isoforms, especially $Na_V1.2$, are observed in epilepsy and could play a role in seizure generation and spread [9, 10]. Thus they are of great importance for the control and suppression of seizures [11].

Since many years our studies have been focused on the search for new anticonvulsant agents among ethosuximide analogues. The previous research from our laboratory has demonstrated diversified anticonvulsant activities among the differently substituted pyrrolidine-2,5-diones [12–16]. The most promising activity was observed among *N*-Mannich bases with an aromatic ring at position 3 and phenylpiperazine moieties (especially with electron-withdrawing chloro atom or trifluoromethyl group) at position 1 of pyrrolidine-2,5-dione (Fig. 2) [17–19].

As a continuation of systematic SAR studies among differently substituted succinimides as potential anticonvulsant agents, in the present work we synthesized a new series of *N*-Mannich bases of 3,3-diphenyl- (**7**–**18**) and 3-ethyl-3-methyl-pyrrolidine-2,5-diones (**19**–**30**). These compounds were designed as analogues of molecules described previously in which a second aromatic ring was introduced at position 3 of pyrrolidine-2,5-dione. Furthermore, to

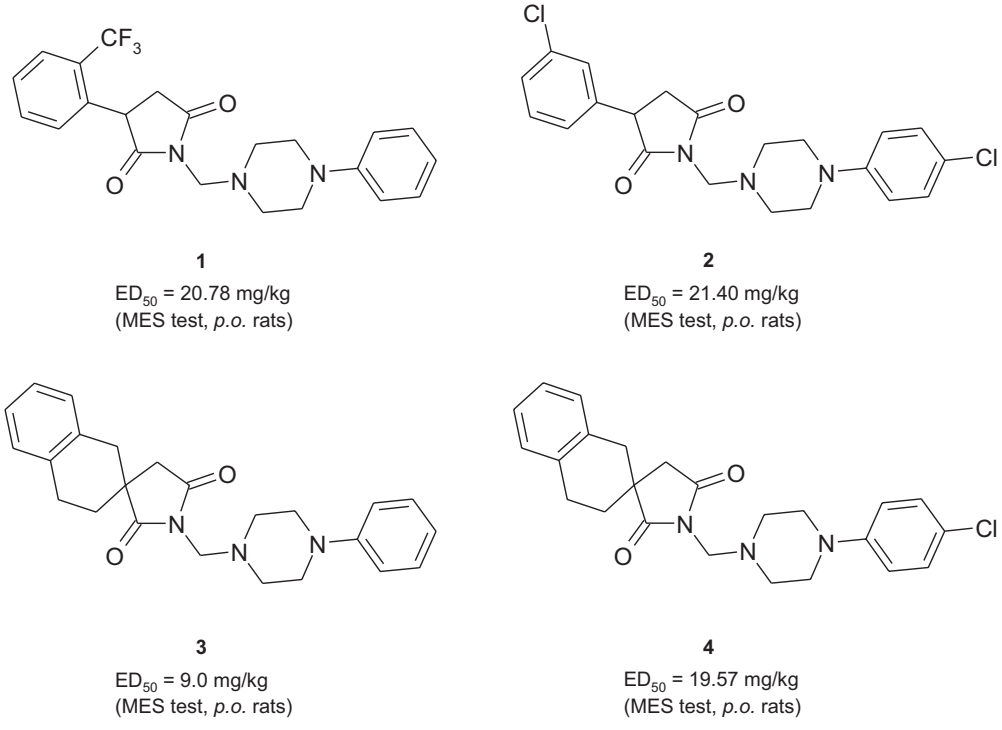


Figure 2. The structures of the model compounds **1**–**4**.

investigate the influence of the above phenyl rings on anticonvulsant activity in the next step of studies, their 3-ethyl-3-methyl-analogues were obtained. Based on the previous results differently substituted 4-phenylpiperazines (both with positive and negative input into anticonvulsant activity), were introduced at the imide nitrogen atom by a methylene spacer. Taking into consideration the structures of compounds **7**–**18** that correspond to molecules active in the MES test (e.g., phenytoin – 5,5-diphenylhydantoin), the assessment of their influence on $Na_V1.2$ sodium channel currents was planned as the most plausible mechanism of anticonvulsant action.

Results and discussion

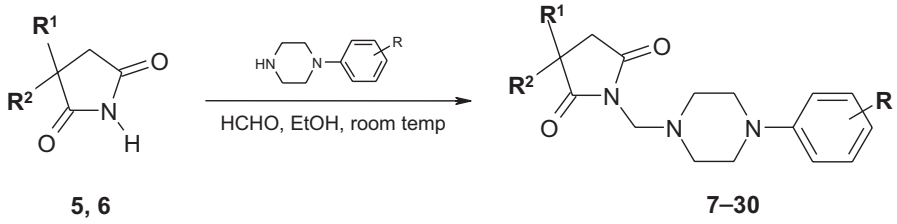
Chemistry

The synthesis of compounds **7**–**30** was accomplished as shown in Scheme 1. The starting material 3,3-diphenyl-phenyl-pyrrolidine-2,5-dione (**5**) was prepared as previously reported [20]. Compounds **19**–**30** were synthesized by use of commercially available 3-ethyl-3-methylsuccinimide (**6**) (Sigma–Aldrich, St. Louis, USA) as reaction substrate. The final compounds **7**–**30** were obtained in the Mannich-type reaction from the 3,3-disubstituted-pyrrolidine-2,5-diones (**5**, **6**), formaldehyde and corresponding 4-phenylpiperazines. The reaction was carried out in ethanol at room temperature for ca. 6–12 h. The crude products were crystallized from 96% ethanol. The final compounds were obtained in yields ranging from 65 to 75%. Their purity was assessed by TLC. The structures of the synthesized compounds were confirmed by both spectral and elemental analysis. The detailed physical and analytical data are listed in the Experimental section.

The synthesis and physicochemical properties of compounds **19** and **24** were described previously [21]. These molecules were not tested for anticonvulsant activity.

Anticonvulsant activity *in vivo* studies

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 [22]. The profile of anticonvulsant activity was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure tests,



5, 6 **7–30**

R1 = **R2** =  compounds **5**, **7**–**18**

R1 = -CH_3, **R2** = -C_2H_5 compounds **6**, **19**–**30**

R	cmpd
H	**7, 19**
2-F	**8, 20**
4-F	**9, 21**
2-Cl	**10, 22**
3-Cl	**11, 23**
4-Cl	**12, 24**
3,4-diCl	**13, 25**
3-CF_3	**14, 26**
2-CH_3	**15, 27**
3-CH_3	**16, 28**
2-OCH_3	**17, 29**
3-OCH_3	**18, 30**

Scheme 1. General method for the synthesis of compounds **7**–**30**.

after intraperitoneal (*i.p.*) injection 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. It should be noticed that despite significant advances that have been made in epilepsy research during the last years, the MES and *sc*PTZ screens 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 [23, 24]. The acute neurological toxicity (NT) was determined in the minimal motor impairment – rotorod screen (NT). The results are shown in Table 1.

Except for compounds **20**–**22** and **29** tested as hydrochloride salts, all other molecules were evaluated as free bases. The compounds tested showed protection against electrically induced seizures (**7**–**9**, **11**, **12**, **14**–**16**, **23**, **24**, and **26**) or were inactive (**10**, **13**, **17**–**22**, **25**, **27**–**30**). All substances were devoid of activity in the pentylenetetrazole (*sc*PTZ) seizures. It was especially surprising in relation to 3-ethyl-3-methyl-pyrrolidine-2,5-diones (**19**–**30**) that are close analogues of ethosuximide which is recognized as model anticonvulsant active in the *sc*PTZ test. The strongest anti-MES activity, indicative of the ability of a substance to prevent seizure spread, was observed for **14** that revealed protection at a dose of 30 mg/kg 4 h after *i.p.* administration. This result was comparable to phenytoin which is known as model AED effective in the MES test. The other molecules were active at dose of 100 mg/kg at the same time point. The activity 4 h after intraperitoneal administration indicates the slow onset and long duration of anticonvulsant action. In the rotorod test for acute NT all of the 3,3-diphenyl-pyrrolidine-2,5-diones (**7**–**18**) and **25** representing series of 3-ethyl-3-methyl-analogues did not show neurotoxicity at the maximum dose administered (300 mg/kg). Among these compounds **7**–**9**, **11**, **12**, **14**–**16** emerged as anticonvulsants without neurotoxic properties. The other molecules exhibited motor impairment at a dose of 100 mg/kg (**19**, **20**, **22**, **28**) or 300 mg/kg (**21**, **23**, **24**, **26**, **27**, **29**, **30**). It should be stressed here that structure–neurotoxic properties analysis showed remarkable growth of neurotoxicity caused by introduction of ethyl and methyl groups in place of phenyl rings at position 3 of pyrrolidine-2,5-dione.

On the basis of mice *i.p.* data seven compounds (**7**, **8**, **11**, **12**, **14**, **23**, and **26**) active in the electrically induced seizures were examined for anticonvulsant activity (MES screen) and neurotoxicity after *p.o.* administration in rats at a dose of 30 mg/kg. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity or neurotoxicity. The results are shown in Table 2.

Table 1. Anticonvulsant and neurotoxicity screening after *i.p.* administration in mice (**7**–**30**).

Compound	MES[a]		NT[b]	
	0.5 h	4 h	0.5 h	4 h
7	–	100	–	–
8	–	100	–	–
9	–	100	–	
10	–	–	–	–
11	–	100	–	–
12	–	100	–	–
13	–	–	–	–
14	100	30	–	–
15	–	100	–	–
16	–	100	–	–
17	–	–	–	–
18	–	–	–	–
19	–	–	100	–
20	–	–	100	–
21	–	–	300	–
22	–	–	100	–
23	–	100	300	–
24	–	100	300	300
25	–	–	–	–
26	–	100	300	300
27	–	–	300	–
28	–	–	100	–
29	–	–	300	
30	–	–	300	300
Phenytoin[c]	30	30	100	–
Ethosuximide[c]	–	–	–	–

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 screening using the rotorod test.

[c] Phenytoin, ethosuximide – reference drugs, tested by use of ADD Program procedures in NIH/NINDS, data from ref. [43].

Table 2. Anticonvulsant activity of selected compounds administrated orally to rats 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	0	2
8	0	0	0	0	0
11	0	0	0	2	3
12	0	0	0	0	0
14	0	0	2	3	4
23	0	0	1	1	1
26	0	1	2	3	3
Phenytoin[b]	1	4	3	3	3

The data indicate the number of rats of four that were protected.

[a] Maximal electroshock test.

[b] Phenytoin, reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from ref. [44].

Among these the most active was compound **14** that protected 100% of animals at 4 h, 75% at 2 h, and 50% at 1 h. This molecule revealed however lower activity than phenytoin – model AED. The others **7**, **11**, **23**, **26** were less active and inhibited convulsions in 75% of rats at 2 h (**26**) or/and 4 h (**11**, **26**); 50% of rats at 1 h (**26**), 2 h (**11**) or 4 h (**7**), and 25% of rats at 0.5 h (**26**), 1 h, 2 h, and 4 h (**23**). All derivatives were non-neurotoxic when given orally (data not indicated in Table 2).

Compound **14** as the most active molecule in previous tests was chosen for phase II evaluation for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative evaluation of the median effective dose (ED_{50}) in the MES seizures and neurotoxic dose (TD_{50}) in the rotorod screen were performed after *p.o.* administration to rats. The studies were carried out at previously estimated TPE – 4 h. Results of the quantitative tests along with the data for the standard drug – phenytoin are shown in Table 3.

As shown in Table 3 compound **14** revealed equivalent activity in comparison to the reference drug – phenytoin. Moreover, it possessed relatively low rotorod toxicity compared with the standard drug that was expressed in 3.6-fold more favorable protection index. These results confirmed also satisfactory absorption from the gastrointestinal tract as well as penetration of the substance into the CNS.

According to the ASP dispositions four compounds **11**, **14**, **23**, **24** were selected randomly and assessed for potential activity against nerve agents using the pilocarpine model of epilepsy as an introductory screen. This model shares many characteristics with nerve agent induced seizures since both initiation and early expression of nerve agent induced seizures are cholinergic followed by the recruitment of other neurotransmitter systems that serve to reinforce recurring seizure activity progressing to status epilepticus (SE). The pilocarpine models are one of the most recognized animal models of SE. The results revealed however that all molecules were inactive in this screen.

In vitro sodium channel electrophysiological studies

Intensive studies into the physiological and biochemical events taking place during epileptic seizures have provided insight into the molecular mechanisms by which these might be controlled. The fundamental role in establishing and regulating excitability of CNS neurons as well as suppression of seizures is ascribed to VGSCs [9]. Thus the brain ion channels are the molecular targets of a number of chemically diverse AEDs from which the most important are phenytoin, lamotrigine, carbamazepine, and oxcarbazepine. These AEDs are sodium channel blockers and have been shown to inhibit $Na_V1.2$ Na channel currents [25–27]. It should be stressed here that such mode of action is characteristic for compounds active in the maximal electroshock seizure (MES) test. Taking into consideration the activity of compounds synthesized in electrically induced seizures, for seven, active compounds **7**, **8**, **9**, **11**, **14**, **23**, and **26** the influence on $Na_V1.2$ sodium channel currents was studied *in vitro*. The results are shown in Table 4.

According to the method applied [28, 29], the percentage of blocking the $Na_V1.2$ channel currents for active compound should be higher than 50%. Taking into consideration the above the most effective blockers were **11** and **26** that showed activity in concentration of 10.0 μM (blocking percentage >50%). These molecules revealed also the highest inhibition in concentration of 100 μM – 91.19% (**11**) and 87.91% (**26**). The other active molecules include **9**, **14**, and **23** (100 μM),

Table 4. Electrophysiological evaluation of selected compounds for efficacy against $Na_v1.2$ current.

Compound	Percentage of blocking $Na_v1.2$ sodium channel currents ($n = 2$)			
	0.1 μM	1.0 μM	10.0 μM	100 μM
7	4.17	9.25	25.87	43.13
8	11.45	14.38	40.78	38.85
9	11.20	4.58	34.09	80.69
11	15.60	28.39	58.47	91.19
14	11.00	4.63	42.48	86.04
23	1.76	2.58	33.47	79.19
26	13.12	16.43	53.90	87.91

The data indicate the percentage of blocking $Na_v1.2$ current. The results are divided as follows:
<20% – inactive compound,
20–50% – compound with marginal activity,
>50% – active compound.

Table 3. Quantification studies of **14** in the MES and neurotoxicity tests (NT) in rats after *p.o.* administration.

Compound	TPE (h)[a]	ED_{50} MES (mg/kg)[b]	TD_{50} (mg/kg)[b]	PI (TD_{50}/ED_{50})[c]
14	4	30.3 (19.40–54.50)	<400	<13.20
Phenytoin[d]	2	28.1 (20.7–35.2)	>100	>3.60

[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] PI, protective index (TD_{50}/ED_{50}).
[d] Phenytoin, reference drug, tested by use of ADD Program procedures in NIH/NINDS, data from ref. [45].

whereas **7** and **8** showed only moderate activity in concentration of 10.0 and 100 μM. Despite of activity the comparison of electrophysiological and *in vivo* data revealed no direct correlation between $Na_V1.2$ channel inhibition and results obtained in animals. It was especially visible for **14** that showed the most potent anti-MES protection in mice and rats however was a weaker $Na_V1.2$ channel blocker than the less active molecules **11** and **26**. Furthermore **7** and **8** which were moderately active on sodium channels possessed equal *in vivo* activity with other compounds tested. These findings suggest that the mechanism of anticonvulsant action may be connected with the influence on other molecular targets beyond sodium channels or interaction with specific sodium channel gating modes such as the slow inactivation mode which is known to be important for AED activity [30].

X-ray structure analysis

As a continuation of discerning structural studies [17, 31–34], the molecular structures of selected compounds **8**, **11**, and **10** were crystallographically confirmed. The presence of the 4-phenylpiperazine moiety influences different properties of the compounds including the ability of intermolecular interactions. In aim to confirm the above assumption the structures of molecules with *ortho*-fluoro- (**8**), *ortho*-chloro- (**10**), and *meta*-chloro- (**11**) substituents were examined. In three structures obtained, similarly to pyrrolidine-2,5-diones described previously [17, 31–34], the main motive for crystal construction is based on C–H...O hydrogen bonds (Fig. 3).

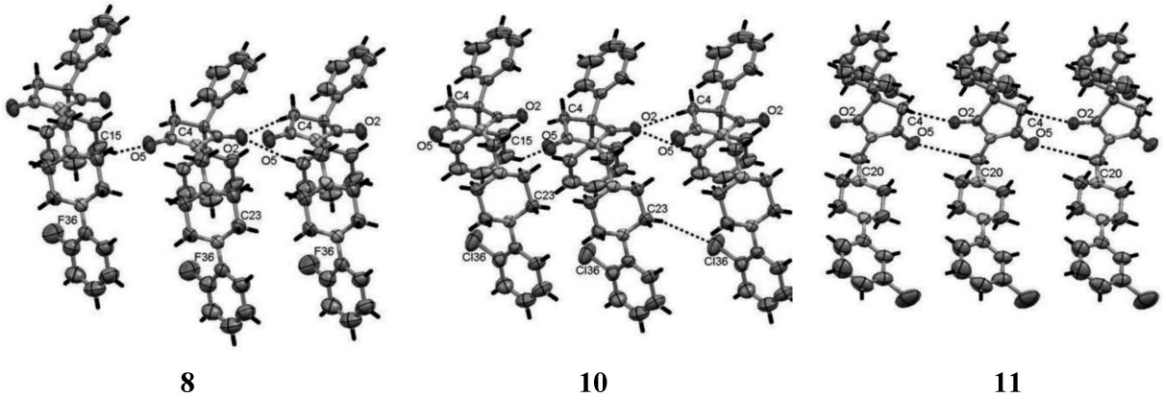
As shown in Table 5, the bond of C_4–H...O_2 (anchored oxygen O_2, imide ring C_4 atom), is the strongest in each structure. Moreover this intermolecular interaction becomes weaker in the following order: *meta*-chloro- (**11**) < *ortho*-fluoro- (**8**) < *ortho*-chloro- (**10**) substituted molecules.

Table 5. H-bonds geometry description.

Compound	X–H...Y	H···Y (Å)	X–H···Y (°)
8	C_4–H_4B...O_2	3.186 (2)	157
	C_{12}–H_{12}...O_2	3.253 (2)	154
	C_{15}–H_{15}...O_5	3.365 (2)	143
10	C_4–H_4B...O_2	3.255 (2)	161
	C_{12}–H_{12}...O_2	3.363 (3)	154
	C_{15}–H_{15}...O_5	3.432 (3)	146
	C_{23}–$H_{23}A$...Cl_{36}	3.795 (2)	149
11	C_4–H_4B...O_2	3.086 (4)	135
	C_{20}–$H_{20}A$...O_5	3.659(4)	174

Structure–activity relationships

The preliminary pharmacological screening revealed that anticonvulsant activity depended strongly on the substituents at position 3 of the imide ring. The most active were compounds with two phenyl rings at position 3 of the pyrrolidine-2,5-dione moiety (**7**–**18**). The exchange of the mentioned phenyl substituents into alkyl – ethyl and methyl groups made the compounds less active or inactive (**19**–**30**). The second factor influencing anticonvulsant activity is the presence of electron-withdrawing atoms especially at position 3 or 4 of the 4-phenylpiperazine moiety. In general, in both series of compounds the most active were the 3-CF_3 derivatives (**14**, **26**) and compounds with a chloro atom located at the *meta*- (**11**, **23**) or *para*-position (**12**, **24**). The respective *ortho*-chloro isomers (**10**, **22**) were devoid of activity. The weaker activity was observed for unsubstituted (**7**, **19**) and fluoro compounds **8**, **9** **20**, and **21**, among which only 3,3-diphenyl-pyrrolidine-2,5-diones (**7**, **8**, **9**) showed anticonvulsant protection. The replacement of electron-withdrawing fragments into electron-donating CH_3 or OCH_3 substituents (**17**, **18**, **27**, **28**, **29**, **30**) yielded inactive molecules, in general (an exception was observed



8 **10** **11**

Figure 3. ORTEP drawing of the **8**, **10**, and **11** molecules.

for active 2-CH_3 (**15**) and 3-CH_3 (**16**) derivatives in series of 3,3-diphenyl-pyrrolidine-2,5-diones).

Summing up, the modifications applied that relied on introduction of two phenyl rings or ethyl and methyl substituents at position 3 of the pyrrolidine-2,5-dione ring did not improve anticonvulsant activity in comparison with model compounds **1**–**4** described previously [17–19]. Therefore it seems that the most favorable for anticonvulsant activity is the presence of one aromatic system in the vicinity of the C-3 imide carbon atom.

Conclusion

Twenty four new *N*-[(4-phenylpiperazin-1-yl)-methyl]- derivatives of 3,3-diphenyl- (**7**–**18**) and 3-ethyl-3-methyl-pyrrolidine-2,5-dione (**19**–**30**) were synthesized and evaluated for their anticonvulsant and neurotoxic properties. The *in vivo* results revealed that 11 compounds were active and revealed protection only in electrically induced seizures. Moreover all effective 3,3-diphenyl-pyrrolidine-2,5-diones did not show acute NT in the rotorod screen. The most active were *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**14**) with ED_{50} of 30.30 mg/kg in rats (*p.o.*). The SAR studies revealed that anticonvulsant activity was closely connected with the presence of phenyl substituents at position 3 of the imide ring as well as electron-withdrawing atoms especially at position 3 or 4 of the phenylpiperazine moiety. The *in vitro* electrophysiological studies demonstrated that compounds active in the MES test had activity against the $Na_V1.2$ sodium channel isoform.

Experimental

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 thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminum sheets (Merck; Darmstadt, Germany), using a developing system consisting of chloroform/acetone (9:1 v/v) (S1) and chloroform/isopropanol/ammonia (9:11:2 by volume) (S2). Spots were detected by their absorption under UV light ($\lambda = 254$ 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 micromethod using the elemental Vario EI III elemental analyzer (Hanau, Germany). The results of the 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, 282, and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as internal standard. The J values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (double doublets), t (triplet), m (multiplet). The mass spectra for compounds **7**–**30** were obtained on a Waters ACQUITY^{TM} 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).

*General procedure for preparation of compounds **7**–**30***

To a mixture of 3,3-diphenyl- (**5**) or 3-ethyl-3-methyl-pyrrolidine-2,5-dione (**6**) (0.01 mol) and 40% solution of formaldehyde (0.01 mol) in 96% ethanol (20 ml), corresponding 4-phenylpiperazines (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 ca. −10°C for 24 h. The precipitated crude products were washed with cold ethanol, separated by filtration, and recrystallized from 96% ethanol. Compounds **20**–**22** and **29** due to their oily character were converted into hydrochloride salts in anhydrous ethanol saturated with HCl gas. The obtained precipitate salts were crystallized from anhydrous ethanol.

*N-[(4-Phenyl-piperazin-1-yl)-methyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**7**)*

White solid. Yield: 79%; mp 160–161°C; TLC: $R_f = 0.81$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.75 (t, 4H, piperazine, $J = 5.0$ Hz), 3.14 (t, 4H, piperazine, $J = 5.0$ Hz), 3.47 (s, 2H, imide), 4.61 (s, 2H, $-CH_2-$), 6.83–6.89 (m, 3H, ArH), 7.22–7.38 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.96, 49.19, 50.56, 57.30, 60.42, 116.19, 119.85, 127.41, 127.76, 128.92, 129.13, 141.64, 151.15, 176.11, 179.49. ESI-MS: 426.18. ($C_{27}H_{27}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{27}N_3O_2$ (425.54): C: 76.21, H: 4.57, N: 9.87. Found C: 76.48, H: 6.38, N: 9.85.

*N-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**8**)*

White solid. Yield: 70%; mp 160–162°C; TLC: $R_f = 0.68$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.77 (t, 4H, piperazine, $J = 4.74$ Hz), 3.05 (t, 4H, piperazine, $J = 4.87$ Hz), 3.50 (s, 2H, imide), 4.60 (s, 2H, $-CH_2-$), 6.88–6.99 (m, 4H, ArH), 7.01–7.39 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.93, 50.20, 50.56, 57.28, 60.37, 115.36, 115.66, 117.87, 117.95, 127.35, 127.75, 128.92, 141.61, 147.77, 155.63, 158.75, 176.05, 179.46. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.40 (s, 1F). ESI-MS: 444.10. ($C_{27}H_{26}N_3O_2F$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{26}N_3O_2F$ (443.53): C: 73.12, H: 5.91, N: 9.47. Found C: 72.85, H: 5.89, N: 9.46.

*N-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**9**)*

White solid. Yield: 68%; mp 106–107°C; TLC: $R_f = 0.75$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.75 (t, 4H, piperazine, $J = 4.87$ Hz), 3.55 (t, 4H, piperazine, $J = 4.74$ Hz), 3.49 (s, 2H, imide), 4.59 (s, 2H, $-CH_2-$), 6.80–6.85 (m, 2H, ArH), 6.91–6.97 (m, 2H, ArH), 7.26–7.37 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.94, 50.19, 50.55, 57.29, 60.38, 115.38, 115.67, 117.89, 117.99, 127.39, 127.76, 128.91, 141.63, 147.78, 155.62, 158.79, 176.09, 179.48. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.38 (s, 1F). ESI-MS: 444.10. ($C_{27}H_{26}N_3O_2F$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{26}N_3O_2F$ (443.53): C: 73.12, H: 5.91, N: 9.47. Found C: 73.22, H: 5.92, N: 9.46.

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

White solid. Yield: 78%; mp 147–149°C; TLC: $R_f = 0.73$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.76 (t, 4H, piperazine, $J = 4.61$ Hz), 2.99 (t, 4H, piperazine, $J = 4.49$ Hz), 3.51 (s, 2H, imide), 4.61 (s, 2H, $-CH_2-$), 6.93–7.01 (m, 2H, ArH), 7.17–7.39 (m, 12H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.91, 48.71, 50.30, 57.31, 60.38, 113.92, 115.80, 119.31, 127.33, 127.76, 128.93, 130.03, 134.92, 141.55, 152.10, 176.02, 179.45. ESI-MS: 460.11. ($C_{27}H_{26}N_3O_2Cl$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{26}N_3O_2Cl$ (459.98): C: 70.50, H: 5.70, N: 9.14. Found C: 70.25, H: 5.71, N: 9.18.

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

White solid. Yield: 74%; mp 105–107°C; TLC: $R_f = 0.76$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.71 (t, 4H, piperazine, $J = 5.0$ Hz), 3.13 (t, 4H, piperazine, $J = 5.0$ Hz), 3.49 (s, 2H, imide), 4.59 (s, 2H, $-CH_2-$), 6.71–6.81 (m, 3H, ArH), 7.14 (t, 1H, ArH, $J = 7.47$ Hz), 7.25–7.37 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.95, 48.69, 50.31, 57.29, 60.31, 113.99, 115.84, 119.36, 127.39, 127.78, 128.92, 130.04, 134.94, 141.59, 152.12, 176.05, 179.49. ESI-MS: 460.11. ($C_{27}H_{26}N_3O_2Cl$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{26}N_3O_2Cl$ (459.98): C: 70.50, H: 5.70, N: 9.14. Found C: 70.58, H: 5.68, N: 9.13.

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

White solid. Yield: 77%; mp 154–155°C; TLC: $R_f = 0.73$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.73 (t, 4H, piperazine, $J = 5.0$ Hz), 3.01 (t, 4H, piperazine, $J = 5.0$ Hz), 3.51 (s, 2H, imide), 4.59 (s, 2H, $-CH_2-$), 6.78 (d, 2H, ArH, $J = 8.97$), 7.18 (d, 2H, ArH, $J = 9.23$), 7.21–7.37 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.96, 48.70, 50.34, 57.28, 60.34, 113.90, 115.85, 119.36, 127.40, 127.77, 128.91, 130.14, 134.90, 141.61, 152.14, 176.08, 179.51. ESI-MS: 460.11. ($C_{27}H_{26}N_3O_2Cl$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{26}N_3O_2Cl$ (459.98): C: 70.50, H: 5.70, N: 9.14. Found C: 70.28, H: 5.71, N: 9.15.

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

White solid. Yield: 65%; mp 143–145°C; TLC: $R_f = 0.68$ (S1); ^{1}H NMR ($CDCl_3$) δ: 2.70 (t, 4H, piperazine, $J = 5.0$ Hz), 3.11 (t, 4H, piperazine, $J = 5.0$ Hz), 3.49 (s, 2H, imide), 4.59 (s, 2H, $-CH_2-$), 6.66–6.70 (dd, 1H, ArH, $J = 2.82$, Hz, $J = 8.97$ Hz), 6.89 (d, 1H, ArH, $J = 2.82$ Hz), 7.23 (s, 1H, ArH), 7.25–7.37 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.98, 48.72, 50.36, 57.30, 60.36, 113.92, 115.87, 119.38, 127.42, 127.80, 128.92, 130.16, 134.91, 141.63, 152.15, 176.12, 179.54. ESI-MS: 494.05 ($C_{27}H_{25}N_3O_2Cl_2$ $[M+H]^+$). Anal. calcd. for $C_{27}H_{25}N_3O_2Cl_2$ (494.43): C: 65.59, H: 5.10, N: 8.50. Found C: 65.95, H: 5.08, N: 8.48.

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

White solid. Yield: 60%; mp 78–80°C; TLC: $R_f = 0.76$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.73 (t, 4H, piperazine, $J = 4.87$ Hz), 3.17 (t, 4H, piperazine, $J = 4.87$ Hz), 3.49 (s, 2H, imide), 4.60 (s, 2H, $-CH_2-$), 6.98–7.08 (m, 4H, ArH), 7.24–7.37 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.95, 48.68, 50.33, 57.29, 60.30, 112.23, 112.28, 115.89, 115.94, 118.80, 127.38, 127.77, 128.92, 129.56, 131.62, 141.58, 151.18, 176.04, 179.48. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −62.71 (s, 3F, CF_3). ESI-MS: 494.11 ($C_{28}H_{26}N_3O_2F_3$ $[M+H]^+$). Anal. calcd. for $C_{28}H_{26}N_3O_2F_3$ (493.54): C: 68.14, H: 5.31, N: 8.51. Found C: 68.10, H: 5.30, N: 8.48.

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

White solid. Yield: 68%; mp 139–141°C; TLC: $R_f = 0.71$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.21 (s, 3H, 3-CH_3), 2.70–2.71 (m, 4H, piperazine), 2.84 (t, 4H, piperazine, $J = 4.74$ Hz), 3.51 (s, 2H, imide), 4.61 (s, 2H, $-CH_2-$), 6.94–6.99 (m, 2H, ArH), 7.15 (t, 2H, ArH, $J = 7.05$), 7.26–7.40 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.76, 44.95, 49.26, 50.59, 57.26, 60.42, 113.30, 117.01, 120.76, 127.40, 127.73, 128.92, 128.98, 138.80, 141.61, 151.19, 176.05, 179.45. ESI-MS: 440.08 ($C_{28}H_{29}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{28}H_{29}N_3O_2$ (439.56): C: 76.51, H: 6.65, N: 9.56. Found C: 76.65, H: 6.67, N: 9.54.

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

White solid. Yield: 72%; mp 139–141°C; TLC: $R_f = 0.82$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.31 (s, 3H, 3-CH_3), 2.74 (t, 4H, piperazine, $J = 4.88$ Hz), 3.13 (t, 4H, piperazine, $J = 5.0$ Hz), 3.49 (s, 2H, imide), 4.61 (s, 2H, $-CH_2-$), 6.67–6.71 (m, 3H, ArH), 7.11–7.17 (m, 1H, ArH), 7.26–7.38 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.78, 44.96, 49.27, 50.61, 57.29, 60.44, 113.33, 117.02, 120.77, 127.41, 127.75, 128.91, 128.97, 138.82, 141.64, 151.22, 176.11, 179.49. ESI-MS: 440.10 ($C_{28}H_{29}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{28}H_{29}N_3O_2$ (439.56): C: 76.51, H: 6.65, N: 9.56. Found C: 76.55, H: 6.63, N: 9.54.

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

White solid. Yield: 66%; mp 123–125°C; TLC: $R_f = 0.70$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.82 (t, 4H, piperazine, $J = 4.75$ Hz), 3.03–3.04 (m, 4H, piperazine), 3.49 (s, 2H, imide), 3.82 (s, 3H, $-OCH_3$), 4.61 (s, 2H, $-CH_2-$), 6.83–7.02 (m, 4H, ArH), 7.26–7.38 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.23, 44.97, 49.29, 50.63, 57.30, 60.46, 113.35, 117.04, 120.79, 127.43, 127.74, 128.92, 128.98, 138.83, 141.65, 151.24, 176.12, 179.50. ESI-MS: 456.18 ($C_{28}H_{29}N_3O_3$ $[M+H]^+$). Anal. calcd. for $C_{28}H_{29}N_3O_3$ (455.56): C: 73.82, H: 6.42, N: 9.22. Found C: 73.68, H: 6.39, N: 9.25.

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

White solid. Yield: 60%; mp 178–180°C; TLC: $R_f = 0.78$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.74 (t, 4H, piperazine, $J = 4.87$ Hz), 3.13 (t, 4H, piperazine, $J = 4.87$ Hz), 3.49 (s, 2H, imide), 3.78 (s, 3H, $-OCH_3$), 4.59 (s, 2H, $-CH_2-$), 6.40–6.43 (m, 2H, ArH), 6.48–6.51 (m, 1H, ArH), 7.13–7.18 (m, 1H, ArH), 7.26–7.38 (m, 10H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.26, 44.98, 49.28, 50.62, 57.30, 60.40, 113.33, 117.03, 120.78, 127.41, 127.75, 128.91, 128.99, 138.84, 141.67, 151.24, 176.13, 179.49. ESI-MS: 456.12 ($C_{28}H_{29}N_3O_3$ $[M+H]^+$). Anal. calcd. for $C_{28}H_{29}N_3O_3$ (455.56): C: 73.82, H: 6.42, N: 9.22. Found C: 73.75, H: 6.39, N: 9.19.

*N-[(4-Phenyl-piperazin-1-yl)-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**19**)*

White solid. Yield: 70%; mp 65–67°C; TLC: $R_f = 0.60$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, 3H, CH_3, $J = 7.44$ Hz), 1.33 (s, 3H, CH_3), 1.54–1.82 (m, 2H, $-CH_2-$), 2.46 (d, 1H, imide, $J = 18.47$ Hz), 2.67 (d, 1H, imide, $J = 18.46$ Hz), 2.79 (brs, 4H, piperazine), 3.18 (brs, 4H, piperazine), 4.53 (s, 2H, $-CH_2-$), 6.91–6.94 (m, 3H, ArH), 7.24–7.29 (m, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.91, 24.25, 31.04, 40.36, 44.36, 49.10, 50.57, 55.15, 59.74, 102.58, 104.59, 108.93, 129.76, 152.58, 160.54, 177.10, 183.89. ESI-MS: 316.09 ($C_{18}H_{25}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{25}N_3O_2$ (315.42): C: 68.54, H: 7.99, N: 13.32. Found C: 68.29, H: 8.02, N: 13.38.

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

White solid. Yield: 78%; mp 165–168°C; TLC: $R_f = 0.80$ (S2); ^{1}H NMR (300 MHz, DMSO): δ 0.78 (t, 3H, CH_3, $J = 7.55$ Hz), 1.24 (s, 3H, CH_3), 1.41–1.89 (m, 2H, $-CH_2-$), 2.39 (d, 1H, imide, $J = 18.21$ Hz), 2.47–2.61 (m, 2H, piperazine), 2.74 (d, 1H, imide, $J = 18.47$ Hz), 3.46 (brs, 2H, piperazine), 3.69 (brs, 4H, piperazine), 4.63 (s, 2H, $-CH_2-$), 6.96–7.20 (m, 4H, ArH), 10.66 (brs, 1H, +NH). ^{13}C NMR (75 MHz, DMSO) δ: 8.94, 24.30, 31.10, 40.38, 44.35, 48.69, 50.40, 59.68, 114.05, 115.85, 119.43, 130.05, 134.89, 152.26, 177.12, 193.88. ESI-MS: 334.15 ($C_{18}H_{24}N_3O_2F$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{24}N_3O_2F \times HCl$ (369.87): C: 58.45, H: 6.54, N: 11.36. Found C: 58.25, H: 6.57, N: 11.38.

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

White solid. Yield: 58%; mp 152–155°C; TLC: $R_f = 0.72$ (S2); ^{1}H NMR (300 MHz, DMSO): δ 0.94 (t, 3H, CH_3, $J = 7.44$ Hz), 1.41 (s, 3H, CH_3), 1.62–1.86 (m, 2H, $-CH_2-$), 2.59 (d, 1H, imide, $J = 18.47$ Hz), 2.74 (d, 1H, imide, $J = 18.47$ Hz), 3.37 (brs, 4H, piperazine), 3.53 (brs, 4H, piperazine), 4.63 (s, 2H, $-CH_2-$), 7.12 (t, 2H, ArH, $J = 8.47$Hz), 7.58 (brs, 2H, ArH), 10.68 (brs, 1H, +NH). ^{13}C NMR (75 MHz, DMSO) δ: 8.93, 24.27, 31.05, 40.38, 44.39, 48.69, 50.41, 59.66, 114.03, 115.88, 119.43, 130.03, 134.88, 152.21, 177.10, 193.81. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.18 (s, 1F). ESI-MS: 334.13 ($C_{18}H_{24}N_3O_2F$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{24}N_3O_2F \times HCl$ (369.87): C: 58.45, H: 6.54, N: 11.36. Found C: 58.55, H: 6.54, N: 11.35.

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

White solid. Yield: 58%; mp 147–149°C; TLC: $R_f = 0.77$ (S2); ^{1}H NMR (300 MHz, DMSO) δ: 0.84 (t, 3H, CH_3, $J = 7.18$ Hz), 1.24 (s, 3H, CH_3), 1.41–1.89 (m, 2H, $-CH_2-$), 2.39 (d, 1H, imide, $J = 18.21$ Hz), 2.75 (d, 1H, imide, $J = 18.46$ Hz), 3.16 (brs, 4H, piperazine), 3.44 (brs, 4H, piperazine), 4.62 (s, 2H, $-CH_2-$), 7.03–7.21 (m, 2H, ArH), 7.27–7.35 (m, 1H, ArH), 7.39–7.46 (m, 1H, ArH), 10.54 (brs, 1H, +NH). ^{13}C NMR (75 MHz, DMSO) δ: 8.93, 24.29, 31.0, 40.35, 44.38, 48.70, 50.41, 59.66, 114.03, 115.86, 119.42, 130.01, 134.88, 152.21, 177.10, 193.87. ESI-MS: 350.10 ($C_{18}H_{24}ClN_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{24}ClN_3O_2 \times HCl$ (386.32): C: 51.14, H: 5.72, N: 9.94. Found C: 51.18, H: 5.70, N: 9.92.

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

White solid. Yield: 78%; mp 105–107°C; TLC: $R_f = 0.65$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, 3H, CH_3, $J = 7.44$ Hz), 1.32 (s, 3H, CH_3), 1.54–1.81 (m, 2H, $-CH_2-$), 2.46 (d, 1H, imide, $J = 18.21$ Hz), 2.70 (d, 1H, imide, $J = 18.21$ Hz), 2.75 (brs, 4H, piperazine), 3.17 (brs, 4H, piperazine), 4.52 (s, 2H, $-CH_2-$), 6.74–6.86 (m, 3H, ArH), 7.12–7.26 (m, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.93, 24.29, 31.03, 40.34, 44.38, 48.73, 50.41, 59.69, 114.04, 115.89, 119.45, 130.0, 134.91, 152.22, 177.10, 193.89. ESI-MS: 350.12 ($C_{18}H_{24}ClN_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{24}N_3O_2Cl$ (349.87): C: 61.79, H: 6.91, N: 12.01. Found C: 62.08, H: 6.92, N: 11.99.

*N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**24**)*

White solid. Yield: 68%; mp 154–155°C; TLC: $R_f = 0.58$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, 3H, CH_3, $J = 7.56$ Hz), 1.33 (s, 3H, CH_3), 1.54–1.81 (m, 2H, $-CH_2-$), 2.46 (d, 1H, imide, $J = 18.21$ Hz), 2.74 (d, 1H, imide, $J = 18.46$ Hz), 2.78 (brs, 4H, piperazine), 3.13 (brs, 4H, piperazine), 4.52 (s, 2H, $-CH_2-$), 6.83–6.85 (m, 2H, ArH), 7.12–7.26 (m, 2H, ArH), $C_{18}H_{24}N_3O_2Cl$ (349.87). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.94, 24.28, 31.01, 40.36, 44.40, 48.71, 50.40, 59.67, 114.02, 115.87, 119.44, 130.02, 134.89, 152.20, 177.11, 193.88. ESI-MS: 350.11 ($C_{18}H_{24}ClN_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{24}N_3O_2Cl$ (349.87): C: 61.79, H: 6.91, N: 12.01. Found C: 61.81, H: 6.92, N: 12.03.

*N-[{4-(3,4-Dichlorophenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**25**)*

White solid. Yield: 75%; mp 82–84°C; TLC: $R_f = 0.64$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, 3H, CH_3, $J = 7.44$ Hz), 1.32 (s, 3H, CH_3), 1.53–1.79 (m, 2H, $-CH_2-$), 2.45 (d, 1H, imide, $J = 18.21$ Hz), 2.67 (d, 1H, imide, $J = 18.21$ Hz), 2.72–2.74 (m, 4H, piperazine), 3.10–3.19 (m, 4H, piperazine), 4.51 (s, 2H, $-CH_2-$), 6.68–6.77 (m, 1H, ArH), 6.91–6.97 (m, 1H, ArH), 7.24–7.28 (m, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.95, 24.29, 31.02, 40.38, 44.43, 48.72, 50.43, 59.69, 114.05, 115.89, 119.47, 130.07, 134.91, 152.22, 177.12, 193.89. ESI-MS: 384.10 ($C_{18}H_{23}N_3O_2Cl_2$ $[M+H]^+$). Anal. calcd. for $C_{18}H_{23}N_3O_2Cl_2$ (384.31): C: 56.26, H: 6.03, N: 10.94. Found C: 56.19, H: 6.04, N: 10.99.

*N-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**26**)*

White solid. Yield: 61%; mp 78–80°C; TLC: $R_f = 0.63$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, 3H, CH_3, $J = 7.44$ Hz), 1.33 (s, 3H, CH_3), 1.54–1.81 (m, 2H, $-CH_2-$), 2.46 (d, 1H, imide, $J = 18.47$ Hz), 2.68 (d, 1H, imide, $J = 18.47$ Hz), 2.74 (brs, 4H, piperazine), 3.25 (brs, 4H, piperazine), 4.53 (s, 2H, $-CH_2-$), 7.02–7.08 (m, 3H, ArH), 7.26–7.36 (m, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.91, 24.29, 31.02, 40.33, 44.38, 48.73, 50.41, 59.68, 112.29, 112.35, 115.96, 116.01, 118.85, 129.53, 151.26, 177.10, 183.88. ESI-MS: 384.10 ($C_{19}H_{24}N_3O_2F_3$ $[M+H]^+$). Anal. calcd. for $C_{19}H_{24}N_3O_2F_3$ (383.43): C: 59.52, H: 6.31, N: 10.96. Found C: 59.63, H: 6.32, N: 11.00.

*N-[{4-(2-Methylphenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**27**)*

White solid. Yield: 64%; mp 73–75°C; TLC: $R_f = 0.67$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, CH_3, $J = 7.44$ Hz), 1.23 (s, 3H, CH_3), 1.53–1.79 (m, 2H, $-CH_2-$), 2.18 (s, 3H, CH_3), 2.45 (d, 1H, imide, $J = 18.21$ Hz), 2.67 (d, 1H, imide, $J = 18.21$ Hz), 2.72–2.74 (m, 4H, piperazine), 3.10–3.19 (m, 4H, piperazine), 4.51 (s, 2H, $-CH_2-$), 6.91–6.97 (m, 2H, ArH), 7.24–7.28 (m, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.90, 21.25, 24.24, 31.06, 40.37, 44.37, 49.28,

19.29, 50.65, 51.57, 59.75, 113.33, 117.05, 120.82, 128.93, 138.77, 151.24, 177.14, 183.89. ESI-MS: 330.17 ($C_{19}H_{27}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{19}H_{27}N_3O_2$ (329.45): C: 69.27, H: 8.26, N: 12.75. Found C: 69.51, H: 8.25, N: 12.71.

*N-[{4-(3-Methylphenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**28**)*

White solid. Yield: 72%; mp 64–66°C; TLC: $R_f = 0.64$ (S1); ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H, CH_3, $J = 10.0$ Hz), 1.23 (s, 3H, CH_3), 1.47–1.67 (m, 2H, $-CH_2-$), 2.18 (s, 3H, CH_3), 2.57 (brs, 1H, imide), 2.61–2.63 (m, 4H, piperazine), 2.69 (brs, 1H, imide), 2.75–2.80 (m, 4H, piperazine), 4.33 (s, 2H, $-CH_2-$), 6.90–6.99 (m, 2H, ArH), 7.09–7.14 (m, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.91, 21.24, 24.26, 31.05, 40.38, 44.36, 49.29, 19.28, 50.66, 51.59, 59.77, 113.32, 117.02, 120.80, 128.94, 138.78, 151.25, 177.12, 183.90. ESI-MS: 330.16 ($C_{19}H_{27}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{19}H_{27}N_3O_2$ (329.45): C: 69.27, H: 8.26, N: 12.75. Found C: 69.18, H: 8.23, N: 12.77.

*N-[{4-(2-Methoxyphenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione monohydrochloride (**29**)*

White solid. Yield: 66%; mp 160–163°C; TLC: $R_f = 0.76$ (S2); ^{1}H NMR (300 MHz, DMSO): δ 0.76–0.86 (m, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.41–1.70 (m, 2H, $-CH_2-$), 2.36–3.17 (m, 6H, 2H, imide, 4H piperazine), 3.28–3.53 (m, 4H, piperazine), 3.79 (s, 3H, OCH_3), 4.67 (s, 2H, $-CH_2-$), 6.86–7.09 (m, 4H, ArH), 11.09 (s, 1H, 1H, +NH). ^{13}C NMR (75 MHz, DMSO) δ: 8.94, 25.22, 28.26, 32.14, 40.65, 44.55, 49.42, 50.30, 50.65, 51.58, 59.78, 113.31, 117.12, 121.10, 128.96, 138.85, 151.34, 177.29, 184.07. ESI-MS: 346.08 ($C_{19}H_{27}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{19}H_{27}N_3O_2 \times HCl$ (381.90): C: 59.76, H: 7.13, N: 11.00. Found C: 59.59, H: 7.14, N: 10.98.

*N-[{4-(3-Methoxyphenyl)-piperazin-1-yl}-methyl]-3-ethyl-3-methyl-pyrrolidine-2,5-dione (**30**)*

White solid. Yield: 65%; mp 103–105°C; TLC: $R_f = 0.54$ (S1); ^{1}H NMR (300 MHz, $CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3, $J = 7.44$ Hz), 1.32 (s, 3H, CH_3), 1.58–1.78 (m, 2H, $-CH_2-$), 2.44 (d, 1H, imide, $J = 18.21$ Hz), 2.66 (d, 1H, imide, $J = 18.21$ Hz), 2.72–2.75 (m, 4H, piperazine), 3.13–3.16 (m, 4H, piperazine), 3.77 (s, 3H, OCH_3), 4.51 (s, 2H, $-CH_2-$), 6.39–6.53 (m, 3H, ArH), 7.13–7.18 (m, 1H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.95, 25.24, 28.28, 32.15, 40.68, 44.58, 49.45, 50.31, 50.66, 51.61, 59.80, 113.36, 117.13, 121.12, 128.98, 138.88, 151.35, 177.31, 184.11. ESI-MS: 346.12 ($C_{19}H_{27}N_3O_2$ $[M+H]^+$). Anal. calcd. for $C_{19}H_{27}N_3O_2$ (345.44): C: 66.06, H: 7.88, N: 12.16. Found C: 66.10, H: 7.78, N: 12.21.

In vivo studies

Compounds **7**–**30** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program, realized in NINDS/NIH, Rockville, USA, using procedures described elsewhere [35, 36].

Male albino mice (CF-1 strain) and male albino rats (Sprague-Dawley) were used as experimental animals. Groups of eight mice or four rats were employed. 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 (**7**, **8**, **11**, **12**, **14**, **23**, and **26**) were administrated orally into rats using four animals at a fixed dose of 30 mg/kg (MES test). 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)

The MES is a model for generalized tonic–clonic seizures and provides an indication of a compound's ability to prevent seizure spread when all neuronal circuits in the brain are maximally active. These seizures are highly reproducible and are electrophysiologically consistent with human seizures. For all tests based on MES convulsions, 60 Hz of alternating current (50 mA in mice and 150 mA in rats) is delivered for 2 s by corneal electrodes which have been primed with an electrolyte solution containing an anesthetic agent (0.5% tetracaine HCl). An animal is considered ''protected'' from MES-induced seizures upon abolition of the hindlimb tonic extensor component of the seizure [37, 38].

Subcutaneous pentylenetetrazole seizure test (scPTZ)

Subcutaneous injection of the convulsant pentylenetetrazole (a $GABA_A$ receptor antagonist) produces clonic seizures in laboratory animals. The *sc*PTZ test detects the ability of a test compound to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure in response to a normally convulsant dose of pentylenetetrazole. Animals are pretreated with various doses of the test compound given by *i.p.* injection or through oral dosing. At various times after dosing with the test compound, the dose of pentylenetetrazole which will induce convulsions in 97% of animals (CD97: 85 mg/kg in mice or 56.4 mg/kg in rats) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress and observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3–5 s, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected [39].

Neurological toxicity (NT)

Neurological toxicity induced by a compound was detected in mice or rats using the standardized rotorod test [40]. When an untreated control mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered neurotoxic if it falls off this rotating rod three times during a 1-min period. In rats, minimal motor impairment is indicated by ataxia, which is manifested by an abnormal, uncoordinated gait. Rats used for evaluating toxicity are examined before the test drug is administered, since individual animals may have peculiarities in gait, equilibrium, placing response, etc., which might be attributed erroneously to the test substance. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response, and changes in muscle tone.

Quantification studies

The quantitative determination of ED_{50} and TD_{50} values was performed at previously estimated TPE after *p.o.* administration into rats. 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 Table 3.

The Pilocarpine Induced Status Prevention (PISP) model

A – Acute toxicity

Acute motor impairment will be assessed following the intraperitoneal (*i.p.*) administration of doses starting at 100 and 300 mg/kg of the test substance. Individual Sprague Dawley rats are evaluated for acute toxicity over several time points following administration of test drugs (unless there is previously obtained *i.p.* toxicity data available). The results obtained from this initial study determine whether any dose adjustments may be required. The behavior of the animals is observed closely and recorded over a 4-h period. Routinely, a minimum number of four (4) rats, two per dose, will be employed in this acute screen.

B – Status intervention

To determine if the test substance can halt the acute pilocarpine-induced status an initial qualitative efficacy screen is performed. A challenge dose of pilocarpine (50 mg/kg) is administered *i.p.* and animals are observed until the first convulsive (e.g., Stage 3, 4, or 5) seizure (time zero). The seizure severity is determined using the well-established Racine scale [41], as follows: (1) immobility, eye closure, twitching of vibrissae, sniffing, and facial clonus; (2) head nodding associated with more severe facial clonus; (3) clonus of one of the fore limbs; (4) rearing often accomplished by bilateral forelimb clonus and (5) all of the above plus loss of balance and falling, accomplished by generalized clonic seizures. At this point a minimally toxic dose of the candidate drug is administered to a group of 8 male albino Sprague Dawley rats (150–180 mg) via the *i.p.* route of administration. Efficacy is defined by the ability of an investigational drug to halt the further expression of pilocarpine induced convulsive seizures (e.g., Stage 3, 4, or 5). The anticonvulsant activity of compounds **11**, **14**, **23**, and **24** was assessed at time zero, namely the time from the first stage III seizures (Test 71). The outcome measures are determination of ''protection'' or ''no protection''.

In vitro sodium channel electrophysiological studies

The radioligand binding studies were performed commercially by Caliper Life Sciences (Hopkinton, MA, USA), using a methodology described elsewhere [28, 29]. The results are shown in Table 4.

X-ray structure analysis

The crystals of **8**, **10**, and **11** were obtained by slow evaporation of solutions in a mixture of methanol and propanol (1:1 v/v). The measurements of the crystals were performed on a KM4-CCD diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) at room temperature. The structures were solved by direct method and refined with SHELXTL [42]. E-maps provided positions for all non-H-atoms. The full-matrix least-squares refinement was carried out on F^2's using anisotropic temperature factors for all non-H-atoms. All C-bound H atoms were placed in idealized locations and refined using a riding model, with C–H = 0.93 Å and $U_{iso}(H) = 1.2U_{eq}(C)$.

Crystal data for **8**: $C_{27}H_{26}N_3O_2F$, $M = 443.51$, monoclinic, space group C2/c, $a = 17.8890(5)$ Å, $b = 6.3931(2)$ Å, $c = 40.1879(11)$ Å, $\beta = 94.293(1)^\circ$, $V = 4583.2(2)$ Å^3, $Z = 8$, $D_x = 1.286$ g/cm^3, $T = 293$ K, $\mu = 0.088$ mm^{-1}, $\lambda = 0.71073$ Å, data/parameters = 3950/299; final $R_1 = 0.0396$.

Crystal data for **10**: $C_{27}H_{26}N_3O_2Cl$, $M = 459.96$, monoclinic, space group C2/c, $a = 18.0187(5)$ Å, $b = 6.5138(2)$ Å, $c = 39.9615(11)$ Å, $\beta = 92.690(4)^\circ$, $V = 4685.1(2)$ Å^3, $Z = 8$, $D_x = 1.308$ g/cm^3, T = 293 K, $\mu = 0.193$ mm^{-1}, $\lambda = 0.71073$ Å, data/parameters = 4163/299; final $R_1 = 0.0501$.

Crystal data for **11**: $C_{27}H_{26}N_3O_2Cl$, $M = 459.96$, monoclinic, space group $P2_1/n$, $a = 17.713(2)$ Å, $b = 6.2223(5)$ Å, $c = 22.0686(17)$ Å, $\beta = 103.330(9)^\circ$, $V = 2366.8(4)$ Å^3, $Z = 4$, $D_x = 1.291$ g/cm^3, $T = 293$ K, $\mu = 0.191$ mm^{-1}, $\lambda = 0.71073$ Å, data/parameters = 4199/299; final $R_1 = 0.0671$.

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