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# Synthesis and Anticonvulsant Properties of New Mannich Bases Derived from 3,3-Disubstituted Pyrrolidine-2,5-diones. Part IV

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A library of 21 new *N*-Mannich bases of 3,3-diphenyl- (**5a–g**), 3-methyl-3-phenyl- (**6a–g**), and 3-ethyl-3-methylpyrrolidine-2,5-diones (**7a–g**) were synthesized and evaluated for their anticonvulsant activity in the maximum electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure tests after intraperitoneal injection into mice. The acute neurological toxicity was determined applying the rotarod screen. The results in mice showed that 13 compounds were effective in the MES or/and *sc*PTZ screen. From these, seven molecules were tested in the MES seizures after oral administration in rats. The quantitative studies showed that *N*-[{4-(2-hydroxyethyl)-piperazin-1-yl}-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6c**) and *N*-[(4-benzylpiperidin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6f**) revealed higher protection in the MES and *sc*PTZ tests than valproic acid or ethosuximide which were used as reference antiepileptic drugs. Four compounds (**5c**, **6c**, **6e**, **6f**) showed high effectiveness in the 6-Hz psychomotor seizure model of partial and therapy resistant epilepsy.

**Keywords:** Anticonvulsant activity / *In vivo* studies / *N*-Mannich bases / Pyrrolidine-2,5-diones / Succinimides

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## Introduction

Epilepsy is a chronic disorder characterized by recurrent unprovoked seizures which are defined as a discrete clinical event arising from transient, hypersynchronous, and abnormal neuronal behavior. Epilepsy then is not a disease but rather a syndrome arising from a group of non-specific, dysfunctional events in the brain. The treatment of patients with epilepsy has been a long-term and consistent administration of anticonvulsant drugs. Unfortunately current drugs are ineffective for approximately 30% of the patients. Many continue to have seizure, while others experience disturbing side effects as drowsiness, dizziness, nausea, and liver damage [1–3]. Thus, there is a need for more efficacious and less toxic drugs.

The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of the majority antiepileptic drugs makes it difficult to use rational methodologies of discovery. Therefore, conceptually, there are two different methods of obtaining new anticonvulsants namely ligand-based and screening approaches [4]. The ligand-based approach relies on the use of different pharmacophores that were established through the analysis of structural characteristics of clinically effective AEDs as well as other anticonvulsant active compounds. The serendipitous approach involves a comprehensive screening process that utilizes rodent models. Traditionally, most screening programs employ mice and rats to assess efficacy against either electrically (e.g., maximal electroshock, MES) or chemically (e.g., pentylenetetrazol, bicuculline, or picrotoxin) induced seizures [5, 6]. The number of new AEDs currently available, or in development, for the management of epilepsy certainly attests to the success of this approach. However, this method may overlook novel compounds that would be uniquely effective in the therapy-resistant population [7]. One example supporting this hypothesis is provided by levetiracetam, which has demonstrated efficacy in refractory human partial epilepsy [5, 8]. It was found to be inactive against MES and PTZ seizures even at high doses, whereas it showed high effectiveness in the 6-Hz psychomo-

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tor seizure model of partial epilepsy [9]. Thus it is suggested that the 6-Hz model might be capable of identifying anti-seizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action.

The past decades have demonstrated many attempts to identify the structural features of compounds crucial for anticonvulsant activity. It is well documented that one of the important core fragments of anticonvulsants is defined by a nitrogen heterocyclic system, usually imide or lactam, and phenyl or alkyl groups attached to the heterocyclic system [10–12]. This common template is present in the structures of old, however well-established anticonvulsants (AEDs), such as ethosuximide and phenytoin, as well as among the newest drugs, e.g., levetiracetam, brivaracetam, or seletracetam (Fig. 1) [13, 14].

Taking into consideration the above our researches are focused on a group of 1,3-substituted pyrrolidine-2,5-diones as targets for new antiepileptic drugs [15–19]. Many of these compounds were effective in the MES or/and subcutaneous pentylenetetrazole (*sc*PTZ) screens that are still recognized as the most popular seizure models in the early stages of the search for new anticonvulsants [20–22]. The structure–activity relationship (SAR) analysis showed that the most active ones were *N*-[(4-phenylpiperazin-1-yl)-methyl]-3-phenyl-pyrrolidine-2,5-diones with different electron withdrawing substituents at both phenyl rings. The structures of chosen, active compounds obtained in previous studies are shown in Fig. 2.

As a continuation of systematic SAR discussion, in the present work we synthesized a series of 21 new *N*-Mannich bases derived from 3,3-diphenyl- (**5a–g**), 3-methyl-3-phenyl-(**6a–g**), and 3-ethyl-3-methyl-(**7a–g**) pyrrolidine-2,5-diones. The diverse substitution modes at position-3 of the imide ring enabled to assess the influence of aromatic or alkyl substituents on the anticonvulsant activity among the compounds obtained. To continue comprehensive SAR analysis, at the imide nitrogen atom we introduced through the methylene spacer various secondary amines such as 4-benzyl-, 4-pyrimid-2-yl-, 4-(2-hydroxyethyl)-, 4-cyclohexyl-, 4-methyl-piperazines, 4-benzylpiperidine, or morpholine.

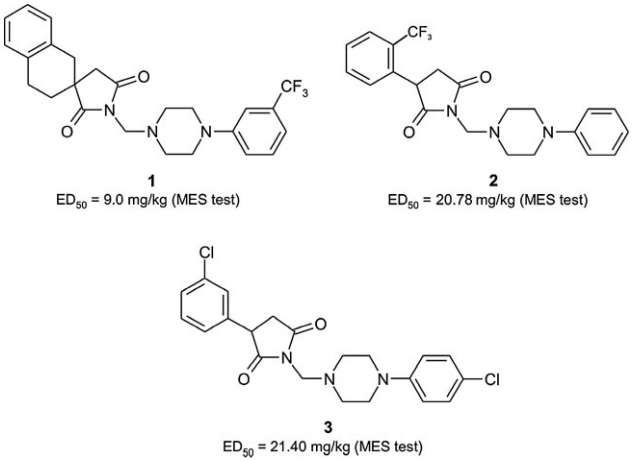



**Figure 2.** Structures of active compounds obtained in previous studies (data in rats after oral administration) [17, 21, 22].

## Results and discussion

### Chemistry

The synthesis of compounds **5a–g**, **6a–g**, and **7a–g** was accomplished as shown in Scheme 1.

The starting materials 2-methyl-2-phenyl- succinic acid (**4**) and 3,3-diphenylpyrrolidine-2,5-dione (**5**) were prepared using methods reported previously [23, 24]. The 3-methyl-3-phenylpyrrolidine-2,5-dione (**6**) was obtained in the cyclization reaction of **4** with the 25% ammonia. The final compounds **5a–g**, **6a–g**, and **7a–g** were synthesized in amino-alkylation reaction of the imide acidic proton, in the presence of formaldehyde and appropriate secondary amines such as 4-substituted piperazines, morpholine, or 4-benzyl-piperidine. The reaction was carried out in ethanol at room temperature for ca. 12 h. Compounds **5a–g**, **6a–g**, and **7a–g** were obtained in yields ranging from 62 to 88%. Their purities were assessed by TLC chromatography. Compounds **6a–g** and **7a–g** were synthesized as racemic mixtures. The chemical structures were confirmed by both

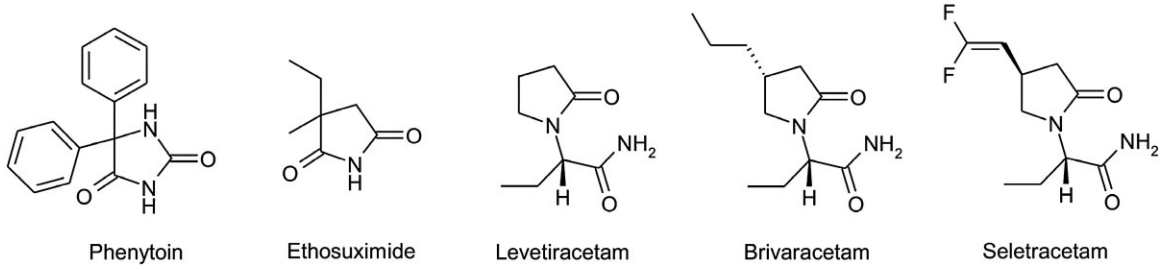
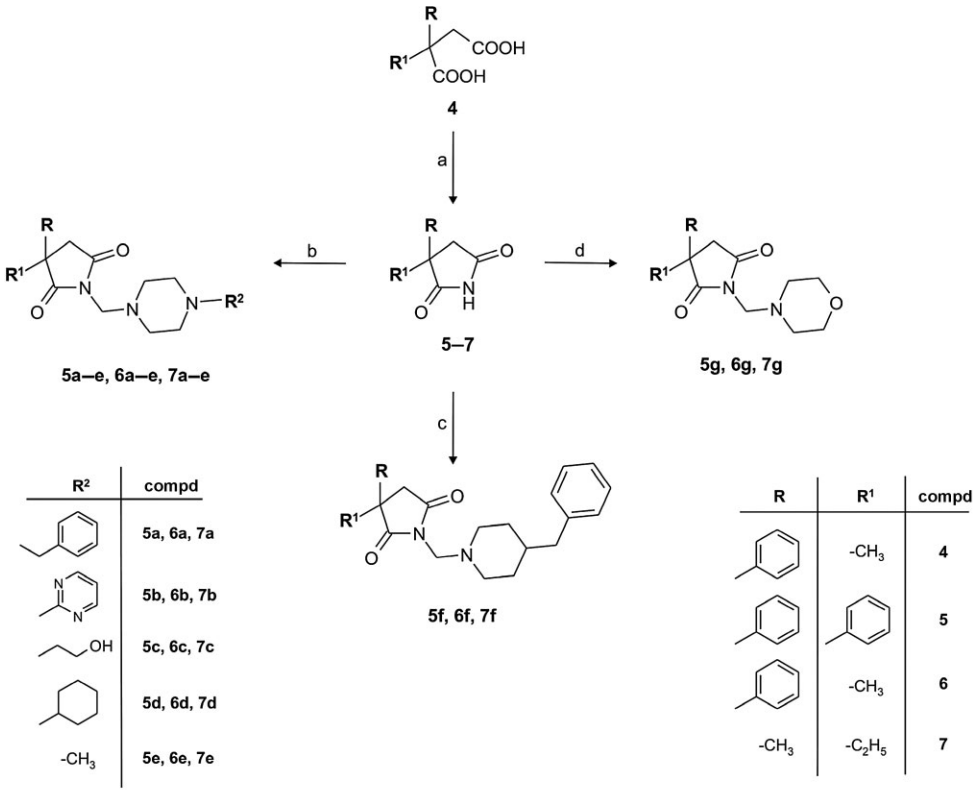



**Figure 1.** Structures of model AEDs.




**Reagents and conditions**: (a) 25% $NH_4OH$, 190°C, 1.5 h, (b) 4-substituted piperazine derivatives, formaldehyde, 96% Et-OH, 12 h r.t., (c) 4-benzylpiperidine, formaldehyde, 96% Et-OH, 12 h r.t., (d) morpholine, formaldehyde, 96% Et-OH, 12 h r.t.

**Scheme 1.** Synthetic procedures for target compounds **5a–g**, **6a–g**, and **7a–g**.

spectral and elemental analysis. The detailed physical and analytical data are listed in the experimental section.

## Anticonvulsant activity

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 *sc*PTZ and the kindling model. From these tests the MES and *sc*PTZ screens are still recognized as the ''gold standards'' in the early stages of testing and enabled to detect compounds affording protection against generalized tonic–clonic seizures and generalized absence seizures, respectively [6].

The profile of anticonvulsant activity of compounds **5a–g**, **6a–g**, and **7a–g** was established in the MES and *sc*PTZ tests, after intraperitoneal (i.p.) injection in mice at doses of 30, 100, and 300 mg/kg. An observation was carried out at two different time intervals – 0.5 and 4 h. The acute neurological toxicity (NT) was determined by use of the minimal motor impairment-rotarod screen (NT). The results are shown in Table 1.

The data obtained revealed that anticonvulsant activity was closely connected with the presence of at least one phenyl ring at position-3 of pyrrolidine-2,5-dione (**5a–g**, **6a–g**), whereas all 3-ethyl-3-methyl- analogs (**7a–g**) were inactive. Except for *N*-[(4-benzyl-piperazin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6a**), all other 3-phenyl-3-methyl- and 3,3-diphenylpyrrolidine-2,5-diones (**5a–g**, **6b–g**) were effective in the MES test and several of them, namely **6b**, **6c**, **6e**, and **6g**, showed protection in the *sc*PTZ screen, additionally. Compounds that revealed protection in the MES test, indicative of the ability of the substance to prevent seizure spread, at a dose of 100 mg/kg included **5a–e**, **5g**, and **6b–g**, whereas molecule **5f** provided anti-MES protection at a dose of 300 mg/kg. Except for compounds **6e** and **6g**, which were active only at 0.5 h after i.p. administration, the other derivatives showed activity at 4 h or in both time intervals (**5a**, **5c–e**, **5g**, **6b**, **6c**, and **6f**), indicating quick onset and long duration of the anticonvulsant activity. Apart from the anti-MES protection,

**Table 1.** The results after i.p. administration into mice.

| Compd | MES[a] | | *sc*PTZ[b] | | NT[c] | |
|---|---|---|---|---|---|---|
| | 0.5 h | 4 h | 0.5 h | 4 h | 0.5 h | 4 h |
| **5a** | 300 | 100 | – | – | – | 300[14] |
| **5b** | – | 100 | – | – | – | – |
| **5c** | 100 | 100 | – | – | 300[14] | 300[14] |
| **5d** | 100 | 100 | – | – | – | 300[14] |
| **5e** | 100 | 100 | – | – | 300[14] | 300[14] |
| **5f** | – | 300 | – | – | – | – |
| **5g** | 100 | 100 | – | – | – | – |
| **6a** | – | – | – | – | 300[1] | – |
| **6b** | 300 | 100 | 100[25] | – | 300 | 300 |
| **6c** | 100 | 300 | 100[25] | – | 300 | – |
| **6d** | – | 100 | – | – | 300[14,1] | – |
| **6e** | 100 | – | 300 | – | – | – |
| **6f** | 100 | 300 | – | – | 300 | – |
| **6g** | 100 | – | 100 | – | – | – |
| **7a** | – | – | – | – | 300[1] | – |
| **7b** | – | – | – | – | 300 | – |
| **7c** | – | – | – | – | – | – |
| **7d** | – | – | – | – | – | – |
| **7e** | – | – | – | – | – | – |
| **7f** | – | – | – | – | 300[14] | – |
| **7g** | – | – | – | – | – | |
| Phenytoin[d] | 30 | 30 | – | – | 100 | 100 |
| Valproic acid[d] | – | – | 300 | – | – | – |
| Ethosuximide[e] | – | – | 100 | 300 | – | – |

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 of the animals. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg). Response comments: [1]death, [14]unable to grasp rotarod, [25]myoclonic jerks.

[a] Maximal electroshock test.

[b] Subcutaneous pentylenetetrazole test.

[c] Neurotoxicity screening – rotarod test.

[d] Reference drugs, data from [29].

[e] Reference drugs, data from [30].

compounds **6b**, **6c**, **6e**, and **6g** were found to be active in the *sc*PTZ test, which identifies substances elevating the seizure threshold. Among these molecules **6b**, **6c**, and **6g** showed anti-*sc*PTZ activity at the dose 100 mg/kg that was equivalent to ethosuximide used as reference anticonvulsant. Unfortunately, despite the protection in PTZ seizures, **6b** and **6c** provoked myoclonic jerks at the same dose. Compound **6d** was less active and inhibited seizures at a dose of 300 mg/kg.

In the rotarod test (NT) for acute neurological toxicity **5b**, **5f**, **5g**, **6e**, **6g** as well as **7c–e** and **7g** did not show neurotoxicity at the maximum dose administered (300 mg/kg). The other derivatives **5a**, **5c–e**, **5g**, **6a–d**, **6f**, **7a**, **7b**, and **7f** showed neurotoxicity at a dose of 300 mg/kg. At the same dose mice were unable to grasp the rotarod after administration of **5a**, **5c–e**, **6d**, and **7f**.

On the basis of data in mice and according to the Anticonvulsant Screening Project disposition, seven compounds **5a**, **5d**, **5e**, **6b–d**, and **6f** were selected and examined for their anticonvulsant activity (MES screen) and neurotoxicity after p.o. administration into rats at a dose of 30 mg/kg. The results obtained are presented in Table 2.

Among the derivatives tested, the most active ones were **6c** and **6f** which showed a peak of 75% protection at 1 h (**6c**) and at 0.5 h (**6f**), respectively. These compounds were also active in 50% of animals at time points 1 h (**6f**), 2 h, and 4 h (**6c**). The latter revealed also 25% protection at 0.5 h. The other molecules were less active and protected up to 25% (**5a**, **5d**, **6b**) or 50% (**6d**) of rats in different time intervals. All compounds given orally did not cause motor impairment in the rotarod screen (data not indicated in Table 2).

Compounds **6c** and **6f** were chosen for quantification of the pharmacological parameters ($ED_{50}$ and $TD_{50}$) after i.p. administration into mice. Quantitative evaluation of the MES and *sc*PTZ median effective dose ($ED_{50}$) and neurotoxic dose ($TD_{50}$) was performed at the previously estimated time of peak effect (TPE). Results of the quantitative tests along with the data for the standard drugs ethosuximide and valproic acid are shown in Table 3.

The quantitative data revealed that **6c** and **6f** were more potent than the reference drugs in the MES test. Furthermore **6c** showed also higher activity in the PTZ seizures in comparison with standard substances. Such *in vivo* profile may indicate potential effectiveness of **6c** in treating different types of seizures including human generalized tonic–clonic, myoclonic, and absence seizures. Both compounds revealed lower rotarod toxicity than valproic acid and ethosuximide that resulted in favorable protection index.

In the next step of pharmacological investigations, compound **6f** was tested in the MES, *sc*PTZ, and rotarod screens after p.o. administration to rats. The quantitative data are shown in Table 4.

**Table 2.** Anticonvulsant activity (MES test) of selected compounds administrated orally to rats (30 mg/kg).

| Compd | 0.25 h | 0.5 h | 1 h | 2 h | 4 h |
|---|---|---|---|---|---|
| **5a** | 0 | 0 | 1 | 0 | 0 |
| **5d** | 1 | 1 | 0 | 0 | 0 |
| **5e** | 0 | 2 | 1 | 0 | 0 |
| **6b** | 0 | 0 | 1 | 0 | 1 |
| **6c** | 0 | 1 | 3 | 2 | 2 |
| **6d** | 0 | 0 | 0 | 2 | 0 |
| **6f** | 0 | 3 | 2 | 0 | 0 |
| Phenytoin[a] | 1 | 4 | 3 | 3 | 3 |

The data in the oral MES screen indicate the number of rats of four that were protected at a dose of 30 mg/kg.

[a] Reference drug, data from [31].

**Table 3.** Quantitative anticonvulsant data in mice after i.p. administration (**6c** and **6f**).

| Compd | TPE (h)[a)] | $ED_{50}$[b)] MES (mg/kg) | $ED_{50}$[b)] *sc*PTZ (mg/kg) | $TD_{50}$[b)] (mg/kg) | PI[c)] ($TD_{50}/ED_{50}$) |
|---|---|---|---|---|---|
| **6c** | 0.5 | 76.15 (60.56–97.80) | 132.90 (106.01–153.76) | 289.28 (251.16–316.50) | 3.8 (MES), 2.17 (*sc*PTZ) |
| **6f** | 0.5 | 95.61 (85.12–111.03) | >195.0 | 212.24 (184.98–259.11) | 2.22 (MES), >1.08 (*sc*PTZ) |
| Valproic acid[d)] | 1.0 | 263 (237–282) | 220 (177–268) | 398 (356–445) | 1.5 (MES), 1.8 (*sc*PTZ) |
| Etosuximide[d)] | 1.0 | >500 | 136 (101–184) | 341 (290–384) | 2.50 (*sc*PTZ) |

[a)] Time to peak effect.
[b)] Results are represented as mean ± SEM at 95% confidence limit (MES, maximal electroshock test; *sc*PTZ, subcutaneous pentylenetetrazole test; neurotoxicity-rotarod screen).
[c)] Protection index ($TD_{50}/ED_{50}$).
[d)] Reference drug, data from [32].

**Table 4.** Quantitative anticonvulsant data in rats after p.o. administration (**6f**).

| Compd | TPE (h)[a)] | $ED_{50}$[a)] MES (mg/kg) | $ED_{50}$[b)] *sc*PTZ (mg/kg) | $TD_{50}$[b)] (mg/kg) | PI[c)] ($TD_{50}/ED_{50}$) |
|---|---|---|---|---|---|
| **6f** | 0.5 | 52.56 (30.21–99.99) | 96.43 (80.06–112.4) | >500 | >9.5 (MES), >5.18 (*sc*PTZ) |
| Valproic acid[d)] | 1.0 | 485 (324–677) | 646 (466–869) | 784 (503–1176) | 1.6 (MES), 1.2 (*sc*PTZ) |
| Etosuximide[d)] | 1.0 | >500 | 167 (116–237) | >500 | >2.99 |

[a)] Time to peak effect.
[b)] Results are represented as mean ± SEM at 95% confidence limit (MES, maximal electroshock test; *sc*PTZ, subcutaneous pentylenetetrazole test; neurotoxicity-rotarod screen).
[c)] Protection index ($TD_{50}/ED_{50}$).
[d)] Reference drug, data from [32].

Compound **6f** given orally to rats showed superior activity and lower neurotoxicity in comparison with model anticonvulsants – valproic acid and ethosuximide. These results confirmed also satisfactory absorption from the gastrointestinal tract as well as penetration of the substance into the central nervous system.

Selected compounds **5c**, **6c**, **6e**, and **6f** were chosen for the evaluation of anticonvulsant activity in the 6-Hz test. The selection was made randomly as a part of the search for molecules providing protection in the 6-Hz model among chemically diversified compounds pursued in the NIH/NINDS. The 6-Hz screen has been validated recently as a model of therapy-resistant epilepsy [9]. The results obtained are shown in Table 5.

**Table 5.** Anticonvulsant activity – i.p. psychomotor seizure test in mice (6-Hz, current 32 mA).

| Compd | 0.25 h | 0.5 h | 1 h | 2 h | 4 h |
|---|---|---|---|---|---|
| **5c** | 4 | 4 | 4 | 4 | 4 |
| **6c** | 3 | 2 | 1 | 1 | 0 |
| **6e** | 4 | 4 | 3 | 2 | 0 |
| **6f** | 1 | 2 | 0 | 0 | 0 |

The data indicate the number of mice of four that were protected at a dose of 100 mg/kg (**5c**, **6c**, **6e**) or 50 mg/kg (**6f**).

As can be seen, the most potent was **5c** which protected 100% of mice in all time intervals at a dose of 100 mg after i.p. administration. Compound **6e** was less active and protected 100% of animals at 0.25 h, 0.5 h; 75% at 1 h and 50% at 2 h, whereas **6c** showed only one peak of 75% protection at 0.25 h. It should be stressed here that compound **6f** revealed 50% effectiveness at 0.5 h, however in contrast with the other molecules, it was tested at a dose of 50 mg/kg. Thus it was selected for quantification of the median effective dose in psychomotor seizure test (6-Hz) after i.p. administration in mice (Table 6).

Compound **6f** revealed higher activity in comparison with valproic acid or ethosuximide however was less active than levetiracetam which is known as model antiepileptic drug effective in 6-Hz seizures.

Summing up, the potency and spectrum of anticonvulsant activity depended mainly on the kind of substituents at position-3 of pyrrolidine-2,5-dine. All the 3,3-diphenyl derivatives (**5a–g**) provided protection only in electrically induced seizures. The exchange of one phenyl ring into the methyl group (compounds **6a–g**) yielded molecules that showed activity in both electrical and chemical (pentylenetetrazole) seizures, in general. The removal of aromatic rings (**7a–g**) caused lack of any anticonvulsant activity. These data enable

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

| Compd | TPE (h)[a)] | $ED_{50}$[b)] 6 Hz (mg/kg) | $TD_{50}$[b)] (mg/kg) | PI[c)] ($TD_{50}/ED_{50}$) |
|---|---|---|---|---|
| **6f** | 0.5 | 90.16 (40.32–167.15) | 275.30 (256.10–300.53) | 3.05 |
| Levetiracetam[d)] | 1 | 19.4 (9.90–36.0) | >500 | >26.0 |
| Ethosuximide[d)] | 1 | 167 (114–223) | 341 (290–384) | 2.5 |
| Valproic acid[d)] | 1 | 126.0 (94.5–152) | 398 (356–445) | 3.2 |

[a)] Time to peak effect.
[b)] Results are represented as mean ± SEM at 95% confidence limit (6-Hz – psychomotor seizure test; neurotoxicity-rotarod screen).
[c)] Protection index ($TD_{50}/ED_{50}$).
[d)] Reference drug, data from [32].

to design new molecules, effective in different types of seizures, in future studies. The *in vivo* results and the structures of the active compounds that correspond to known AEDs such as ethosuximide, phensuximide, or phenytoin may suggest that the most possible mechanism of anticonvulsant action is related with the influence on voltage-depended sodium or/and calcium channels.

## Conclusion

In the present studies a library of twenty one new *N*-Mannich bases of 3,3-diphenyl-, 3-methyl-3-phenyl-, and 3-ethyl-3-methyl-pyrrolidine-2,5-diones were synthesized and evaluated for their anticonvulsant activity in the maximum electroshock (MES) and *sc*PTZ seizure tests. The results of *in vivo* studies revealed that the majority of compounds exhibited anticonvulsant activity in the MES test, which is an animal model of human generalized tonic–clonic seizures. Several molecules showed activity in the *sc*PTZ and 6-Hz screens that may indicate their potential usefulness in absence seizures as well as partial and therapy resistant epilepsy, respectively. From the whole series the most promising compound was *N*-[(4-benzylpiperidin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6f**), which revealed $ED_{50}$ values of 95.61 mg/kg (MES, i.p. mice), 52.56 (MES, p.o. rats), 96.43 (*sc*PTZ, p.o. rats), and 90.16 mg/kg (6-Hz test, i.p. mice).

## Experimental protocol

### Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and homogeneity of the compounds were assessed with TLC. The thin-layer chromatography (TLC) was performed on Merck silica gel 60 $F_{254}$ aluminum sheets (Merck; Darmstadt, Germany), using developing systems consisting of $S_1$ chloroform/acetone (9:1 v/v), $S_2$ – chloroform/isopropanol/ammonia (9:11:2 by volume).

Spots were detected by their absorption under UV light ($\lambda = 254$ nm). Elemental analysis for C, H, and N were carried out by a micro-method using the elemental Vario EI III Elemental analyzer (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 $CDCl_3$ or DMSO, operating at 300 MHz. Chemical shifts are reported in $\delta$ values (ppm) relative to TMS $\delta$: 0 (1H), 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), t (triplet), and m (multiplet).

The starting materials 2-methyl-2-phenyl-succinic acid (**4**) and 3,3-diphenylpyrrolidine-2,5-dione (**5**) were prepared using methods described previously [23, 24].

Compounds **7a–g** were obtained using as a starting material commercially available 3-ethyl-3-methyl-pyrrolidine-2,5-dione (**7**) (Sigma–Aldrich, USA).

#### *General procedure for the synthesis of 3-methyl-3-phenylpyrrolidine-2,5-dione (**6**)*

A total of 0.05 mol of the 2-methyl-2-phenyl-succinic acid (**4**) was dissolved in 50 mL of water and 0.05 mol of the 25% ammonia was gradually added. The mixture was heated in a sand bath with simultaneous distillation of water. After the water was completely removed, the temperature of the reaction rose up to 190°C and was maintained for 1.5 h. The solid product was separated by crystallization from isopropanol (Scheme 1).

#### *3-Methyl-3-phenylpyrrolidine-2,5-dione (**6**)*

White powdery crystals. Yield: 70%; mp 77–78°C; TLC: $R_f = 0.51$ ($S_1$); $^1$H NMR ($CDCl_3$): $\delta$ 1.76 (s, 3H, $CH_3$), 2.90

(d, 1H, imide, $J = 18.21$ Hz), 3.17 (d, 1H, imide, $J = 18.46$ Hz), 7.26–7.39 (m, 5H, ArH), 8.00 (brs, 1H, NH); $C_{11}H_{11}O_2N$ (189.22).

### *General procedure for the synthesis of compounds **5a–f**, **6a–f**, and **7a–f***

The mixture of 3,3-disubstituted-succinimide (0.01 mol) (**5**, **6**, **7**), 40% formaldehyde solution (0.01 mol) and corresponding 4-substituted piperazine (0.01 mol), morpholine (0.01 mol), or 4-benzyl-piperidine in 96% ethanol (40 mL) was left for ca. 12 h at room temperature and was then refrigerated at ca. −10°C for 24 h. The products were washed with cold ethanol and the solid products (**5a–g**, **6a–g**, and **7a–g**) were separated by filtration and recrystallized from 96% ethanol. Compounds **5c**, **6a**, **6c**, **6e**, **7a**, **7c**, and **7e** 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-Benzyl-piperazin-1-yl)-methyl]-3,3-diphenylpyrrolidine-2,5-dione (**5a**)*

White powdery crystals. Yield: 78%; mp 82–84°C; TLC: $R_f = 0.69$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.41 (brs, 4H, piperazine), 2.61 (brs, 4H, piperazine) 3.47 (s, 4H, 2H, $CH_2$, 2H, imide), 4.54 (2H, $CH_2$), 7.21–7.39 (m, 15H, ArH); $C_{28}H_{29}O_2N_3$ (439.56).

### *N-[(4-Pyrimid-2-yl-piperazin-1-yl)-methyl]-3,3-diphenylpyrrolidine-2,5-dione (**5b**)*

White powder-y crystals. Yield: 69%; mp 153–154°C; TLC: $R_f = 0.68$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.61 (t, 4H, piperazine $J = 5.13$ Hz), 3.46 (s, 2H, imide), 3.76 (t, 4H piperazine $J = 5.00$ Hz), 4.59 (s, 2H, $CH_2$), 6.48 (t, 1H, pyrimidine, $J = 4.74$ Hz), 7.22–7.34 (m, 10H, ArH), 8.28 (d, 2H, pyrimidine, $J = 4.61$ Hz); $C_{25}H_{25}O_2N_5$ (427.50).

### *N-{[4-(2-Hydroxyethyl)-piperazin-1-yl]-methyl}-3,3-diphenylpyrrolidine-2,5-dione dihydrochloride (**5c**)*

White powdery crystals. Yield: 77%; mp 121–124°C; TLC: $R_f = 0.82$ ($S_2$); $^1$H NMR (300 MHz, DMSO): $\delta$ 2.66–3.10 (m, 6H, 2H, $CH_2$, 4H, piperazine), 3.24 (t, 1H, OH, $J = 4.75$ Hz), 3.39–3.80 (m, 8H, 2H, imide, 2H, $CH_2$, 4H, piperazine), 4.40 (s, 2H, $CH_2$), 7.26–7.41 (m, 10H, ArH), 11.37 (brs, 1H, HCl), 11.56 (brs, 1H, HCl); $C_{23}H_{29}O_3N_3Cl_2$ (466.42).

### *N-[(4-Cyclohexyl-piperazin-1-yl)-methyl]-3,3-diphenylpyrrolidine-2,5-dione (**5d**)*

White powdery crystals. Yield: 79%; mp 116–118°C; TLC: $R_f = 0.67$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.10–1.24 (m, 5H, cyclohexane), 1.58–1.63 (m, 1H, cyclohexane), 1.75–1.85 (m, 4H, cyclohexane), 2.18–2.19 (m, 1H, cyclohexane), 2.54–2.55 (m, 4H, piperazine), 2.62–2.65 (m, 4H, piperazine), 3.46 (s, 2H, imide), 4.54 (s, 2H, $CH_2$), 7.24–7.37 (m, 10H, ArH); $C_{27}H_{33}O_2N_3$ (431.58).

### *N-[(4-Methyl-piperazin-1-yl)-methyl]-3,3-diphenylpyrrolidine-2,5-dione (**5e**)*

White powdery crystals. Yield: 75%; mp 103–105°C; TLC: $R_f = 0.67$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.25 (s, 3H, $CH_3$), 2.39 (brs, 4H, piperazine), 2.66 (brs, 4H, piperazine), 3.46 (s, 2H, imide), 4.55 (s, 2H, $CH_2$), 7.25–7.37 (m, 10H, ArH); $C_{22}H_{25}O_2N_3$ (363.46).

### *N-[(4-Benzylpiperidin-1-yl)-methyl]-3,3-diphenylpyrrolidine-2,5-dione (**5f**)*

White powdery crystals. Yield: 78%; mp 101–103°C; TLC: $R_f = 0.69$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.13–1.26 (m, 2H, piperidine), 1.28–1.37 (m, 1H, piperidine), 1.55–1.58 (m, 2H, piperidine), 2.00–2.17 (m, 2H, piperidine), 2.47 (d, 2H, piperidine, $J = 6.67$ Hz), 2.94 (d, 2H, $CH_2$, $J = 12.06$ Hz), 3.45 (s, 2H, imide), 4.52 (s, 2H, $CH_2$), 7.08–7.38 (m, 10H, ArH); $C_{29}H_{30}O_2N_2$ (438.57).

### *N-(Morpholin-1-yl)-methyl-3,3-diphenylpyrrolidine-2,5-dione (**5g**)*

White powdery crystals. Yield: 80%; mp 66 – 67°C; TLC: $R_f = 0.66$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 2.56 (t, 4H, morpholine, $J = 4.75$ Hz), 3.49 (s, 2H, imide), 3.63 (t, 4H morpholine, $J = 4.75$ Hz), 4.52 (2H, $CH_2$), 7.25–7.39 (m, 10H, ArH); $C_{21}H_{22}O_3N_2$ (350.41).

### *N-[(4-Benzyl-piperazin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione hydrochloride (**6a**)*

White powdery crystals. Yield: 70%; mp 208–210°C; TLC: $R_f = 0.85$ ($S_2$); $^1$H NMR (300 MHz, DMSO): $\delta$ 1.65 (s, 3H, $CH_3$), 2.77–3.05 (m, 8H, piperazine), 3.20–3.24 (m, 2H, imide), 4.26 (s, 2H, $CH_2$), 4.39 (s, 2H, $CH_2$), 7.24–7.29 (m, 1H, ArH), 7.35–7.45 (m, 7H, ArH), 7.60–7.63 (m, 2H, ArH), 11.38 (brs, 1H, HCl); $C_{23}H_{28}O_2N_3Cl$ (413.95).

### *N-[(4-Pyrimid-2-yl-piperazin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6b**)*

White powdery crystals. Yield: 82%; mp 99–101°C; TLC: $R_f = 0.39$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.72 (s, 3H, $CH_3$), 2.66 (t, 4H, piperazine, $J = 4.36$ Hz), 2.86 (d, 1H, imide, $J = 18.21$ Hz), 3.15 (d, 1H, imide, $J = 18.21$ Hz), 3.78 (t, 4H, piperazine, $J = 5.13$ Hz), 4.59 (s, 2H, $CH_2$), 6.48 (t, 1H, pyrimidine, $J = 4.75$ Hz), 7.24–7.36 (m, 5H, ArH), 8.29 (d, 2H, pyrimidine, $J = 4.62$ Hz); $C_{20}H_{23}O_2N_5$ (365.44).

### *N-{[4-(2-Hydroxyethyl)-piperazin-1-yl]-methyl}-3-methyl-3-phenylpyrrolidine-2,5-dione hydrochloride (**6c**)*

White powdery crystals. Yield: 78%; mp 159–161°C; TLC: $R_f = 0.83$ ($S_2$); $^1$H NMR (300 MHz, DMSO): $\delta$ 1.66 (s, 3H,

$CH_3$), 2.77–3.12 (m, 8H, 2H, imide, 2H, $CH_2$, 4H, piperazine), 3.25 (brs, 1H, OH), 3.42–3.80 (m, 6H, 2H, $CH_2$, 4H, piperazine), 4.39 (s, 2H, $CH_2$), 7.25–7.31 (m, 1H, ArH), 7.35–7.43 (m, 4H, ArH), 11.38 (brs, 1H, HCl); $C_{18}H_{26}O_3N_3Cl$ (367.88).

### *N-[(4-Cyclohexyl-piperazin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6d**)*

White powdery crystals. Yield: 81%; mp 101–103°C; TLC: $R_f = 0.53$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.14–1.24 (m, 5H, cyclohexane), 1.54–1.63 (m, 1H, cyclohexane), 1.72 (s, 3H, $CH_3$), 1.75–1.85 (m, 4H, cyclohexane), 2.20–2.21 (m, 1H, cyclohexane), 2.55–2.56 (m, 4H, piperazine), 2.65–2.66 (m, 4H, piperazine), 2.86 (d, 1H, imide, $J = 17.95$ Hz), 3.13 (d, 1H imide, $J = 18.21$ Hz), 4.53 (s, 2H, $CH_2$), 7.24–7.38 (m, 5H, ArH); $C_{22}H_{31}O_2N_3$ (369.51).

### *N-[(4-Methyl-piperazin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione dihydrochloride (**6e**)*

White powdery crystals. Yield: 86%; mp 189–191°C; TLC: $R_f = 0.84$ ($S_2$); $^1$H NMR (300 MHz, DMSO): $\delta$ 1.65 (s, 3H $CH_3$), 2.67–2.79 (m, 5H, 3H, $CH_3$, 2H, piperazine), 2.92–3.00 (m, 5H, 4H, piperazine, 1H, imide), 3.29–3.32 (m, 3H, 2H piperazine, 1H, imide), 4.40 (s, 2H, $CH_2$), 7.25–7.32 (m, 1H, ArH), 7.35–7.41 (m, 4H, ArH), 11.04 (brs, 1H, HCl), 11.38 (brs, 1H, HCl); $C_{17}H_{24}O_2N_3Cl$ (337.85).

### *N-[(4-Benzylpiperidin-1-yl)-methyl]-3-methyl-3-phenylpyrrolidine-2,5-dione (**6f**)*

White powdery crystals. Yield: 67%; mp 77–78°C; TLC: $R_f = 0.57$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.17–1.29 (m, 2H, piperidine), 1.34–1.41 (m, 1H, piperidine), 1.58–1.62 (m, 2H, piperidine), 1.72 (s, 3H, $CH_3$), 2.04–2.14 (m, 2H, piperidine), 2.49 (d, 2H, piperidine, $J = 6.93$ Hz), 2.85 (d, 1H, imide, $J = 18.21$ Hz), 2.95–2.99 (m, 2H, $CH_2$), 3.14 (d, 1H, imide, $J = 18.21$ Hz), 4.51 (s, 2H, $CH_2$), 7.09–7.38 (m, 10H, ArH); $C_{24}H_{28}O_2N_2$ (376.50).

### *N-(Morpholin-1-yl)-methyl-3-methyl-3-phenylpyrrolidine-2,5-dione (**6g**)*

White powdery crystals. Yield: 82%; mp 63–64°C; TLC: $R_f = 0.55$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.75 (s, 3H, $CH_3$), 2.60 (t, 4H, morpholine, $J = 4.62$ Hz), 2.89 (d, 1H, imide, $J = 18.21$ Hz), 3.17 (d, 1H, imide, $J = 18.21$ Hz), 3.65 (t, 4H, morpholine, $J = 4.62$ Hz), 4.51 (2H, $CH_2$), 7.25–7.40 (m, 10H, ArH); $C_{16}H_{20}O_3N_2$ (288.34).

### *N-[(4-Benzyl-piperazin-1-yl)-methyl]-3-ethyl-3-methylpyrrolidine-2,5-dione hydrochloride (**7a**)*

White powdery crystals. Yield: 62%; mp 69–70°C; TLC: $R_f = 0.90$ ($S_2$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.80 (t, 3H, $CH_3$, $J = 7.44$ Hz), 1.20 (s, 3H, $CH_3$), 1.46–1.63 (m, 2H, $CH_2$), 2.42–2.49 (m, 3H, 2H, piperazine, 1H, imide), 2.49–2.73 (m, 3H, 2H piperazine, 1H imide), 2.90 (d, 4H piperazine, $J = 12.05$ Hz), 3.20 (d, 2H, $CH_2$ $J = 11.03$ Hz), 4.28 (s, 2H, $CH_2$), 7.41–7.45 (m, 3H, ArH), 7.58–7.60 (m, 2H, ArH), 10.99 (brs, 1H, HCl); $C_{19}H_{27}O_2N_3$ (329.45).

### *N-[(4-Pyrimid-2-yl-piperazin-1-yl)-methyl]-3-ethyl-3-methylpyrrolidine-2,5-dione (**7b**)*

White powdery crystals. Yield: 88%; mp 69–71°C; TLC: $R_f = 0.034$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.87 (t, 2H, $CH_2$, $J = 7.44$ Hz), 1.29 (s, 3H, $CH_3$), 1.60–1.78 (m, 2H, $CH_2$), 2.42 (d, 1H, imide, $J = 18.21$ Hz), 2.60–2.68 (m, 5H, 4H, piperazine, 1H, imide), 3.79 (t, 4H, piperazine, $J = 4.87$ Hz), 4.51 (s, 2H, $CH_2$), 6.47 (t, 1H, pyrimidine, $J = 4.74$ Hz), 8.28 (d, 2H, pyrimidine, $J = 4.87$ Hz); $C_{16}H_{23}O_2N_5$ (317.39).

### *N-{[4-(2-Hydroxyethyl)-piperazin-1-yl]-methyl}-3-ethyl-3-methylpyrrolidine-2,5-dione dihydrochloride (**7c**)*

White powdery crystals. Yield: 68%; mp 140–142°C; TLC: $R_f = 0.93$ ($S_2$), $^1$H NMR (300 MHz, DMSO): $\delta$ 0.79 (t, 3H, $CH_3$, $J = 7.44$ Hz), 1.22 (s, 3H, $CH_3$), 1.47–1.65 (m, 2H $CH_2$), 2.50 (d, 1H imide, $J = 18.21$ Hz), 2.68 (d, 1H, imide, $J = 18.21$ Hz), 2.80 (d, 2H, piperazine, $J = 11.80$ Hz), 2.93–3.03 (m, 4H, piperazine), 3.11 (t, 2H, $CH_2$, $J = 5.00$ Hz), 3.23 (t, 1H, OH, $J = 5.00$ Hz), 3.44 (d, 2H, piperazine, $J = 11.80$ Hz), 3.73 (t, 2H, $CH_2$, $J = 5.13$ Hz), 4.34 (s, 2H $CH_2$), 10.58 (brs, 1H, HCl); $C_{14}H_{26}O_3N_3Cl$ (319.83).

### *N-[(4-Cyclohexyl-piperazin-1-yl)-methyl]-3-ethyl-3-methylpyrrolidine-2,5-dione (**7d**)*

White powdery crystals. Yield: 81%; mp 77–79°C; TLC: $R_f = 0.52$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.88 (t, 3H, CH3, $J = 7.44$ Hz) 1.04–1.23 (m, 5H, cyclohexane), 1.30 (s, 3H, $CH_3$), 1.57–1.84 (m, 7H, 5H, cyclohexane, 2H, $CH_2$), 2.17–2.21 (m, 1H, cyclohexane), 2.41 (d, 1H, imide, $J = 18.21$ Hz), 2.54–2.55 (m, 4H, piperazine), 2.58–2.64 (m, 5H, 4H, piperazine, 1H, imide), 4.45 (s, 2H, $CH_2$); $C_{18}H_{31}O_2N_3$ (321.46).

### *N-[(4-Methyl-piperazin-1-yl)-methyl]-3-ethyl-3-methylpyrrolidine-2,5-dione dihydrochloride (**7e**)*

White powdery crystals. Yield: 86%; mp 178–181°C; TLC: $R_f = 0.92$ ($S_2$); $^1$H NMR (300 MHz, DMSO): $\delta$ 0.75–0.84 (m, 3H, $CH_3$), 1.14 (s, 3H, $CH_3$), 1.21 (s, 3H, $CH_3$), 1.41–1.64 (m, 2H, $CH_2$), 2.39 (d, 1H, imide, $J = 18.21$ Hz), 2.54 (d, 1H, imide, $J = 18.21$ Hz), 2.65–2.92 (m, 8H, piperazine), 4.30 (s, 2H, $CH_2$), 11.07 (brs, 2H, HCl); $C_{13}H_{25}O_2N_3Cl_2$ (326.27).

### *N-[(4-Benzylpiperidin-1-yl)-methyl]-3-ethyl-3-methylpyrrolidine-2,5-dione (**7f**)*

White powdery crystals. Yield: 67%; mp 92–94°C; TLC: $R_f = 0.70$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.88 (t, 3H, $CH_3$, $J = 7.57$ Hz), 1.29 (s, 1H, $CH_3$), 1.32–1.37 (m, 2H, piperidine),

1.39–1.43 (m, 1H, piperidine), 1.53–1.79 (m, 4H, 2H, $CH_2$, 2H, piperidine), 2.07–2.15 (m, 2H, piperidine), 2.42 (d, 1H, imide, $J = 18.21$ Hz), 2.50 (d, 2H, piperidine, $J = 6.67$ Hz), 2.63 (d, 1H, imide, $J = 18.21$ Hz), 2.99 (d, 2H, $CH_2$, $J = 10.77$ Hz), 4.44 (s, 2H, $CH_2$), 7.09–7.38 (m, 5H, ArH); $C_{20}H_{28}O_2N_2$ (328.46).

### *N-(Morpholin-1-yl)-methyl-3-ethyl-3-methylpyrrolidine-2,5-dione (**7g**)*

White powdery crystals. Yield: 82%; mp 48–50°C; TLC: $R_f = 0.53$ ($S_1$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.91 (t, 3H, $CH_3$, $J = 7.44$ Hz), 1.32 (s, 3H, $CH_3$), 1.55–1.79 (m, 2H, $CH_2$), 2.45 (d, 1H, imide, $J = 18.47$ Hz), 2.57 (t, 4H, morpholine, $J = 4.75$ Hz), 2.66 (d, 1H, imide, $J = 18.47$ Hz), 3.65 (t, 4H, morpholine, $J = 4.75$ Hz), 4.43 (2H, $CH_2$); $C_{12}H_{20}O_3N_2$ (240.30).

## Pharmacology

Compounds **3–24** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program in the Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere [25, 26].

Phase I studies of the investigated compounds involved three tests: MES, *sc*PTZ and rotorod test for NT. Male albino mice (CF#1 strain, weighing 18–25 g) and male albino rats (Sprague–Dawley) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The compounds were injected intraperitoneally into mice as a suspension in 0.5% methylcellulose/water mixture at dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h intervals after administration. Promising derivatives from phase I underwent phase VIa in which they were administered orally to rats at a fixed dose of 30 mg/kg for both MES and the rotorod toxicity tests or 50 mg/kg in the *sc*PTZ screen. Groups of eight mice or four rats were employed.

### *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) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

### *Subcutaneous pentylenetetrazole seizure test (scPTZ)*

The *sc*PTZ test utilizes a dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) that produces clonic seizures lasting for a period of at least 5 s in 97% ($CD_{97}$) of the animals tested. At the anticipated time of testing the pentylenetetrazole is administrated subcutaneously.

### *Neurological toxicity (NT)*

The neurological toxicity induced by a compound was detected in mice using the standardized rotorod test [27]. 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 the animal to maintain equilibrium for 1 min in each of three successive trials.

### *Quantification studies*

Quantitative determination of the median effective dose ($ED_{50}$) and the median neurotoxic dose ($TD_{50}$) values was performed at previously estimated TPE after i.p. administration into mice and p.o. administration into rats. Groups of eight rats received various doses of the compound until at least three points were established in the range of 10–90% seizure protection and 0% 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.

### *The 6-Hz model*

The 6-Hz model 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 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 [28].

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