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Design, synthesis and anticonvulsant properties of new *N*-Mannich bases derived from 3-phenylpyrrolidine-2,5-diones

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ABSTRACT

The synthesis and anticonvulsant properties of new *N*-Mannich bases of 3-phenyl- (**9a–d**), 3-(2-chlorophenyl)- (**10a–d**), 3-(3-chlorophenyl)- (**11a–d**) and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones (**12a–d**) were described. The key synthetic strategies involve the formation of 3-substituted pyrrolidine-2,5-diones (**5–8**), and then aminoalkylation reaction (Mannich-type) with formaldehyde and corresponding secondary amines, which let to obtain the final compounds **9a–d**, **10a–d**, **11a–d** and **12a–d** in good yields. Initial anticonvulsant screening was performed in mice (ip) using the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizures tests. The most effective compounds in mice were tested after oral administration in rats. The acute neurological toxicity was determined applying the minimal motor impairment rotarod test. The in vivo results revealed that numerous compounds were effective especially in the MES test (model of human tonic-clonic seizures). The most active in the MES seizures in rats was 1-[(4-benzyl-1-piperidyl)methyl]-3-(2-chlorophenyl)pyrrolidine-2,5-dione (**10c**) which showed ED_{50} value of 37.64 mg/kg. It should be stressed that this molecule along with **9a**, **9d** and **10d** showed protection in the psychomotor seizure test (6-Hz), which is known as an animal model of therapy-resistant epilepsy. Furthermore compounds **9a**, **9d** and **10d** were also tested in the pilocarpine-induced status prevention (PISP) test to assess their potential effectiveness in *status epilepticus*. For the most promising molecule **9d** an influence on human CYP3A4 isoform of P-450 cytochrome was studied in vitro.

1. Introduction

Epilepsy is the most prevalent neurological disorder, affecting approximately 50 million people worldwide.[1,2] It is characterized by recurrent seizures of cerebral origin with episodes of sensory, motor or autonomic phenomena, which proceed with or without loss of consciousness. Even though significant advances have been made in epilepsy research, convulsions in almost one third of patients are inadequately controlled by standard drug therapy.[3] Furthermore, compliance is often limited by adverse side effects most notably related to central nervous system exposure like diminished attention, executive function, intelligence, language skills, memory and processing speed.[4] In recent times several new drugs, for example, levetiracetam, felbamate, lamotrigine, gabapentin, and topiramate, have been approved to treat epilepsy. Although these drugs have been shown to be effective in epileptic syndromes in a number of patients, their efficacy does not appear to be superior to that of the established antiepileptic drugs. Therefore the ideal antiepileptic should prevent different types of seizures without producing side effects that affect adversely patients' quality of life. Additionally such substance should possess antiepileptogenic properties. The currently used antiepileptic drugs (AEDs) can be classified into four categories on the basis of the main molecular mechanisms of action, as follow: modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, enhancement of GABA-mediated inhibition or other effect on the GABA system, inhibition of synaptic excitation mediated by ionotropic glutamate receptors and modulation of synaptic release.[5]

The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority AEDs make it difficult to use rational methodologies of drug discovery, which are based on three-dimensional structure of biological target. Thus the most useful for the design of new anticonvulsants is ligand-based approach that relies on the use of different pharmacophores established through the analysis of structural characteristics of clinically effective AEDs as well as other anticonvulsant active molecules.[6] 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 important core fragment is defined by nitrogen heteroatomic system, usually imide or lactam, at least of one carbonyl group and phenyl or alkyl substituents attached to the heterocyclic system.[7,8] This template is noticeable in the structures of the first generation AEDs such

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as ethosuximide and phenytoin which act as T-type calcium channel or sodium channel blockers, respectively, as well as between the newest drugs, for example, levetiracetam, brivaracetam or seletracetam with novel mechanism of action that relies on the binding to a synaptic vesicle glycoprotein SV2A, and also inhibition of presynaptic calcium channels (Fig. 1).

The previous studies from our laboratory demonstrated diversified anticonvulsant activities among variously substituted pyrrolidine-2,5-diones.[9–12] Nevertheless, the most promising were *N*-Mannich bases with an aromatic area at position-3 and the phenylpiperazines, especially those with electron-withdrawing chlorine and fluorine atoms or the trifluoromethyl group at position-1 of pyrrolidine-2,5-dione (Fig. 2).[13–16]

Following these findings, in aim to search for new, effective anticonvulsants as well as to continue systematic structure–activity relationship studies among differently substituted succinimides, in the current work we have synthesized the focused library of new *N*-Mannich bases derived from 3-phenyl-, 3-(2-chlorophenyl)-, 3-(3-chlorophenyl)- and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones. In relation to compounds described earlier,[13–16] the main modification relied on the different N-containing heterocyclic ring functions, namely phenylpiperazines moieties have been changed into 1-(2-pyrimidyl)piperazine, 1-cyclohexylpiperazine, 1-benzylpiperidine or morpholine.

2. Results and discussion

2.1. Chemistry

The series of *N*-Mannich bases of 3-phenyl- (**9a–d**), 3-(2-chlorophenyl)- (**10a–d**), 3-(3-chlorophenyl)- (**11a–d**) and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones (**12a–d**) was synthesized according to Scheme 1.

The starting 2-phenyl- (**1**), 2-(2-chlorophenyl)- (**2**), 2-(3-chlorophenyl)- (**3**) and 2-(4-chlorophenyl)- (**4**) succinic acids were prepared according to the method described by Miller and Long.[17] The cyclocondensation reaction of dicarboxylic acids **1–4** with 25% ammonia yielded in 3-phenyl- (**5**), 3-(2-chlorophenyl)- (**6**), 3-(3-chlorophenyl)- (**7**) and 3-(4-chlorophenyl) (**8**) pyrrolidine-2,5-diones. The physicochemical and spectral data of intermediates **5–8** have been described in a separate paper.[18] Final compounds **9a–d**, **10a–d**, **11a–d** and **12a–d** were obtained in the Mannich-type reaction from equimolar amounts of the imides **5–8**, formaldehyde and corresponding secondary amines. The reaction was carried out in ethanol at room temperature for ca. 12 h. The crude products were crystallized from 96% ethanol. The final compounds were obtained in good yields (58–82%). All compounds were prepared as racemic mixtures. Their purity and homogeneity were assessed by TLC and gradient HPLC chromatography. The structures of compounds synthesized were confirmed by both spectral (^{1}H NMR, ^{13}C NMR, LC–MS) and elemental (C, H, N) analyses. The detailed physical and analytical data are listed in the Section 4.

2.2. Biological studies

The initial anticonvulsant evaluation was performed within the Antiepileptic Drug Development (ADD) Program in the Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA. The development of new chemical agents for the treatment of epilepsy is based mainly on the use of predictable animal models. Such models fall into two main categories, namely models of acute seizures (non-epileptic animals induced to have a seizure by an electrical or chemical stimulus) and models of chronic epilepsy (animals induced to have enhanced seizure susceptibility or spontaneous seizures). At the present time there are three in vivo screens used routinely that include the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole (*sc*PTZ) and the kindling model. From these tests, the MES and *sc*PTZ screens are still recognized as the 'gold standard' in the early stages of testing of new anticonvulsants. Furthermore, the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests are claimed to detect compounds affording protection against human generalized tonic–clonic seizures and generalized absence seizures, respectively.[19]

Bearing in mind the above, the anticonvulsant activity of compounds **9a–d**, **10a–d**, **11a–d** and **12a–d** was studied in the MES and *sc*PTZ tests after intraperitoneal injection in mice at doses of 30, 100 or 300 mg/kg. The observation was carried out at two different time intervals, namely 0.5 and 4 h. The acute neurological toxicity (NT) was determined in the minimal motor impairment rotorod screen. The results are shown in Table 1.

Except for compounds **9a**, **9b** and **12a**, which were devoid of activity, all other compounds showed protection in the MES screen (**9c**, **10a**, **10b**, **11a–d**, **12b–d**) or were effective in both tests applied—**9d**, **10c** and **10d**. Compounds that revealed protection in the MES test, indicating the ability of a substance to prevent seizure spread, at a dose of 100 mg/kg included **9c**, **9d**, **10a–d**, **11a**, **11c**, **11d** and **12b–d**, whereas **11b** provided anti-MES protection at a dose of 300 mg/kg. In this series, **9d** was active only at 0.5 h after ip administration whereas **10a**, **10b**, **11a**, **12b** and **12c** showed protection only at time point 4 h. Nevertheless, majority of the compounds, namely **9c**, **10c**, **10d**, **11b–d** and **12d** showed activity in both time intervals, indicating quick onset and long duration of the anticonvulsant activity.

Apart from the anti-MES protection, compounds **9d**, **10c** and **10d** were found to inhibit seizures in the *sc*PTZ test, which identifies substances elevating the seizure threshold. Among these molecules, **10c** and **10d** showed activity at the dose of 100 mg/kg at time points 4 and 0.5 h, respectively. It should be stressed here that compound **10d** revealed equivalent protection to ethosuximide, the model antiepileptic drug effective in the *sc*PTZ test, and furthermore was active in the MES test at dose of 100 mg/kg in both time intervals. Thus **10d** joins the biological properties of phenytoin and ethosuximide. Compound **9d** was less active and showed protection at a dose of 300 mg/kg.

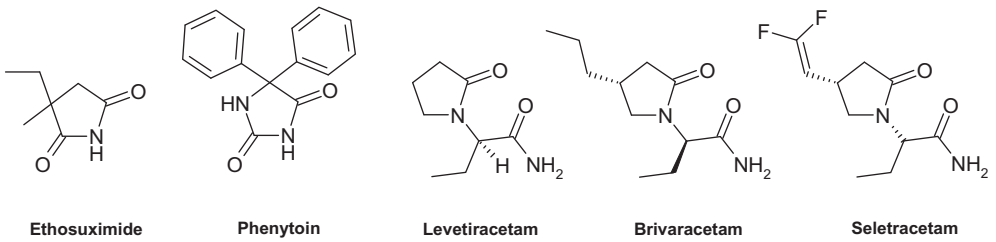


Figure 1. Structures of known AEDs containing nitrogen heterocyclic ring.

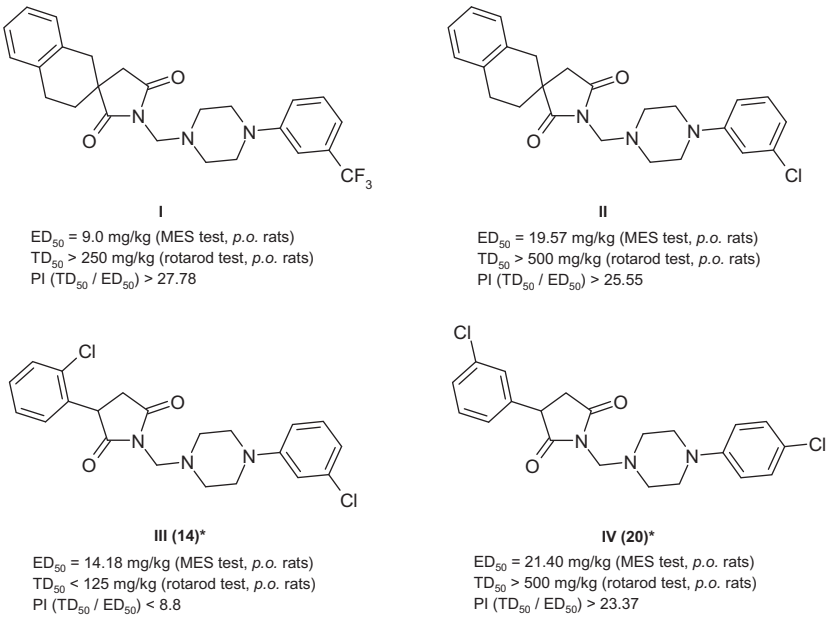
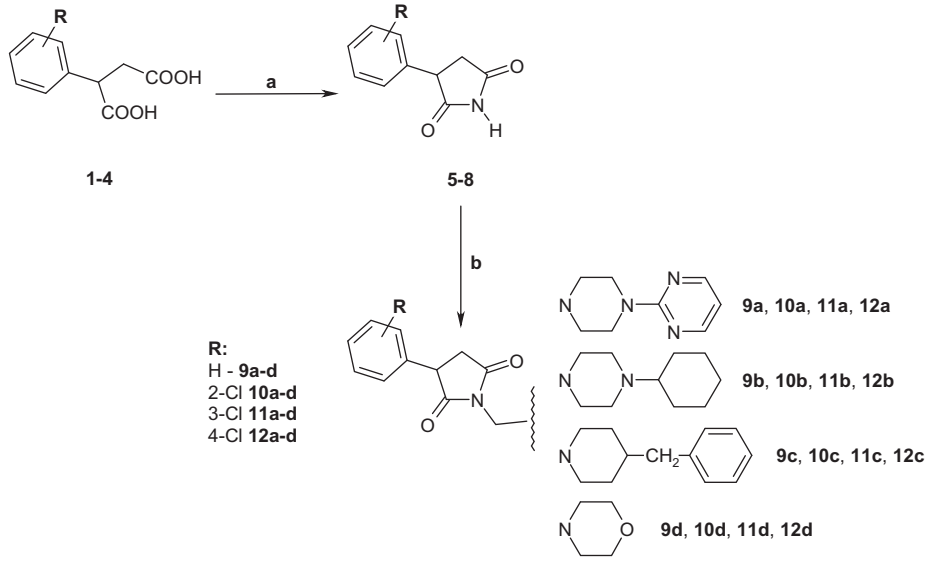


Figure 2. Model compounds **I**–**IV** obtained in the previous studies.[14–16] (*The numbers of compounds **III** and **IV** in Table 1).



Scheme 1. Synthesis of intermediates **5**–**8** (Ref. 18), and final compounds **9a**–**d**, **10a**–**d**, **11a**–**d** and **12a**–**d**. Reagents and conditions: (a) 25% NH_4OH, 180 °C, 1 h (b) 1-(2-pyrimidyl)piperazine, 1-cyclohexylpiperazine, 1-benzylpiperidine or morpholine, formaldehyde, 96% ethyl alcohol, room temperature ca. 12 h.

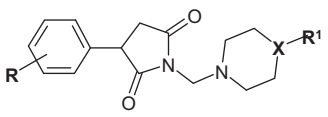
In the rotorod test (NT) for acute neurological toxicity, compounds **9d**, **10a**, **10c**, **10d**, **11d** and **12a** showed motor impairment in the maximum dose administered (300 mg/kg). The rest of molecules were devoid of neurotoxic properties. It should be emphasized that eight molecules, namely **9c**, **10b**, **11a**–**c** and **12b**–**d** emerged as anticonvulsants without neurotoxic activity.

A valuable property of the candidate anticonvulsant is its ability to inhibit convulsions when it is given by the oral route. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity or neurotoxicity. On the basis of the ip screening data in mice, and according to Anticonvulsant Screening Project (ASP) disposition, eight compounds **9a**, **9d**, **10a**, **10c**, **10d**, **11a**, **11c** and **11d** were selected and examined in the MES test as well as rotorod screen after *p.o.* administration in rats at a dose of 30 mg/kg (Table 2).

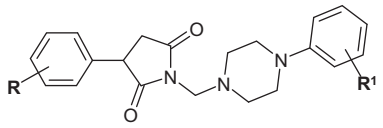
As shown in Table 2 the most active was compound **10c**, which protected 75% of animals at 0.5 and 1 h. This compound was also active in twenty-five percent of animals at time points 0.25 and 2 h. The other molecules were less active and protected 50% of animals at 0.25 and 2 h (**11c**) or at 0.5, 1, 2 and 4 h (**11d**). 25% protection was observed for compounds **11c** at 1 h and **10d** at 0.5 and 1 h. All derivatives tested did not cause motor impairment when they were given orally. The in vivo data in rats confirmed that compounds tested are absorbed from gastrointestinal tract and are also capable to penetrate to central nervous system.

On the basis of the preliminary data in rats, compound **10c** was chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}) after *p.o.* application. The quantitative evaluation of the MES median effective dose (ED_{50}) and toxic dose (TD_{50}) were performed at previously estimated TPE. Results of the quantitative

Table 1
Anticonvulsant and neurotoxicity screening after ip administration in mice



9a-d, **10a-d**, **11a-d**, **12a-d**



13-27

No.	R	X	R^1	Intraperitoneal administration in mice[a]					
				MES[b]		*sc*PTZ[c]		NT[d]	
				0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
9a	H	N	2-Pyrimidinyl	—	—	—	—	—	—
9b	H	N	Cyclohexyl	—	—	—	—	—	—
9c	H	C	Benzyl	100	300	—	—	—	—
9d	H	O	—	100	—	300	300	300	—
10a	2-Cl	N	2-Pyrimidinyl	—	100	—	—	—	300[41]
10b	2-Cl	N	Cyclohexyl	—	100	—	—	—	—
10c	2-Cl	C	Benzyl	300	100	—	100[25]	300	—
10d	2-Cl	O	—	100	100	100	300	300[19]	300
11a	3-Cl	N	2-Pyrimidinyl	—	100	—	—	—	—
11b	3-Cl	N	Cyclohexyl	300	300	—	—	—	—
11c	3-Cl	C	Benzyl	300	100	—	—	—	—
11d	3-Cl	O	-	100	100	—	—	300	—
12a	4-Cl	N	2-Pyrimidinyl	—	—	—	—	300	—
12b	4-Cl	N	Cyclohexyl	—	100	—	—	—	—
12c	4-Cl	C	Benzyl	—	100	—	—	—	—
12d	4-Cl	O	—	100	300	—	—	—	—
13[g]	2-Cl	—	2-Cl	—	—	—	—	—	—
14[h,i]	2-Cl	—	3-Cl	—	100	—	—	100	300
15[g]	2-Cl	—	4-Cl	—	300	—	—	—	—
16[g]	2-Cl	—	3-CF_3	—	100	—	—	—	—
17[g]	3-Cl	—	2-F	—	100	—	—	300	—
18[g]	3-Cl	—	2-Cl	—	—	—	—	—	—
19[g]	3-Cl	—	3-Cl	—	—	—	—	—	—
20[gi]	3-Cl	—	4-Cl	300	100	—	—	—	—
21[g]	3-Cl	—	3-CF_3	100	100	—	—	300	300
22[g]	3-Cl	—	3-CH_3	—	30	—	—	100	-
23[g]	3-Cl	—	2-OCH_3	—	—	—	—	300	300
24[g]	4-Cl	—	2-Cl	—	—	—	—	300	300
25[g]	4-Cl	—	3-Cl	—	100	—	—	300	300
26[g]	4-Cl	—	4-Cl	—	—	—	—	—	—
27[g]	4-Cl	—	3-CF_3	—	100	—	—	300	—
Phenytoin[e]				30	30	—	—	100	100
Ethosuximide[f]				—	—	100	300	—	—

Response comments: [19]sedated, [25]myoclonic jerks, [41]diarrhea.

[a] Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby bioactivity was demonstrated in half or more animals. The animals were examined at 0.5 and 4.0 h. A dash indicates the absence of anticonvulsant activity or neurotoxicity at the maximum dose administered (300 mg/kg).

[b] Maximal electroshock test.

[c] Subcutaneous pentylenetetrazole test.

[d] Neurotoxicity screening using rotorod test.

[e] Phenytoin, reference antiepileptic drug tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 30.

[f] Ethosuximide, reference antiepileptic drug tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 30.

[g] Compounds **13**, **15**–**27** data from Ref. 15.

[h] Compound **14**, data from Ref. 16.

[i] Compounds **14** and **20** are shown in Figure 2 as **III** and **IV**, respectively.

tests along with the data for the standard AEDs—valproic acid, ethosuximide and phenytoin, are shown in Table 3.

The quantitative data showed that **10c** had comparable ED_{50} with phenytoin (MES test) and ethosuximide (*sc*PTZ test), and furthermore was distinctly more potent than valproic acid in both screens. These resulted also in more favorable protection indexes in comparison with model anticonvulsants.

The previous studies among differently substituted pyrrolidine-2,5-diones revealed that the most plausible mechanism of action for these type of molecules is connected with the inhibition of sodium channel current.[14]

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. 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. One example supporting this hypothesis is provided by levetiracetam, which has demonstrated efficacy in refractory human partial epilepsies. It was found to be inactive against MES and *sc*PTZ seizures even at high doses, whereas showed high effectiveness in the 6-Hz psychomotor seizure model of partial epilepsy.[20] Thus it is suggested that the 6-Hz model might be capable for identifying anti-seizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action.

Taking into consideration the above, and in accordance with ASP dispositions, six compounds **9a**, **9c**, **9d**, **10c**, **10d** and **12c** were

Table 2
Test results in rats after oral administration at a dose of 30 mg/kg

No.	MES[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
9a	0	0	0	0	0
9d	0	0	0	0	0
10a	0	0	0	0	0
10c	1	3	3	1	0
10d	0	1	1	0	0
11a	0	0	0	0	0
11c	2	0	1	2	0
11d	0	2	2	2	2
Phenytoin[b]	1	4	3	3	3

The data indicate the number of rats out of four that were protected.
[a] Maximal electroshock test.
[b] Phenytoin, reference antiepileptic drug, tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 31.

selected for the evaluation of anticonvulsant activity in the 6-Hz test in mice (ip). The results obtained are presented in Table 4.

In the 6-Hz model the most potent was compound **10d** which protected 100% of mice in all time intervals at dose of 100 mg/kg. This molecule showed also 100% protection (0.25 h) and 75% (0.5, 1, 2 h) at dose of 50 mg/kg. The other molecules **9a**, **9d**, **10c** and **12c** showed one peak of 75% protection in different time points. It is worthy of note that **9a** was inactive in mice MES and *sc*PTZ tests, however showed activity in the psychomotor seizures. Finally, the weakest protection—25% (at 1 and 2 h) was observed for compound **9c**.

On the basis of preliminary screening four molecules **9a**, **9d**, **10c** and **10d** were selected for quantification of ip ED_{50} values in the psychomotor seizure test (6-Hz) in mice. At the same time the TD_{50} values were determined in the rotorod test (Table 5).

As it is shown in Table 5 compounds **9a**, **9d**, **10c** and **10d** showed on the one hand lower activity and on the other hand higher neurotoxicity in comparison with the results observed for levetiracetam (a model anticonvulsant active in the 6-Hz seizures).

In continuation of the biological studies, compounds **9a**, **9d** and **10d** were additionally assessed for potential activity against nerve agents using the pilocarpine-induced status prevention (PISP) test. The results are presented in Table 6.

The PISP 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 status epilepticus (SE).

In the PISP test two compounds **9a** and **9d** were found to be active and protected, respectively 5 and 4 rats at time-zero using the dose of 200 mg/kg (ip route of administration). Compound **9d** showed also protection in a sustained seizure model (time 0.5 h) at a dose of 400 mg/kg. For **9d** which revealed activity in more than 50% of animals the ED_{50} value was determined at time point 0.5 h and the data are shown in Table 7.

Table 4
Psychomotor seizure test in mice (6-Hz, current 32 mA)

No.	Dose (mg/kg)	Intraperitoneal injection[a]				
		0.25 h	0.5 h	1 h	2 h	4 h
9a	100	3	2	2	2	0
9c	100	0	0	1	1	0
9d	100	3	0	1	0	0
10c	75	0	3	0	1	0
10d	50	4	3	3	3	0
10d	100	4	4	4	4	4
12c	100	0	0	0	3	1

[a] The data indicate the number of mice out of four that were protected.

Many drug–drug interactions are metabolism based and mediated primarily via the microsomal cytochrome P450 (CYP) family of enzymes. Ten CYP isoforms are expressed in a typical human liver, and six of them (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) appear to be the most commonly responsible for the metabolism of most drugs and the associated drug–drug interactions. The inhibition of these enzymes may decrease the metabolic clearance of a co-administered drug resulting in elevated blood concentration, which may cause adverse drug effects or toxicity. As detailed in the FDA's Draft Guidance document for Drug–Drug Interactions,[21] the FDA has placed emphasis on evaluating the inhibition potential of a new chemical entity (NCE) at an earlier stage in drug development in order to avoid developing compounds with the potential to yield adverse drug interactions.

Taking into consideration the above, according to the ASP disposition, the most promising 1-(morpholinomethyl)-3-phenyl-pyrrolidine-2,5-dione (**9d**) (active in the MES, *sc*PTZ, 6-Hz and PISP test), was tested in the human cytochrome P450 inhibition studies. The studies were carried out for CYP3A4 subtype of the cytochrome P450, and the outcome was the inhibition of metabolite formation, namely 6-β-OH-testosterone from the probe substrate—testosterone. The results are shown in Table 8.

The data revealed that compound **9d** showed only slight inhibition of CYP3A4 activity in vitro and is unlikely to show inhibitory interactions with other CYP3A4 metabolized drugs in vivo.

2.3. Structure activity relationships

The results of the preliminary screening in mice (see Table 1), enabled to draw some general conclusions about the relationships between structure and anticonvulsant properties. Taking into consideration the substitution of the 3-phenyl ring, the highest

Table 3
Quantification data in rats after p.o. administration

No.	TPE[a] (h)	ED_{50} MES[b] (mg/kg)	ED_{50} *sc*PTZ[b] (mg/kg)	TD_{50} NT[b] (mg/kg)	PI[c] (TD_{50}/ED_{50})
10c	1	37.64 (22.86–58.51)	106.83 (71.14–164.65)	>500	>13.28 (MES) >4.68 (*sc*PTZ)
Valproic acid[d]	1	485 (324–677)	646 (466–869)	784 (503–1176)	1.6 (MES) 1.2 (*sc*PTZ)
Ethosuximide[d]	1	>500	167 (116–237)	>500	>2.99 (*sc*PTZ)
Phenytoin[d]	1	28.10 (20.7–35.2)	>500	>100	>3.6 (MES)

[a] Time to peak effect.
[b] Results are presented as mean ± SEM at 95% confidence limit (MES-maximal electroshock test; *sc*PTZ—subcutaneous pentylenetetrazole test; NT-neurotoxicity, rotarod test).
[c] Protection index (TD_{50}/ED_{50}).
[d] Reference antiepileptic drugs tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 19.

Table 5
Quantification data—psychomotor seizure tests (6-Hz, current 32 mA) after ip injection into mice

No.	TPE[a] (h)	ED_{50} 6 Hz[b] (mg/kg)	TD_{50} NT[b] (mg/kg)	PI[c] (TD_{50}/ED_{50})
9a	1	103.05 (79.0–147.25)	nd	—
9d	0.25	77.20 (66.3–81.6)	292.90 (273.3–336)	3.79
10c	0.5	84.59 (54.96–148.65)	275.33 (226.98–325.02)	3.26
10d	0.25	31.06 (27.46–36.48)	nd	—
10d (Current 44 mA)	0.5	55.63 (51.33–66.74)	nd	—
Levetiracetam[d]	1	19.40 (9.90–36.0)	>500	>25.77

Nd—no data.
[a] Time to peak effect.
[b] Results are presented as mean ± SEM at 95% confidence limit (6 Hz- psychomotor seizure tests; NT-neurotoxicity, rotarod test).
[c] Protection index (TD_{50}/ED_{50}).
[d] Levetiracetam, reference antiepileptic drug tested by use of ADD Program procedures in NIH/NINDS, data from Ref. 19.

Table 6
Pilocarpine induced status prevention (PISP) model results

No.	Dose (mg/kg)	Time[a] (h)	Protected rats	Non-protected rats	Average weight change ± SEM[b] (g)	
					Protected rats	Non-protected rats
9a	200	0	5	8	11.7 ± 4.1	—
9d	200	0	4	6	10.0 ± 2.5	10.0 ± 0.0
9d	400	0.5	5	7	23.0 ± 1.6	25.0 ± 2.5
10d	100	0.5	0	8	—	20.6 ± 1.5

[a] Post first stage III seizure.
[b] Weight change 24 h Post first stage III seizure.

Table 7
Pilocarpine induced status prevention (PISP) model, quantification data for compound **9d**

No.	Time (h)	ED_{50} PISP[a] (mg/kg)
9d	0.5	342.2 (265.4–436.6)

[a] Result is presented as mean ± SEM at 95% confidence limit.

Table 8
Human cytochrome P450 inhibition studies for compound **9d**

Compound **9d** concentration (μM)	[S] = 200 μM	[S] = 50 μM
0	4,225.1[a] (0)	1,839.7 (0)
500	3,417.8 (19)	1,532.6 (17)

[a] Activities are reported in protein/min. In parentheses below each activity is (%) inhibition relative to the activity determined at the same substrate concentration [S] in the absence of **9d** (0 μM).

activity in the maximal electroshock (MES) test was observed for 2-chlorophenyl- (**10a–d**) and 3-chlorophenyl (**11a–d**) derivatives whereas weaker protection revealed 4-chlorophenyl- analogs (**12a–d**) and unsubstituted molecules (**9a–d**). Moreover, the 3-chlorophenyl- (**11a–d**) and 4-chlorophenyl-pyrrolidine-2,5-diones (**12a–d**) were active only in the MES screen, whereas several compounds representing morpholine (**9d**, **10d**) or 4-benzyl-1-piperidine (**10c**) derivatives from the remaining series were also effective in the pentylenetetrazole seizures (*sc*PTZ). These molecules showed additionally potent activity in the psychomotor (6-Hz) seizures.

The weaker protection was observed for 2-pyrimidinyl (**9a–12a**) and cyclohexyl (**9b–12b**) derivatives that showed protection at the most in two in vivo screens, namely MES, 6-Hz or PISP. It should be noticed, as described previously,[15] the 4-phenylpiperazine derivatives of 3-(2-chlorophenyl)-, 3-(3-chlorophenyl)- and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones were active exclusively in the maximal electroshock test (the data for selected compounds **13–27** from Refs. 15,16 are shown in Table 1).

3. Conclusion

In the current studies the focused library of sixteen new *N*-Mannich bases of 3-phenyl-pyrrolidine-2,5-diones were synthesized and assessed for their anticonvulsant activity in several animal models of epilepsy. The results revealed that majority of compounds showed protection in the maximal electroshock tests (MES). Several compounds were also active in the pentylenetetrazole (*sc*PTZ) and psychomotor (6-Hz) seizures. The most active 1-(morpholinomethyl)-3-phenyl-pyrrolidine-2,5-dione (**9d**) was effective in the MES, *sc*PTZ, 6-Hz tests as well as in the pilocarpine-induced status prevention (PISP) screen which may indicate its potential usefulness in the tonic-clonic, absence and refractory epilepsy, and also *status epilepticus*. This molecule as the most promising from the whole series showed only slight inhibition of CYP3A4 activity in vitro and is unlikely to show inhibitory interactions with other CYP3A4 metabolized drugs in vivo.

4. Experimental

4.1. General

All chemicals and solvents were purchased from Sigma–Aldrich (St. Louis, USA) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil,

Switzerland) and are uncorrected. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminum sheets (Merck; Darmstadt, Germany), using subsequent developing systems: S_1—chloroform:methanol (9:0.2, *v*/*v*), S_2—chloroform:2-propanol:25% ammonia (9:11:2, *v*/*v*/*v*). Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol I_2 in 10% HCl. HPLC analyses were run on a HPLC Waters® 2695 Separation Module equipped with photodiode array detector (Waters® 2998). A Chromolith RP-18 SpeedROD column (4.6 × 50 mm) was used. Conditions applied were as follow: eluent A (water/0.1% TFA), eluent B (acetonitrile/0.1% TFA); flow rate of 5 ml/min, gradient of 0–100% B over 3 min were used, injection volume was 10 μL. Standard solutions (1 mg/ml) of each compound were prepared in analytical grade acetonitrile and analysed at wavelengths 214 nm and 254 nm. Retention times (t_R) are given in minutes. Elemental analysis for C, H, and N were carried out by a micro method using the elemental Vario EI III Elemental analyser (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values. ^{1}H NMR and ^{13}C NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$ or DMSO, operating at 300 MHz and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to TMS δ = 0 (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). The mass spectra for compounds **9a**–**d**, **10a**–**d**, **11a**–**d** and **12a**–**d** were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, USA). The ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm column was used (Waters, Milford, USA).

The starting 2-phenyl- (**1**), 2-(2-chlorophenyl)- (**2**), 2-(3-chlorophenyl)- (**3**) and 2-(4-chlorophenyl)- (**4**) succinic acids were prepared by use of method described by Miller and Long.[17]

4.2. Chemical synthesis

4.2.1. General procedure for the synthesis of 3-phenyl-, 3-(2-chlorophenyl)-, 3-(3-chlorophenyl)- and 3-(4-chlorophenyl)-pyrrolidine-2,5-diones (5–8)

A total of 0.05 mol of the 2-phenyl- (**1**), 2-(2-chlorophenyl)- (**2**), 2-(3-chlorophenyl)- (**3**) or 2-(4-chlorophenyl)- (**4**) succinic acids were suspended in 20 ml of water and 0.05 mol of the 25% ammonia was gradually added. The mixture was heated in a term-regulated sand bath (ST 72 Roth, Karlsruhe, Germany) with simultaneous distillation of water. After complete removal of water, the temperature of the reaction mixture raised up to 180 °C and was maintained for 1 h. The crude products were recrystallized from methanol. The physicochemical and spectral data of intermediates **5**–**8** have been described in a separate paper.[18]

4.2.2. General procedure for the synthesis of final compounds 9a–d, 10a–d, 11a–d and 12a–d

The mixture of appropriately substituted 3-phenyl-succinimide (**5**–**8**) (0.01 mol), 40% formaldehyde solution (0.01 mol) and 1-(2-pyrimidyl)piperazine, 1-cyclohexylpiperazine, 1-benzylpiperidine or morpholine in 96% ethanol (20 ml) was stirred for ca. 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. Compound **10a** was obtained as light oil which was converted into solid hydrochloride salt in anhydrous ethanol saturated with HCl gas. The obtained precipitate was crystallized from anhydrous ethanol.

4.2.2.1. 3-Phenyl-1-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}pyrrolidine-2,5-dione (9a). White solid. Yield: 70%; mp 118–120 °C; R_f = 0.46 (S_1), R_f = 0.91 (S_2); HPLC (t_R 0.769 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.70 (br s, 4H, piperazine), 2.86 (dd, 1H, H_b, imide, *J* = 5.00 Hz), 3.22 (dd, 1H_a, imide, *J* = 9.62 Hz), 3.82 (br s, 4H, piperazine), 4.04 (q, 1H_c, imide, *J* = 4.88 Hz), 4.60 (s, 2H, CH_2), 6.50 (t, 1H, ArH, *J* = 4.74 Hz), 7.19–7.39 (m, 5H, ArH), 8.30 (d, 2H, ArH, *J* = 4.87 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.98, 43.63, 46.00, 20.59, 60.41, 109.97, 127.30, 127.99, 129.24, 137.03, 157.69, 176.96. ESI-MS: 352.2 ($C_{19}H_{21}N_5O_2$ $[M+H]^+$). Anal. Calcd for $C_{19}H_{21}N_5O_2$ (351.41): C, 64.93; H, 6.02; N, 19.93. Found: C, 64.59; H, 6.08; N, 19.48.

4.2.2.2. 1-[(4-Cyclohexylpiperazin-1-yl)methyl]-3-phenylpyrrolidine-2,5-dione (9b). White solid. Yield: 75%; mp 164–166 °C; R_f = 0.59 (S_1), R_f = 0.90 (S_2); HPLC (t_R 0.721 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (t, 5H, cyclohexane, *J* = 9.75 Hz), 1.62 (d, 1H, cyclohexane, *J* = 11.28 Hz), 1.57–1.69 (m, 4H, cyclohexane), 2.19 (t, 1H, cyclohexane, *J* = 6.62 Hz), 2.57 (br s, 4H, piperazine), 2.67 (br s, 4H, piperazine), 2.84 (dd, 1H, H_a imide, *J* = 5.39 Hz), 3.20 (dd, 1H, H_b, imide, *J* = 9.74 Hz), 4.02 (q, 1H, H_c, imide, *J* = 5.02 Hz), 4.55 (s, 2H, CH_2), 7.22–7.40 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.83, 26.26, 28.83, 28.94, 36.60, 45.48, 48.74, 51.09, 60.40, 63.45, 125.55, 127.88, 128.21, 130.45, 134.99, 138.65, 176.52, 177.97. ESI-MS: 356.2 ($C_{21}H_{29}N_3O_2$ $[M+H]^+$); Anal. Calcd for $C_{21}H_{29}N_3O_2$ (355.47): C, 70.95; H, 8.22; N, 11.82. Found: C, 70.94; H, 8.31; N, 11.73.

4.2.2.3. 1-[(4-Benzyl-1-piperidyl)methyl]-3-phenyl-pyrrolidine-2,5-dione (9c). White solid. Yield: 68%; mp 132–134 °C; R_f = 0.58 (S_1), R_f = 0.86 (S_2); HPLC (t_R 0.769 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27 (br s, 2H, piperidine), 1.59–1.64 (m, 5H, piperidine), 2.50 (d, 2H, CH_2, *J* = 6.92 Hz), 2.85 (dd, 1H, H_b, imide, *J* = 4.87 Hz), 2.98 (br s, 2H, piperidine), 3.21 (dd, 1H, H_a, imide, *J* = 9.62 Hz), 4.02 (q, 1H, H_c, imide, *J* = 4.79 Hz), 4.52 (s, 2H, CH_2), 7.10–7.40 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.05, 32.12, 36.99, 37.31, 43.01, 46.01, 51.40, 51.45, 60.86, 125.81, 127.32, 127.95, 128.15, 129.07, 129.22, 137.29, 140.48, 177.06, 178.64. ESI-MS: 363.2 ($C_{23}H_{26}N_2O_2$ $[M+H]^+$); Anal. Calcd for $C_{23}H_{26}N_2O_2$ (362.47): C, 76.20; H, 7.23; N, 7.73. Found: C, 76.35; H, 7.38; N, 7.70.

4.2.2.4. 1-(Morpholinomethyl)-3-phenyl-pyrrolidine-2,5-dione (9d). White solid. Yield: 65%; mp 106–107 °C; R_f = 0.40 (S_1), R_f = 0.92 (S_2); HPLC (t_R 0.698 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.63 (q, 4H, morpholine, *J* = 5.26 Hz), 2.89 (dd, 1H, H_b, imide, *J* = 4.87 Hz), 3.24 (dd, 1H, H_a imide, *J* = 9.62 Hz), 3.67 (t, 4H, morpholine, *J* = 4.62 Hz), 4.06 (q, 1H, H_c, imide, *J* = 4.87 Hz), 4.62 (s, 2H, CH_2), 7.22–731 (m, 2H, ArH), 7.33–7.42 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.24, 44.97, 51.08, 60.60, 66.89, 127.58, 129.53, 130.17, 130.39, 133.62, 134.82, 176.59, 178.07. ESI-MS: 274.1 ($C_{15}H_{18}N_2O_3$ $[M+H]^+$); Anal. Calcd for $C_{15}H_{18}N_2O_3$ (274.32): C, 65.68; H, 6.61; N, 10.21. Found: C, 65.43; H, 6.56; N, 10.11.

4.2.2.5. 3-(2-Chlorophenyl)-1-[(4-pyrimidin-2-ylpiperazin-1-yl)methyl]pyrrolidine-2,5-dione monohydrochloride (10a). White solid. Yield: 80%; mp 239–241 °C; R_f = 0.67 (S_1), R_f = 0.72 (S_2); HPLC (t_R 1.068 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.72 (t, 4H, piperazine, *J* = 5.64 Hz), 2.82 (dd, 1H, H_b, imide, *J* = 6.15 Hz),3.23 (dd, 1H, H_a, imide, *J* = 9.87 Hz), 3.82 (t, 4H, piperazine, *J* = 5.13 Hz), 4.32 (q, 1H, H_c, imide, *J* = 5.95 Hz), 4.62 (s, 2H, CH_2), 7.16–7.19 (m, 1H, ArH), 7.20–7.37 (m, 3H, ArH), 7.38–7.42 (m, 1H, pyrimidine), 8.29 (d, 2H, pyrimidine, *J* = 4.62 Hz), 11.09 (br s, 1H, 1H, +NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.99, 43.62, 46.01, 20.60, 60.43, 109.99, 127.32, 128.01, 129.26, 137.05, 157.70, 176.98. ESI-MS: 386.1 ($C_{19}H_{20}ClN_5O_2$ $[M+H]^+$); Anal. Calcd

for $C_{19}H_{21}Cl_2N_5O_2$ (422.30): C, 54.04; H, 5.01; N, 16.58. Found: C, 54.14; H, 5.19; N, 16.49.

4.2.2.6. 3-(2-Chlorophenyl)-1-[(4-cyclohexylpiperazin-1-yl) methyl]pyrrolidine-2,5-dione (10b). White solid. Yield: 82%; mp 159–160 °C; R_f = 0.55 (S_1), R_f = 0.72 (S_2); HPLC (t_R 0.82 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (m, 5H, cyclohexane), 1.62 (d, 1H, cyclohexane, J = 7.18 Hz), 1.57–1.68 (m, 4H, cyclohexane), 2.21 (d, 1H, cyclohexane, J = 7.18 Hz), 2.57–2.69 (m, 4H, piperazine), 2.71 (br s, 4H, piperazine), 2.76 (dd, 1H, H_b, imide, J = 6.03 Hz), 3.23 (dd, 1H, H_a, imide, J = 9.87 Hz), 4.35 (q, 1H, H_c, imide, J = 6.03 Hz), 4.57 (s, 2H, CH_2), 7.18–7.28 (m, 3H, ArH), 7.38–7.44 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.82, 26.27, 28.85, 28.95, 36.61, 45.49, 48.76, 51.10, 60.42, 63.47, 125.57, 127.89, 128.24, 130.46, 135.01, 138.68, 176.55, 177.98. ESI-MS: 390.2 ($C_{21}H_{28}ClN_3O_2$ [M+H]$^+$); Anal. Calcd for $C_{21}H_{28}ClN_3O_2$ (389.92): C, 64.68; H, 7.24; N, 10.77. Found: C, 64.45; H, 7.32; N, 10.79.

4.2.2.7. 1-[(4-Benzyl-1-piperidyl)methyl]-3-(2-chlorophenyl) pyrrolidine-2,5-dione (10c). White solid. Yield: 70%; mp 71–73 °C; R_f = 0.49 (S_1), R_f = 0.86 (S_2); HPLC (t_R 1.055 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23–1.32 (m, 2H, piperidine), 1.45–1.65 (m, 5H, piperidine), 2.52 (d, 2H, CH_2, J = 7.18 Hz), 2.81 (dd, 1H, H_b, imide, J = 5.90 Hz), 3.02 (d, 2H, piperidine, J = 12.05 Hz) 3.22 (dd, 1H, H_a, imide, J = 9.87 Hz), 4.31 (q, 1H, H_c, imide, J = 6.03 Hz), 4.53 (s, 2H, CH_2), 7.11–7.29 (m, 4H, ArH), 7.30–7.39 (m, 4H, ArH), 7.41–7.43 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.06, 32.12, 36.98, 37.32, 43.01, 46.02, 51.41, 51.46, 60.86, 125.82, 127.33, 127.96, 128.14, 129.08, 129.23, 137.28, 140.49, 177.07, 178.64. ESI-MS: 397.1 ($C_{23}H_{25}ClN_2O_2$ [M+H]$^+$); Anal. Calcd for $C_{23}H_{25}ClN_2O_2$ (396.92): C, 69.59; H, 6.35; N, 7.06. Found: C, 69.38; H, 6.54; N, 7.23.

4.2.2.8. 3-(2-Chlorophenyl)-1-(morpholinomethyl)pyrrolidine-2,5-dione (10d). White solid. Yield: 78%; mp 99–101 °C; R_f = 0.42 (S_1), R_f = 0.91 (S_2); HPLC (t_R 0.951 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.60–2.72 (m, 4H, morpholine), 2.86 (dd, 1H, H_b, imide, J = 6.03 Hz), 3.25 (dd, 1H, H_a, imide, J = 10.00 Hz) 3.68 (t, 4H, morpholine, J = 4.74 Hz), 4.34 (q, 1H, H_c, imide, J = 5.90 Hz), 4.53 (s, 2H, CH_2), 7.19–7.24 (m, 1H, ArH), 7.28–7.32 (m, 2H, ArH), 7.40–7.44 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.25, 44.98, 51.08, 60.61, 66.90, 127.59, 129.55, 130.18, 130.40, 133.63, 134.83, 176.60, 178.08. ESI-MS: 309.1 ($C_{15}H_{17}ClN_2O_3$ [M+H]$^+$); Anal. Calcd for $C_{15}H_{17}ClN_2O_3$ (308.76): C, 58.35; H, 5.55; N, 9.07. Found: C, 58.52; H, 5.45; N, 9.15.

4.2.2.9. 3-(3-Chlorophenyl)-1-[(4-pyrimidin-2-ylpiperazin-1-yl) methyl]pyrrolidine-2,5-dione (11a). White solid. Yield: 82%; mp 118–120 °C; R_f = 0.40 (S_1), R_f = 0.80 (S_2); HPLC (t_R 1.1044 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.49–2.58 (m, 4H, piperazine) 2.86 (dd, 1H, H_b, imide, J = 6.10 Hz), 3.18 (dd, 1H, H_a, imide, J = 9.86 Hz), 3.70 (t, 4H, piperazine, J = 5.12 Hz), 4.27 (q, 1H, H_c, imide, J = 5.89 Hz), 4.38 (s, 2H, CH_2), 6.60 (t, 1H, pyrimidine, J = 4.62 Hz), 7.24–7.42 (m, 3H, ArH), 8.34 (d, 2H, pyrimidine, J = 4.78 Hz), 7.59–7.63 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.98, 43.63, 46.02, 20.60, 60.44, 109.99, 127.33, 128.02, 129.26, 137.06, 157.71, 176.99. ESI-MS: 386.1 ($C_{19}H_{20}ClN_5O_2$ [M+H]$^+$); Anal. Calcd for $C_{19}H_{20}ClN_5O_2$ (385.85): C, 59.12; H, 5.22; N, 18.14. Found: C, 59.24; H, 5.18; N, 18.00.

4.2.2.10. 3-(3-Chlorophenyl)-1-[(4-cyclohexylpiperazin-1-yl)methyl]pyrrolidine-2,5-dione (11b). White solid. Yield: 69%; mp 130–131 °C; R_f = 0.36 (S_1), R_f = 0.77 (S_2); HPLC (t_R 1.033 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.09–1.24 (m, 5H, cyclohexane), 1.62 (d, 1H, cyclohexane,J = 11.80 Hz), 1.76–1.86 (m, 4H, cyclohexane), 2.21 (br s, 1H, cyclohexane), 2.57 (br s, 4H, piperazine), 2.66 (br s, 4H, piperazine), 2.81 (dd, 1H, H_b, imide, J = 5.51 Hz), 3.20 (dd, 1H, H_a, imide, J = 9.74 Hz), 4.01 (q, 1H, H_c, imide, J = 5.37 Hz), 4.55 (s, 2H, CH_2), 7.11–7.16 (m, 1H, ArH), 7.24–7.34 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.83, 26.27, 28.87, 28.97, 36.62, 45.48, 48.77, 51.13, 60.40, 63.46, 125.58, 127.89, 128.23, 130.47, 135.00, 138.69, 176.56, 177.99. ESI-MS: 390.2 ($C_{21}H_{28}ClN_3O_2$ [M+H]$^+$); Anal. Calcd for $C_{21}H_{28}ClN_3O_2$ (389.92): C, 64.68; H, 7.24; N, 10.77. Found: C, 64.50; H, 7.30; N, 10.83.

4.2.2.11. 1-[(4-Benzyl-1-piperidyl)methyl]-3-(3-chlorophenyl) pyrrolidine-2,5-dione (11c). White solid. Yield: 58%; mp 81–83 °C; R_f = 0.53 (S_1), R_f = 0.68 (S_2); HPLC (t_R 1.056 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21–1.30 (m, 2H, piperidine), 1.31–1.48 (m, 1H, piperidine), 1.59–1.64 (m, 2H, piperidine), 2.06–2.20 (m, 2H, piperidine), 2.51 (d, 2H, CH_2, J = 6.92 Hz), 2.82 (dd, 1H, H_b, imide, J = 4.87 Hz), 2.95–3.00 (m, 2H, piperidine), 3.20 (dd, 1H, H_a, imide, J = 9.74 Hz), 4.00 (q, 1H, H_c, imide, J = 4.87 Hz), 4.51 (s, 2H, CH_2), 7.10–7.32 (m, 9H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.05, 32.12, 36.99, 37.33, 43.00, 46.01, 51.42, 51.47, 60.85, 125.81, 127.35, 127.96, 128.15, 129.07, 129.24, 137.29, 140.48, 177.06, 178.65. ESI-MS: 397.2 ($C_{23}H_{25}ClN_2O_2$ [M+H]$^+$); Anal. Calcd for $C_{23}H_{25}ClN_2O_2$ (396.92): C, 69.59; H, 6.35; N, 7.06. Found: C, 69.41; H, 6.44; N, 7.20.

4.2.2.12. 3-(3-Chlorophenyl)-1-(morpholinomethyl)pyrrolidine-2,5-dione (11d). White solid. Yield: 70%; mp 98–100 °C; R_f = 0.46 (S_1), R_f = 0.77 (S_2); HPLC (t_R 0.998 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.55–2.66 (m, 4H, morpholine), 2.85 (dd, 1H, H_b, imide, J = 5.13 Hz) 3.24 (dd, 1H, H_a, imide, J = 9.40 Hz), 3.66 (t, 4H, morpholine, J = 4.62 Hz), 4.04 (q, 1H, H_c, imide, J = 5.13 Hz), 4.51 (s, 2H, CH_2), **7**.11 (t, 1H, ArH, J = 2.31 Hz), 7.13–7.16 (m, 1H, ArH), 7.26–7.36 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.24, 44.97, 51.08, 60.60, 66.91, 127.58, 129.55, 130.18, 130.41, 133.62, 134.83, 176.61, 178.07. ESI-MS: 309.2 ($C_{15}H_{17}ClN_2O_3$ [M+H]$^+$); Anal. Calcd for $C_{15}H_{17}ClN_2O_3$ (308.76): C, 58.35; H, 5.55; N, 9.07. Found: C, 58.49; H, 5.42; N, 9.16.

4.2.2.13. 3-(4-Chlorophenyl)-1-[(4-pyrimidin-2-ylpiperazin-1-yl)methyl]pyrrolidine-2,5-dione (12a). White solid. Yield: 65%; mp 154–155 °C; R_f = 0.63 (S_1), R_f = 0.72 (S_2); HPLC (t_R 1.061 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.60–2.70 (m, 4H, piperazine), 2.81 (dd, 1H, H_b, imide, J = 5.13 Hz), 3.21 (dd, 1H, H_a, imide, J = 9.74 Hz), 3.79 (t, 4H, piperazine, J = 5.13 Hz), 4.01 (q, 1H, H_c, imide, J = 5.13 Hz), 4.58 (s, 2H, CH_2), 6.49 (t, 1H, pyrimidine, J = 4.74 Hz), 7.13–7.17 (m, 2H, ArH), 7.29–7.33 (m, 2H, ArH), 8.29 (d, 2H, pyrimidine, J = 4.62 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.82, 26.27, 28.88, 28.97, 36.61, 45.47, 48.78, 51.14, 60.41, 63.45, 125.57, 127.88, 128.24, 130.46, 135.02, 138.68, 176.58, 177.98. ESI-MS: 386.2 ($C_{19}H_{20}ClN_5O_2$ [M+H]$^+$); Anal. Calcd for $C_{19}H_{20}ClN_5O_2$ (385.85): C, 59.12; H, 5.22; N, 18.14. Found: C, 59.25; H, 5.20; N, 18.10.

4.2.2.14. 3-(4-Chlorophenyl)-1-[(4-cyclohexylpiperazin-1-yl) methyl]pyrrolidine-2,5-dione (12b). White solid. Yield: 68%; mp 134–136 °C; R_f = 0.83 (S_1), R_f = 0.90 (S_2); HPLC (t_R 1.054 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15–1.25 (m, 5H, cyclohexane), 1.60–1.64 (m, 1H, cyclohexane), 1.77–1.87 (m, 4H, cyclohexane), 2.24 (br s, 1H, cyclohexane), 2.58 (br s, 4H, piperazine), 2.67 (br s, 4H piperazine), 2.80 (dd, 1H, H_b, imide, J = 5.51 Hz), 3.20 (dd, 1H, H_a, imide, J = 9.74 Hz), 4.00 (q, 1H, H_c, imide, J = 5.26 Hz), 4.54 (s, 2H, CH_2), 7.19 (d, 2H, ArH, J = 8.46 Hz), 7.35 (d, 2H, ArH, J = 8.46 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 25.84, 26.28, 28.88, 28.97, 36.63, 45.49, 48.78, 51.14, 60.41, 63.46, 125.59, 127.90, 128.24, 130.48, 135.01, 138.68, 176.57, 177.97.

ESI-MS: 390.1 ($C_{21}H_{28}ClN_3O_2$ $[M+H]^+$); Anal. Calcd for $C_{21}H_{28}ClN_3O_2$ (389.92): C, 64.68; H, 7.24; N, 10.77. Found: C, 64.57; H, 7.31; N, 10.82.

4.2.2.15. 1-[(4-Benzyl-1-piperidyl)methyl]-3-(4-chlorophenyl) pyrrolidine-2,5-dione (12c). White solid. Yield: 78%; mp 118–120 °C; R_f = 0.47 (S_1), R_f = 0.73 (S_2); HPLC (t_R 1.049 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18–1.29 (m, 2H, piperidine), 1.38–1.43 (m, 1H, piperidine), 1.61 (d, 2H, piperidine, J = 12.57 Hz), 2.04–2.18 (m, 2H, piperidine), 2.50 (d, 2H, CH_2, J = 6.92 Hz), 2.80 (dd, 1H, H_b, imide, J = 5.13 Hz), 2.96 (br s, 2H, piperidine), 3.20 (dd, 1H, H_a, imide, J = 9.75 Hz), 4.00 (q, 1H, H_c, imide, J = 5.13 Hz), 4.50 (s, 2H, CH_2), 7.10–7.20 (m, 4H, ArH), 7.24–7.29 (m, 3H, ArH), 7.33–7.37 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.06, 32.13, 36.98, 37.34, 43.01, 46.02, 51.42, 51.46, 60.87, 125.82, 127.35, 127.97, 128.16, 129.08, 129.24, 137.28, 140.48, 177.07, 178.66. ESI-MS: 397.1 ($C_{23}H_{25}ClN_2O_2$ $[M+H]^+$); Anal. Calcd for $C_{23}H_{25}ClN_2O_2$ (396.92): C, 69.59; H, 6.35; N, 7.06. Found: C, 69.45; H, 6.41; N, 7.15.

4.2.2.16. 3-(4-Chlorophenyl)-1-(morpholinomethyl)pyrrolidine-2,5-dione (12d). White solid. Yield: 68%; mp 94–96 °C; R_f = 0.38 (S_1), R_f = 0.79 (S_2); HPLC (t_R 0.997 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.54–2.66 (m, 4H, morpholine), 2.84 (dd, 1H, H_b, imide, J = 5.14 Hz), 3.24 (dd, 1H, H_a, imide, J = 9.62 Hz), 3.66 (t, 4H, morpholine, J = 4.62 Hz), 4.04 (q, 1H, H_c, imide, J = 5.13 Hz), 4.51 (s, 2H, CH_2), 7.16–7.24 (m, 2H, ArH), 7.26–7.39 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 36.25, 44.98, 51.08, 60.61, 66.90, 127.58, 129.56, 130.19, 130.40, 133.63, 134.84, 176.63, 178.08. ESI-MS: 309.1 ($C_{15}H_{17}ClN_2O_3$ $[M+H]^+$); Anal. Calcd for $C_{15}H_{17}ClN_2O_3$ (308.76): C, 58.35; H, 5.55; N, 9.07. Found: C, 58.42; H, 5.47; N, 9.15.

4.3. Biological studies

4.3.1. Animals and routes of administration

The initial anticonvulsant evaluation was performed within the Antiepileptic Drug Development (ADD) Program in the Epilepsy Branch, Neurological Disorders Program, National Institutes of Health, National Institute of the Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere.[22–24]

Male albino mice (CF-1 strain) and male albino rats (Sprague-Dawley) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The compounds were suspended in 0.5% methylcellulose/water mixture. The ASP (Anticonvulsant Screening Project) initially evaluates anticonvulsant activity for newly submitted compounds following intraperitoneal (ip) administration in mice and oral administration in rats. Groups of eight mice or four rats were employed. Phase I studies in mice involved two convulsant tests: maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ) and rotorod test for neurological toxicity (NT).

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. Selected derivatives were administrated orally into rats using four animals at a fixed dose of 30 mg/kg (MES test). This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity. For both doses the motor impairment was studied in parallel. Rats were tested at five time periods ranging from one quarter to 4 h post substance administration. The results of preliminary anticonvulsant screening are shown in Tables 1 and 2.

4.3.1.1. Maximal electroshock seizure 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.

4.3.1.2. Subcutaneous pentylenetetrazole seizure test (scPTZ). This screen utilizes a dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) that produces clonic seizures lasting for a period of at least five seconds in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously.

4.3.1.3. Neurological toxicity (NT). The neurological toxicity induced by a compound was detected in mice or rats using the standarized rotorod test.[25] 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.

4.3.1.4. The 6-Hz psychomotor seizure model. This screen was carried out according to the protocol originally described by Brown et al.[26] and more recently by Barton et al.[20] and Kaminski et al.[27] 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 observation cage immediately after the current application. The seizures manifested 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 (Table 4).

4.3.1.5. The pilocarpine-induced status prevention (PISP) model. Male albino rats (Sprague-Dawley, 150–180 g) were used as experimental animals. The compounds were administrated via the ip route of administration. Then, a challenge dose of pilocarpine is given observing the treatment-effects of the substance tested. The seizure severity is determined using the well-known Racine scale as follows: (I) immobility, eye closure, twitching of vibrissae, sniffing, and facial clonus; (II) head nodding associated with more several facial clonus; (III) clonus of one of the forelimbs; (IV) rearing often accomplished by bilateral forelimb clonus, and (V) all of the above plus loss of balance and falling, accomplished by generalized clonic seizures.[28] The anticonvulsant activity of compound was assessed at time zero, namely the time from the first-stage III seizures (Test 71). The outcome measures are the determination of 'protection' or 'no protection' (Table 6).

4.3.1.6. Quantification studies. Quantitative determination of the median effective dose (ED_{50}) and the median neurotoxic dose (TD_{50}) values was performed at previously estimated time of peak effect (TPE) after *p.o.* administration into rats. Groups of minimum eight animals received various doses of the compound until at least three points were established in the range of 10–90% seizure protection or minimal motor impairment. From the plot of the data obtained, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line and standard error of the slope were calculated by means of a computer program written at NINDS/NIH. The results are shown in Tables 3, 5 and 7.

4.3.2. Cytochrome P450 inhibition studies

The cytochrome P450 inhibition studies were carried out in National Institutes of Health, National Institute of the Neurological Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere.[29] Compound tested was incubated together with probe substrate (testosterone) in the presence of NADPH, and the inhibition of metabolite formation, namely 6-β-OH-testosterone by CYP3A4 subtype of the cytochrome P450 was determined. The results are shown in Table 8.

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