Full Paper

Synthesis and Anticonvulsant Activity of New *N*-Mannich Bases Derived from 5-Cyclopropyl-5-phenyl-hydantoins

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Synthesis, physicochemical and anticonvulsant properties of new *N*-Mannich bases **3–24** derived from 5-cyclopropyl-5-phenyl- and 5-cyclopropyl-5-(4-chlorophenyl)-hydantoins were described here. Initial anticonvulsant screening was performed using intraperitoneal (*i.p.*) maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizures tests. Selected derivatives were also screened in the 6-Hz test. The neurotoxicity was determined applying the rotorod test. The pharmacological results revealed that the majority of compounds were effective in MES and/or *sc*PTZ tests. The quantitative studies after oral administration into rats showed that several molecules were more potent than phenytoin and ethosuximide which were used as reference antiepileptic drugs. From the whole series the most active was 3-[(4-phenylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**3**) with the ED_{50} value of 5.29 mg/kg in the MES test.

Keywords: Anticonvulsant activity / 5-Cyclopropyl-5-phenyl-hydantoins / Imidazolidine-2,4-diones / *N*-Mannich bases

Received: August 24, 2010; Revised: October 13, 2010; Accepted: October 15, 2010

DOI 10.1002/ardp.201000241

Introduction

Epilepsy is a common neurological disorder with chronic and progressive course that affects about 1% of the world's population. It has numerous causes, may occur at any age, typically require lifelong treatment and may significantly limit patient autonomy and quality of life. Despite the increasing understanding of the pathogenesis of seizures up to 30% of epilepsies are poorly treated with the available antiepileptic drugs (AEDs) [1]. Moreover, a large number of new AEDs marked during recent years, did not change the proportion of patients responding to the treatment and many of those medications cause serious side effects, which include ataxia, nausea, mental dulling and hepatotoxicity [2, 3]. The ideal antiepileptic would prevent seizures without producing side effects that adversely affect the patient's quality of life. Therefore with all of above findings in mind the continued search for safer and more effective AEDs is urgently necessary.

The rational design of new anticonvulsants is based 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. Their appearance in the structure may determine or enhance anticonvulsant properties. Thus one of the important core fragments is defined by a nitrogen heteroatomic system, usually a cyclic imide, at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system [4–6]. This common template is present in the structures of old generation of AEDs such as phenobarbital (**I**), ethosuximide (**II**) or phenytoin (**III**) as well as in the newest drugs *e.g.* levetiracetam (**IV**) and its analogues, being currently under the clinical trials, namely brivaracetam (**V**) or seletracetam (**VI**) [7–9] (Fig. 1).

Previous researches in our group have identified the differently substituted pyrrolidine-2,5-diones or imidazolidine-2,4-diones as targets for new antiepileptic drugs. Many of these compounds were effective in the maximal electroshock (MES) or/and subcutaneous pentylenetetrazole (*sc*PTZ) screens that are the most popular seizure models in the early stages of testing of new anticonvulsants. The SAR studies have demonstrated the potent anticonvulsant activity among the *N*-Mannich bases containing the differently substituted arylpiperazines as amine function [10–12] (Fig. 2). These amines

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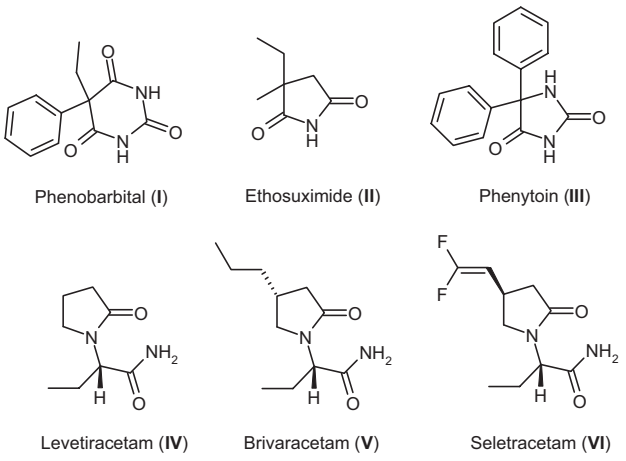


Figure 1. Structures of known anticonvulsants.

were presented in structures of many other anticonvulsant active agents [13–15].

Furthermore the comparison of pharmacological results obtained for pyrrolidine-2,5-diones and respective imidazolidine-2,4-dione analogues revealed higher activity of compounds bearing in their structures the latter core fragment [13].

Taking into consideration the above findings in the present study a library of twenty four new *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl- or 5-cyclopropyl-5-(4-chlorophenyl)-hydantoins containing the 4-substituted piperazines as amine function has been synthesized. The results of anticonvulsant screening enabled the evaluation of structure-activity relationships in this series of derivatives.

Results and discussion

Chemistry

The synthesis of compounds **1–24** was accomplished as shown in Scheme 1. The starting 5-cyclopropyl-5-phenyl- (**1**) and 5-cyclopropyl-5-(4-chlorophenyl)-hydantoins (**2**) were obtained from the cyclopropyl-phenyl- or cyclopropyl-4-chlorophenyl-ketones by means of the Bűcherer–Berg reaction with modification described by Goodson et al. [16]. In the next step twenty four new Mannich bases with the 5-cyclopropyl-5-phenyl- (**3–10**, **19–21**) and 5-cyclopropyl-5-(4-chlorophenyl)-hydantoin (**11–18**, **22–24**) as a core fragment were prepared. The aminoalkylation of the acidic proton (N_3H) in the imidazolidine-2,4-dione ring was carried out in the presence of formaldehyde (40% solution) and variously substituted 4-aryl- (**3–6**, **11–14**) or 4-benzyl-piperazines (**7–10**, **15–18**). The 4-arylpiperazine fragment was also replaced by 4-methyl- (**19**, **22**), 4-ethyl- (**20**, **23**), and 4-(2-hydroxy)-ethyl-pipreazines (**21**, **24**). The reaction was carried out in ethanol at a room temperature for ca. 6–12 h. The crude products, as racemic mixtures, were crystallized from ethanol giving the final compound in yield about 70%. Their purities were assessed by TLC chromatography and the structures were confirmed by both spectral and elemental analysis. The detailed physical and analytical data are listed in the experimental section.

Anticonvulsant activity

The preclinical discovery and development of new chemical agents for the treatment of epilepsy are 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 recognized as the ''gold standards'' in the early stages of testing. Furthermore, the MES and *sc*PTZ tests are claimed to detect compounds affording protection

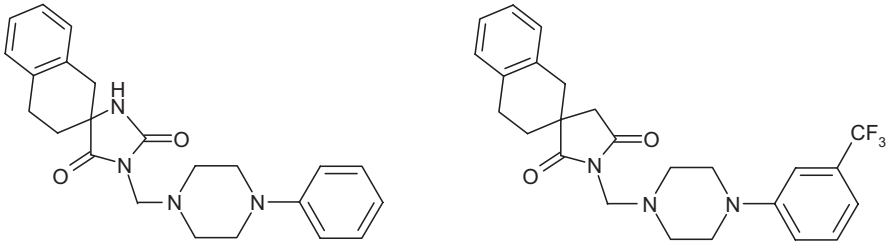
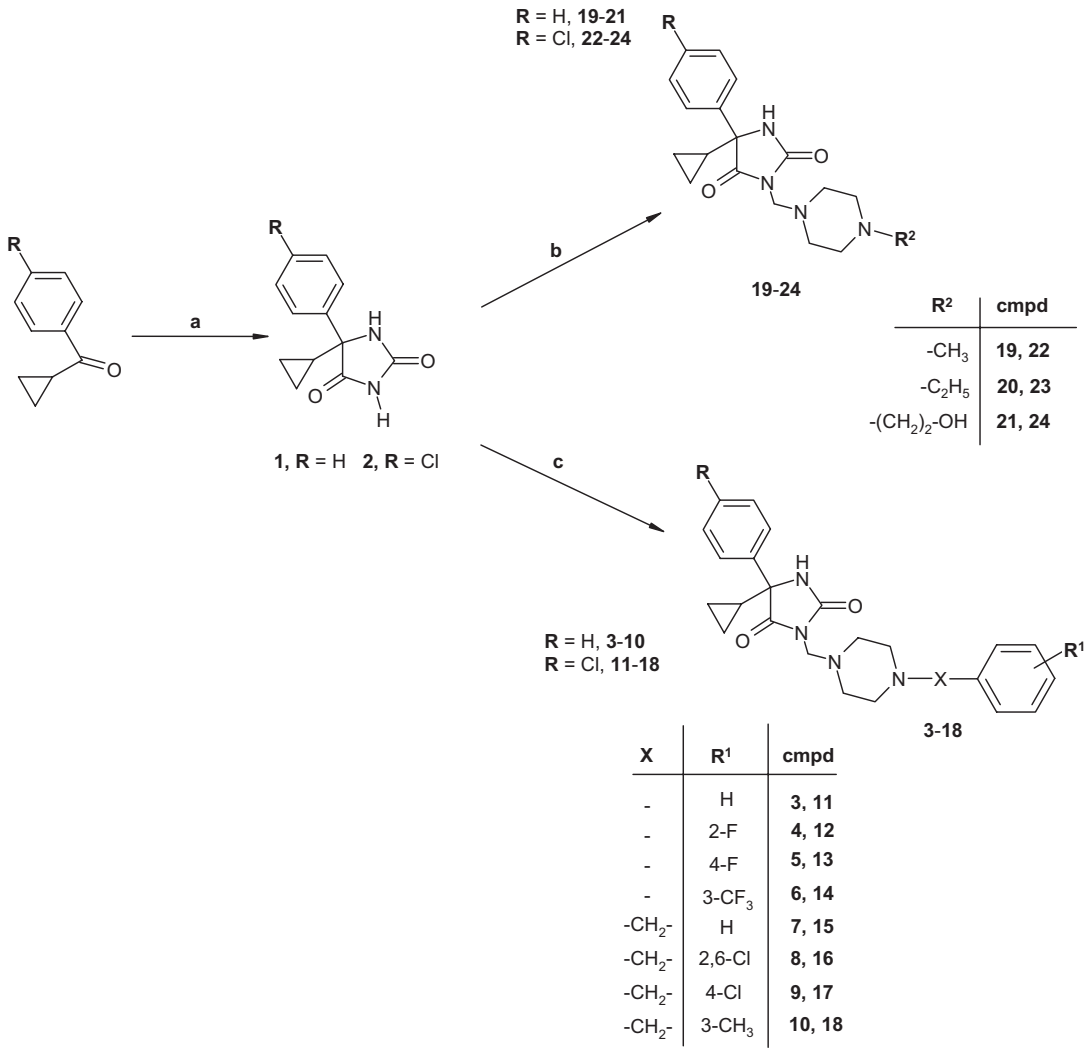


Figure 2. Structures of active compounds **VII** and **VIII** obtained in previous studies.



Reagents and reaction conditions: (a) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol; (b, c) 4-substituted piperazines, formaldehyde, 96% ethyl alcohol, 6–12 h room temperature.

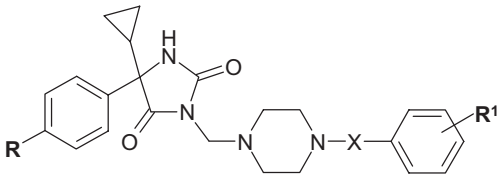
Scheme 1. Synthetic procedures of compounds **3–24**.

against generalized tonic-clonic seizures and generalized absence seizures, respectively [17, 18].

The profile of anticonvulsant activity of all examined compounds (**3–24**) was established in the MES and *sc*PTZ tests, after intraperitoneal (*i.p.*) injection into mice at doses of 30, 100, and 300 mg/kg. An observation was carried out at two different time intervals, namely 0.5 h and 4 h. The acute neurological toxicity (NT) was determined in the minimal motor impairment–rotorod screen. The results are shown in Tables 1 and 2.

The anticonvulsant evaluation showed that majority of compounds were effective in MES or/and *sc*PTZ screens. In the whole series only **16** and **22** were inactive in both tests used. The activity in the MES test indicates the ability of substance to prevent seizure spread. The anti-MES protection was observed at a dose of 30 mg/kg: **5** and **7**; 100 mg/kg: **3**, **4**, **6**, **8–11**, **13–15**, and **19–21** or 300 mg/kg: **17**, **18**, **23**, and **24**. Compounds **3**, **5–8**, **10**, **15**, **18**, **20**, **21**, **23**, and **24** were found to be active in the *sc*PTZ test which identifies substances elevating seizure threshold. Among these **3**, **5–8**, **10**, **15**, **18**, **20**, **21**, **23**, and **24** were active at a dose of 100 mg/kg or 300 mg/kg comparable to ethosuximide used as reference anticonvulsant. The activity in MES or *sc*PTZ screens was observed mainly at 4 h or in both time intervals that indicates long duration of anticonvulsant action.

Table 1. The results after intraperitoneal administration to mice (**3–18**).



3-18

Comp.	R	R^1	X	MES[a]		*sc*PTZ[b]		NT[c]	
				0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
3	H	H	–	–	100	300	–	–	–
4	H	2-F	–	–	100	–	–	–	–
5	H	4-F	–	300	30	–	100[25]	100	100
6	H	3-CF_3	–	–	100	–	300[25]	100	300
7	H	H	$-CH_2$	100	30	100	100	300	300[14]
8	H	2,6-Cl	$-CH_2-$	100	100	–	300[25]	–	300[14]
9	H	4-Cl	$-CH_2-$	–	100	–	–	300[1,14]	300[14,33]
10	H	3-CH_3	$-CH_2-$	300	100	100	300	–	–
11	Cl	H	–	–	100	–	–	300	300[14]
12	Cl	2-F	–	–	300	–	–	300	–
13	Cl	4-F	–		100	–	–	–	–
14	Cl	3-CF_3	–		100	–	–	–	300
15	Cl	H	$-CH_2$	–	100	–	300	300	300[14]
16	Cl	2,6-Cl	$-CH_2$	–	–	–	–	–	–
17	Cl	4-Cl	$-CH_2-$	–	300	–	–	–	300[14,33]
18	Cl	3-CH_3	$-CH_2-$	–	300	–	300[25]	30	100
PHT[d]				30	30	–	–	100	100
ETX[d]				–	–	100	300	–	–

Doses of 30, 100, and 300 mg/kg were administrated intraperitoneally in mice. The figures indicate the minimum dose whereby anticonvulsant activity or neurotoxicity was demonstrated in 100% of the animals. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg). [a] Maximal electroshock test. [b] Subcutaneous pentylenetetrazole test. [c] Neurotoxicity screening using rotorod test. [d] PHT – phenytoin, ETX – ethosuximide reference drugs, data from [23]. Response comments: [1] death, [14] unable to grasp rotorod, [25] myoclonic jerks, [33] tremors.

In the neurotoxicity screen (NT), active compounds **3**, **4**, **10**, **13**, and two inactive derivatives **16**, **22** did not show neurotoxicity at the highest dose administered (300 mg/kg). Compounds **5–9**, **11**, **12**, **14**, **15**, **17**, **19–21**, and **23** exhibited motor impairment at a dose of 100 mg/kg and/or 300 mg/kg, whereas **18** and **24** at 30 mg/kg.

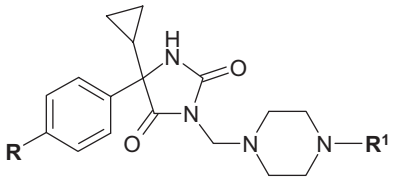
A valuable property of a candidate anticonvulsant is its ability to inhibit convulsions when given by the oral route. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity or neurotoxicity. Therefore on the basis of the data obtained in *i.p.* screen in mice and according to the anticonvulsant screening project (ASP) disposition, twelve compounds **3**, **5**, **7–11**, **13–15**, **20**, and **24** were examined for their activity (MES test) and neurotoxicity after *p.o.* administration into rats at a dose of 30 mg/kg. The results obtained are presented in Table 3.

Compounds **3**, **5**, **7**, **8**, **10**, and **14** protected 100% of rats at 2 h or/and 4 h and from 25% to 75% of animals in other time intervals. These molecules showed comparable activity to phenytoin used as reference drug. Satisfactory protection was also observed for **11** and **20**. Except of compound **5** all other were non-neurotoxic when given orally.

Compounds **7** and **10** both active in pentylenetetrazole seizures in mice were tested in the *sc*PTZ screen after *p.o.* administration in rats (dose of 50 mg/kg). As shown in Table 4 these molecules revealed comparable, however, longer protection to the reference anticonvulsant ethosuximide. These compounds did not exhibit neurotoxicity at the tested dose of 50 mg/kg.

Compounds **3**, **7**, and **10** have been chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}) in rats after oral application (Table 5). The quantitative evaluation of the median effective dose (ED_{50}) in the MES and *sc*PTZ

Table 2. The results after intraperitoneal administration to mice (**19–24**).



19-24

Comp.	R	R^1	MES[a]		*sc*PTZ[b]		NT[c]	
			0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
19	H	$-CH_3$	100	100	–	–	100	300[14]
20	H	$-C_2H_5$	100	100	300	30	300[14]	–
21	H	$-(CH_2)_2$-OH	100	100	300	300	100	300[14]
22	Cl	$-CH_3$	–	–	–	–	–	–
23	Cl	$-C_2H_5$	–	300	300	300	300[14]	–
24	Cl	$-(CH_2)_2$-OH	300	300	300	–	30	100[14]
PHT[d]			30	30	–	–	100	100
ETX[d]			–	–	100	300	–	–

For [a], [b], [c], [d], see Table 1. Response comments: [14] unable to grasp rotorod.

tests as well as toxic dose (TD_{50}) were performed at previously estimated time of peak effect (TPE) after *p.o.* administration. The quantitative *p.o.* data revealed that all compounds tested showed higher activity than phenytoin in the electrically induced seizures. Moreover **7** and **10** were more active than ethosuximide in the *sc*PTZ screen. These data suggest potential effectiveness of **7** and **10** in treating both generalized tonic-clonic epilepsy and absence seizures. The range of doses applied enabled the determination of TD_{50} values only for compounds **3** and **7**. These molecules displayed higher protection index in comparison to reference antiepileptic drugs.

According NIH/NINDS disposition compounds **4** and **10** were screened in the 6-Hz test. The selection was made randomly as a part of the search of molecules providing anti-6-Hz protection among chemically diversified compounds. The 6-Hz model was not used widely because of its lack clinical

Table 3. Anticonvulsant activity (MES test) of selected compounds administrated orally to rats.

Comp.	MES[a]					NT[b]				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
3	1	2	3	4	4	0	0	0	0	0
5	2	2	2	4	4	1	3	2	1	1
7	0	3	4	4	4	0	0	0	0	0
8	0	3	3	3	4	0	0	0	0	0
9	1	1	3	2	3	0	0	0	0	0
10	0	3	2	4	4	0	0	0	0	0
11	2	0	1	1	4	0	0	0	0	0
13	0	0	1	1	3	0	0	0	0	0
14	0	0	2	4	4	0	0	0	0	0
15	0	1	0	1	0	0	0	0	0	0
20	0	4	1	4	2	0	0	0	0	0
24	0	2	0	0	3	0	0	0	0	0
PHT[c]	1	4	3	3	3	ND	ND	ND	ND	ND

[a] The data indicate the number of rats of four that were protected at a dose of 30 mg/kg. [b] Neurotoxicity screening using rotorod test. The data in indicate the number of rats of four in which neurotoxicity was observed at a dose of 30 mg/kg. [c] PHT – phenytoin, reference drug, data from [23]. ND – No data

Table 4. Anticonvulsant activity (*sc*PTZ test) of selected compounds administrated orally to rats.

Comp.	Oral administration to rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
7	2	1	3	1	2
10	0	0	0	2	2
ETX[b]	0	2	1	1	0

[a] Dose of 50 mg/kg was administrated. The data indicate the number of rats out of four that were protected. [b] ETX – ethosuximide, reference drug, data from [23].

Table 6. Psychomotor seizure 6-Hz test (dose of 100 mg/kg, current −32 mA).

Comp.	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
4	0	0	2	1	4
10	0	1	0	0	1

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

validity since the hydantoins such as phenytoin failed to show protective activity. However, it is worthy of note that the newest anticonvulsants such as levetiracetam, which is not active in the conventional MES test, does exhibit protective activity in the 6-Hz model. This suggested that the 6-Hz model might be capable for identifying anti-seizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action. The results are shown in Table 6.

As can be seen from the data above, compound **4** administrated intraperitoneally at a dose of 100 mg/kg into mice protected 100% of animals tested at 4 h. This molecule was also effective in 50% of mice at 1 h and 25% at 2 h. Compound **10** were less active and protected only 25% of animals at 0.5 h and 4 h.

Structure activity relationships

The results of preliminary anticonvulsant screening enable to draw general conclusions about the relationships between structure and anticonvulsant activity. The spectrum and duration of activity depended on kind of the substituents at position-4 of piperazine moiety *e.g.*, phenyl, benzyl, alkyl or hydroxyalkyl as well as the presence of a chloro atom at the *para*-position of the 5-phenyl ring at the imidazolidine-2,4-dione. The SAR studies have shown higher activity of the 5-cyclopropyl-5-phenyl-hydantoins (**3–10** and **19–21**) in comparison to respective 5-(4-chlorophenyl) analogues **11–18** and **22–24**. In the series of 5-cyclopropyl-5-phenyl-hydantoins two compounds **4** and **9** were active only in the maximal electroshock test (MES) whereas all other derivatives revealed protection in both MES and *sc*PTZ screens in mice. The majority of these molecules were active at 0.5 h and 4 h after *i.p.* administration that means rapid onset and long duration of anticonvulsant action. An exception was observed for **4**, **6**, and **9** that were active only at time point 4 h. The structure–activity relationships were more pronounced for 5-cyclopropyl-5-(4-chlorophenyl)-hydantoins. In this series, all 4-phenyl-piperazine derivatives (**11–14**) were effective only in the MES test. The introduction of methylene spacer between piperazine and phenyl ring among 4-benzylpiperazine derivatives (**15–18**) caused activity in both screens (**15**, **18**) made compound less active (**17**) or inactive (**16**). The 4-phenyl- or 4-benzylpiperazines showed activity only at 4 h after *i.p.* administration that means delayed onset however long anticonvulsant action. In this series different pharmacological

Table 5. Quantification studies of selected compounds in the MES, scPTZ, and neurotoxicity tests after oral administration in rats.

Comp.	TPE (h)[a]	MES ED_{50}[b] (mg/kg)	*sc*PTZ ED_{50}[b] (mg/kg)	TD_{50}[b] (mg/kg)	PI[c]
3	4	5.29 (3.48–7.74)	>250	114.4 (81.75–138.57)	21.62 (MES)
7	4 (MES) 1 (*sc*PTZ)	13.11 (8.21–21.78)	17.58 (8.88–28.46)	150	11.44 (MES) 8.53 (*sc*PTZ)
10	2 (MES) 1 (*sc*PTZ)	12.06 (6.47–23.32)	30.78 (12.0–108.36)	>100	>8.29 (MES) >3.25 (*sc*PTZ)
PHT[d]	1	28.1 (27.7–35.20)	>500	>100	>3.60 (MES)
ETX[d]	2	>500	167.00 (116.00–237.00)	>500	>3.0 (*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; TD_{50} – neurotoxicity). [c] Protection index (TD_{50}/ED_{50}). [d] PHT – phenytoin, ETX – ethosuximide reference drugs, data from [24].

properties were observed for compounds with the alkyl or hydroxyalkyl substituents at the piperazine ring (**22–24**). Excluding inactive methyl derivative **22** the other showed activity in MES and additionally in *sc*PTZ screens in both time intervals 0.5 h and 4 h. Similar results were observed for 5-cyclopropyl-5-phenyl analogues (**19–21**). It proves that the introduction of alkyl or hydroxyalkyl groups at the piperazine moiety is especially important for protection against pentylenetetrazole seizures.

Conclusion

The library of twenty two new *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione has been synthesized and tested for anticonvulsant activity. The results of anticonvulsant screening revealed that majority of derivatives were effective in the MES or/and *sc*PTZ screens, the most widely employed seizure models for early identification of candidate anticonvulsants. The quantitative studies in rats after oral administration showed that three compounds were more potent than phenytoin in the maximal electroshock test. The highest activity was observed for **3** with ED_{50} of 5.29 mg/kg (MES), **7** with ED_{50} of 13.11 mg/kg (MES), 17.58 mg/kg (*sc*PTZ) and **10** with ED_{50} of 12.06 mg/kg (MES), 30.78 mg/kg (*sc*PTZ). These molecules were more active in comparison with standard anticonvulsants - phenytoin or/and ethosuximide.

Experimental protocol

Chemistry

All chemicals and solvents were obtained from Merck (Darmstadt, Germany) and were used without purification. 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 the developing system: S_1 benzene/ethylacetate/acetone in the ratio of 10:5:1 (v/v/v) and S_2 methanol/25% ammonia in the ratio of 10:1.5 (v/v). The structures were confirmed by both spectral (^{1}H-NMR, LC/MS) and elemental analysis and the data were within ±0.4% of the theoretical values. Elemental analyses for C, H, N were carried out with an Elementar Vario EL III (Hanau, Germany). ^{1}H-NMR spectra were obtained in a Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, with TMS as an internal standard. Chemical shifts are reported in *d* values (ppm) and *J* values in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Additionally, the liquid chromatography/mass spectrometry (LC/MS) spectra for chosen compounds were obtained on Applied Biosystem/MDS-SCIEX API 2000 with Agilent HPLCs.

*General procedure for the synthesis of 5-cyclopropyl-5-phenyl- (**1**) and 5-cyclopropyl-5-(4-chlorophenyl)-hydantoins (**2**) [16]*

A solution of cyclopropyl-phenyl or cyclopropyl-(4-chlorophenyl)-ketone (0.33 mol) and ammonium carbonate (1 mol) in 50% ethanol was warmed to 50°C, at which time potassium cyanide (0.35 mol) dissolved in 50 mL of water was dropped in over a period of 15 min. The mixture was heated under reflux at 56–60°C for more than 20 h. The reflux condenser was then replaced by an air condenser and the temperature rose to 80°C for 1 h to remove the excess ammonium carbonate. Then the reaction solution was cooled and acidified. The precipitated solids were filtered off, washed with water, and recrystallized from a mixture of ethanol and water.

*5-Cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**1**)*

White powdery crystals. Yield 70%; mp 216–217°C; TLC: $R_f = 0.53$ (S_1); ^{1}H-NMR (300 MHz, DMSO) δ 0.28–0.56 (m, 4H, cyclopropane), 1.54–1.63 (m, 1H, cyclopropane), 7.32–7.55 (m, 5H, ArH), 8.33 (m, 1H, N_1H), 10.76 (s, 1H, N_3H). $C_{12}H_{12}N_2O_2$ (216.42).

*5-Cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**2**)*

White powdery crystals. Yield 64%; mp 255–256°C; TLC: $R_f = 0.59$ (S_1); ^{1}H-NMR (300 MHz, DMSO) δ 0.29–0.52 (m, 4H, cyclopropane), 1.52–1.61 (m, 1H, cyclopropane), 7.45–7.48 (m, 2H, ArH), 7.52–7.57 (m, 2H, ArH), 8.39 (s, 1H, N_1H) 10.93 (s, 1H, N_3H). $C_{12}H_{11}N_2O_2Cl$ (250.68).

*General procedure for the preparation of compounds **3–24***

To a mixture of 5-cyclopropyl-5-phenyl- (**1**) or 5-cyclopropyl-5-(4-chlorophenyl)-hydantoin (**2**) (0.01 mol), 40% solution of formaldehyde (0.01 mol) and corresponding 4-substituted piperazines (0.01 mol) dissolved in 96% ethanol, were added. The mixture was left for ca. 6–12 h at room temperature and then refrigerated at ca. −10°C for 24 h. The precipitated crude products were washed with cold ethanol and separated by filtration. The final compounds were purified by and crystallization from 96% ethanol.

*[R,S]3-[(4-Phenylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**3**)*

White powdery crystals. Yield: 70%; mp 188–190°C; TLC: $R_f = 0.45$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.32–0.41 (m, 1H, cyclopropane), 0.52–0.78 (m, 3H, cyclopropane), 1.68–1.77 (m, 1H, cyclopropane), 2.76–2.80 (t, 4H, piperazine, $J = 4.99$ Hz), 3.15–3.18 (t, 4H, piperazine, $J = 5.00$ Hz), 4.57 (s, 2H, $-CH_2-$), 6.00 (s, 1H, N_1H), 6.82–6.91 (m, 3H, ArH), 7.21–7.42 (m, 5H, ArH), 7.55–7.59 (m, 2H, ArH). $C_{23}H_{26}N_4O_2$ (390.49), $[M + H]^+ = 391.8$.

*[R,S]3-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**4**)*

White powdery crystals. Yield: 81%; mp 188–190°C; TLC: $R_f = 0.61$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.34–0.42 (m, 1H, cyclopropane), 0.54–0.78 (m, 3H, cyclopropane), 1.68–1.78 (m, 1H, cyclopropane), 2.79–2.82 (t, 4H, piperazine, $J = 5.10$ Hz), 3.05–3.08 (t, 4H, piperazine, $J = 5.10$ Hz), 4.55 (s, 2H, $-CH_2-$), 6.19 (s, 1H, N_1H), 6.88–7.07 (m, 5H, ArH), 7.32–7.44 (m, 2H, ArH), 7.56–7.61 (m, 2H, ArH). $C_{23}H_{25}N_4O_2F$ (408.48).

*[R,S]3-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**5**)*

White powdery crystals. Yield: 69%; mp 157–159°C; TLC: $R_f = 0.54$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.33–0.41 (m, 1H, cyclopropane), 0.52–0.77 (m, 3H, cyclopropane), 1.63–1.76 (m, 1H, cyclopropane), 2.78–2.92 (t, 4H, piperazine, $J = 5.02$ Hz), 3.05–3.08 (t, 4H, piperazine, $J = 4.90$ Hz), 4.58 (s, 2H, $-CH_2-$), 5.90–5.98 (brs, 1H, N_1H), 6.78–6.88 (m, 2H, ArH), 6.90–6.98 (m, 2H, ArH), 7.33–7.43 (m, 3H, ArH), 7.52–7.54 (m, 2H, ArH). $C_{23}H_{25}N_4O_2F$ (408.48).

*[R,S]3-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**6**)*

White powdery crystals. Yield: 67%; mp 143–147°C; TLC: $R_f = 0.59$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.33–0.41 (m, 1H, cyclopropane), 0.51–0.72 (m, 3H, cyclopropane), 1.65–1.74 (m, 1H, cyclopropane), 2.68–2.72 (t, 4H, piperazine, $J = 4.97$ Hz), 3.18–3.28 (m, 4H, piperazine), 4.58 (s, 2H, $-CH_2-$), 6.55 (s, 1H, N_1H), 6.98–7.15 (m, 3H, ArH), 7.25–7.39 (m, 4H, ArH),7.45–7.54 (m, 2H, ArH). $C_{24}H_{25}N_4O_2F_3$ (458.49).

*[R,S]3-[(4-Benzylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**7**)*

White powdery crystals. Yield 55%; mp 161–163°C; TLC: $R_f = 0.27$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.33–0.41 (m, 1H, cyclopropane), 0.54–0.75 (m, 3H, cyclopropane), 1.66–1.75 (m, 1H, cyclopropane), 2.42 (brs, 4H, piperazine), 2.65 (brs, 4H, piperazine), 3.48 (s, 2H, $-CH_2-$), 4.49 (s, 2H, $-CH_2-$), 6.32 (s, 1H, N_1H), 7.21–7.42 (m, 8H, ArH), 7.55–7.59 (m, 2H, ArH). $C_{24}H_{28}N_4O_2$ (405.50), $[M + H]^+ = 405.6$.

*[R,S]3-[{4-(2,6-Dichlorobenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**8**)*

White powdery crystals. Yield 46%; mp 75–77°C; TLC: $R_f = 0.89$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.25–0.38 (m, 1H, cyclopropane), 0.50–0.78 (m, 3H, cyclopropane), 1.68–1.74 (m, 1H, cyclopropane), 2.46–2.57 (m, 8H, piperazine), 3.74 (s, 2H, $-CH_2-$), 4.46 (s, 2H, $-CH_2-$), 5.95 (brs, 1H, N_1H), 7.12 (t, 1H, ArH, $J = 7.80$ Hz), 7.26–7.40 (m, 5H, ArH), 7.53–7.57 (m, 2H, ArH). $[M + H]^+$: 473.8. $C_{24}H_{26}N_4O_2Cl_2$ (473.41), $[M + H]^+ = 473.8$.

*[R,S]3-[{4-(4-Chlorobenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**9**)*

White powdery crystals. Yield 67%; m.p. 169–171°C; TLC: $R_f = 0.80$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.36–0.48 (m, 1H, cyclopropane), 0.58–0.79 (m, 3H, cyclopropane), 1.69–1.79 (m, 1H, cyclopropane), 2.42 (brs, 4H, piperazine), 2.68 (m, 4H, piperazine), 3.42 (s, 2H, $-CH_2-$), 4.52 (s, 2H, $-CH_2-$), 6.02 (s, 1H, N_1H), 7.23–7.32 (m, 3H, ArH), 7.36–7.46 (m, 4H, ArH), 7.59–7.62 (m, 2H, ArH). $C_{24}H_{27}N_4O_2Cl$ (438.96).

*[R,S]3-[{4-(3-Methylbenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**10**)*

White powdery crystals. Yield 79%; mp 165–167°C; TLC: $R_f = 0.47$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.36–0.43 (m, 1H, cyclopropane), 0.61–0.79 (m, 3H, cyclopropane), 1.71–1.77 (m, 1H, cyclopropane), 2.36 (s, 3H, $-CH_3$), 2.47 (t, 4H, piperazine, $J = 5.00$ Hz), 2.69 (t, 4H, piperazine, $J = 4.85$ Hz), 3.49 (s, 2H, $-CH_2-$), 4.53 (s, 2H, $-CH_2-$), 6.62 (s, 1H, N_1H), 7.07–7.22 (m, 4H, ArH), 7.38–7.44 (m, 3H, ArH), 7.59–7.60 (m, 2H, ArH). $C_{25}H_{30}N_4O_2$ (418.54).

*[R,S]3-[(4-Phenyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**11**)*

White powdery crystals. Yield 54%; mp 185–187°C; TLC: $R_f = 0.59$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.34–0.38 (m, 1H, cyclopropane), 0.63–0.73 (m, 3H, cyclopropane), 1.63–1.72 (m, 1H, cyclopropane), 2.75 (t, 4H, piperazine, $J = 4.70$ Hz), 3.16 (t, 4H, piperazine, $J = 4.78$ Hz), 4.56 (s, 2H, $-CH_2-$), 6.64 (s, 1H, N_1H), 6.88–6.91 (m, 3H, ArH), 7.25–7.26 (m, 2H, ArH) 7.35–7.38 (m, 2H, ArH), 7.51–7.54 (m, 2H, ArH). $C_{23}H_{25}N_4O_2Cl$ (424.93), $[M + H]^+ = 425.7$.

*[R,S]3-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**12**)*

White powdery crystals. Yield 76%; mp 190–192°C; TLC: $R_f = 0.52$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.35–0.38 (m, 1H, cyclopropane), 0.57–0.73 (m, 3H, cyclopropane), 1,65–1.71 (m, 1H, cyclopropane), 2.79 (t, 4H, piperazine, $J = 4.61$ Hz), 3.00 (t, 4H, piperazine, $J = 4.85$ Hz), 4.55 (s, 2H, $-CH_2-$), 6.13–6,18 (brs, 1H, N_1H), 6.91–7.04 (m, 4H, ArH), 7.38–7.39 (d, 2H ArH, $J = 2.05$ Hz) 7.52–7.53 (d, 2H, ArH, $J = 2.05$ Hz). $C_{23}H_{24}N_4O_2ClF$ (442.92).

*[R,S]3-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**13**)*

White powdery crystals. Yield 81%; mp 188–190°C; TLC: $R_f = 0.49$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.33–0.38 (m,

1H, cyclopropane), 0.56–0.73 (m, 3H, cyclopropane), 1,64–1.69 (m, 1H, cyclopropane), 2.76 (t, 4H piperazine, $J = 4.80$ Hz), 3.07(t, 4H piperazine, $J = 4.88$ Hz), 4.55 (s, 2H, $-CH_2-$), 5.97 (s, 1H, N_1H), 6.83–6.86 (m, 2H, ArH), 6.91–9.95 (m, 2H, ArH) 7.38 (d, 2H, ArH, $J = 2.31$ Hz), 7.51 (d, 2H, ArH, $J = 2.31$ Hz). $C_{23}H_{24}N_4O_2ClF$ (442.92).

*[R,S]3-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**14**)*

White powdery crystals. Yield 63%; mp 155–156°C; TLC: $R_f = 0.31$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.35–0.38 (m, 1H, cyclopropane), 0.65–0.72 (m, 3H, cyclopropane), 1,65–1.72 (m, 1H, cyclopropane), 2.75 (t, 4H piperazine, $J = 4.70$ Hz), 3.07 (t, 4H piperazine, $J = 4.70$ Hz), 4.55 (s, 2H, $-CH_2-$), 6.66 (s, 1H, N_1H), 7.01–7.07 (m, 3H, ArH), 7.30–7.38 (m, 3H, ArH) 7.45–7.54 (m, 2H, ArH). $C_{24}H_{24}N_4O_2ClF_3$ (492.93).

*[R,S]3-[(4-Benzylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**15**)*

White powdery crystals. Yield 52%; mp 156–158°C; TLC: $R_f = 0.43$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.34–0.40 (m, 1H, cyclopropane), 0.56–0.61 (m, 1H, cyclopropane), 0.65–0.73 (m, 2H, cyclopropane), 1.63–1.69 (m, 1H, cyclopropane), 2.43 (brs, 4H, piperazine), 2.64 (brs, 4H, piperazine), 3.49 (s, 2H, $-CH_2-$), 4.43 (s, 2H, $-CH_2-$), 5.90 (s, 1H, N_1H), 7.20–7.36 (m, 4H, ArH), 7.39–7.42 (m, 2H, ArH) 7.50–7.55 (m, 3H, ArH). $C_{24}H_{27}N_4O_2Cl$ (438.95), $[M + H]^+ = 439.4$.

*[R,S]3-[{4-(2,6-Dichlorobenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**16**)*

White powdery crystals. Yield 71%; mp 159–161°C; TLC: $R_f = 0.59$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.30–0.34 (m, 1H, cyclopropane), 0.56–0.71 (m, 3H, cyclopropane), 1,61–1.64 (m, 1H, cyclopropane), 2.54–2.56 (m, 8H, piperazine), 3.72 (s, 2H, $-CH_2-$), 4.47 (s, 2H, $-CH_2-$), 5.93 (s, 1H, N_1H), 7.26–7.34 (m, 3H, ArH), 7.36–7.378 (d, 2H, ArH, $J = 2.05$ Hz) 7.49–7.50 (d, 2H, ArH, $J = 2.31$ Hz). $C_{24}H_{25}N_4O_2Cl_3$ (507.85).

*[R,S]3-[{4-(4-Chlorobenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**17**)*

White powdery crystals. Yield 70%; mp 158–160°C; TLC: $R_f = 0.84$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.31–0.38 (m, 1H, cyclopropane), 0.58–0.61 (m, 1H, cyclopropane), 0.63–0.79 (m, 2H, cyclopropane), 1.61–1.70 (m, 1H, cyclopropane), 2.41 (brs, 4H, piperazine), 2.63 (brs, 4H, piperazine), 3.46 (s, 2H, $-CH_2-$), 4.52 (s, 2H, $-CH_2-$), 5.93 (s, 1H, N_1H), 7.24–7.35 (m, 4H, ArH), 7.40 (d, 2H, ArH, $J = 4.50$ Hz) 7.54 (d, 2H, ArH, $J = 4.50$ Hz). $C_{24}H_{25}N_4O_2Cl_2$ (472.40).

*[R,S]3-[{4-(3-Methylbenzyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**18**)*

White powdery crystals. Yield 43%; mp 138–140°C; TLC: $R_f = 0.42$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.32–0.38 (m, 1H, cyclopropane), 0.54–0.61 (m, 1H, cyclopropane), 0.62–0.76 (m, 2H, cyclopropane), 1,60–1.69 (m, 1H, cyclopropane), 2.33 (s, 3H, $-CH_3$), 2.43 (brs, 4H, piperazine), 2.64 (brs, 4H, piperazine), 3.46 (s, 2H, $-CH_2-$), 4.47 (s, 2H, $-CH_2-$), 6.20 (s, 1H, N_1H), 7.04–7.21 (m, 4H, ArH), 7.40 (d, 2H, ArH, $J = 4.60$ Hz) 7.55 (d, 2H, ArH, $J = 4.60$ Hz). $C_{25}H_{29}N_4O_2Cl$ (452.99), $[M + H]^+ = 453.5$.

*[R,S]3-[(4-Methyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**19**)*

White powdery crystals. Yield 53%; mp 62–64°C; TLC: $R_f = 0.59$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.36–0.38 (m, 1H, cyclopropane), 0.52–0.59 (m, 1H, cyclopropane), 0.60–0.76 (m, 2H, cyclopropane), 1.64–1.74 (m, 1H, cyclopropane), 2.26 (s, 3H, $-CH_3$), 2.42 (brs, 4H, piperazine), 2.68 (brs, 4H, piperazine), 4.45 (s, 2H, $-CH_2-$), 6.02 (s, 1H, N_1H), 7.35–7.60 (m, 5H, ArH). $C_{18}H_{24}N_4O_2$ (328.42).

*[R,S]3-[(4-Ethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**20**)*

White powdery crystals. Yield 77%; mp 78–80°C; TLC: $R_f = 0.72$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.31–0.39 (m, 1H, cyclopropane), 0.51–0.59 (m, 1H, cyclopropane), 0.60–0.73 (m, 2H, cyclopropane), 1.04 (t, 3H, $-CH_3$, $J = 6.0$ Hz), 1.63–1.72 (m, 1H, cyclopropane), 2.35–2.42 (m, 6H, 4H, piperazine, 2H $-CH_2-$), 2.67 (t, 4H, piperazine, $J = 5.00$ Hz), 4.42 (s, 2H, $-CH_2-$), 6.20 (s, 1H, N_1H), 7.31–7.58 (m, 5H, ArH). $C_{19}H_{26}N_4O_2$ (342.45).

*[R,S]3-[(4-Hydroxyethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**21**)*

White powdery crystals. Yield 48%; mp 168–170°C; TLC: $R_f = 0.44$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 0.32–0.40 (m, 1H, cyclopropane), 0.52–0.77 (m, 3H, cyclopropane), 1.67–1.76 (m, 2H, 1H, cyclopropane, 1H, OH), 2.48–2.52 (m, 6H, 4H, piperazine, 2H, $-CH_2-$), 2.64 (t, 4H, piperazine, $J = 5.02$ Hz), 3.55 (t, 2H, $-CH_2-$, $J = 7.0$ Hz), 4.50 (s, 2H, $-CH_2-$), 6.09 (s, 1H, N_1H), 7.32–7.43 (m, 3H, ArH), 7.54–7.58 (m, 2H, ArH). $C_{19}H_{26}N_4O_3$ (358.44).

*[R,S]3-[(4-Methyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**22**)*

White powdery crystals. Yield 92%; mp 68–71°C; TLC: $R_f = 0.84$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.38–0.39 (m, 1H, cyclopropane), 0.53–0.58 (m, 1H, cyclopropane), 0.67–0.69 (m, 2H, cyclopropane), 1.61–1.67 (m, 1H, cyclopropane),

2.35 (s, 3H, $-CH_3$), 2.56 (brs, 4H, piperazine), 2.74 (brs, 4H, piperazine), 4.49 (s, 2H, $-CH_2-$), 6.42–6.52 (brs, 1H, N_1H), 7.36 (d, 2H, ArH, $J = 8.72$ Hz), 7.51–7.54 (m, 2H, ArH). $C_{18}H_{23}N_4O_2Cl$ (362.86), $[M + H]^+ = 363.4$.

*[R,S]3-[(4-Ethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**23**)*

White powdery crystals. Yield 57%; mp 83–86°C; TLC: $R_f = 0.39$ (S_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.37–0.38 (m, 1H, cyclopropane), 0.53–0.57 (m, 1H, cyclopropane), 0.66–0.69 (m, 2H, cyclopropane), 1.09–1.15 (m, 3H, $-CH_3$), 1.61–1.66 (m, 1H, cyclopropane), 2.54–2.65 (m, 6H, 4H, piperazine, 2H, $-CH_2$), 2.74 (brs, 4H, piperazine), 4.48 (s, 2H, $-CH_2-$), 6.44 (s, 1H, N_1H), 7.35–7.38 (d, 2H, ArH, $J = 8.72$ Hz), 7.52–7.55 (m, 2H, ArH). $C_{19}H_{25}N_4O_2Cl$ (376.89).

*[R,S]3-[(4-Hydroxyethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**24**)*

White powdery crystals. Yield: 63%; mp 189–191°C; TLC: $R_f = 0.46$ (S_1); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.31–0.38 (m, 1H, cyclopropane), 0.68–0.77 (m, 3H, cyclopropane), 1.62–1.71 (m, 2H, 1H, cyclopropane, 1H, OH), 2.49–2.51 (m, 6H, 4H, piperazine, 2H, $-CH_2-$), 2.58 (t, 4H, piperazine, $J = 4.90$ Hz), 3.57 (t, 2H $-CH_2-$, $J = 5.20$ Hz), 4.49 (s, 2H, $-CH_2-$), 6.01 (s, 1H, N_1H), 7.37–7.42 (m, 2H, ArH), 7.52–7.60 (m, 2H, ArH). $C_{19}H_{25}N_4O_3Cl$ (392.89).

Pharmacology

Compounds **3–24** were pharmacologically pre-evaluated within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NIH/NINDS), Rockville, MD, USA, by using procedures described elsewhere [19, 20].

Phase I studies of the investigated compounds involved three tests: Maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ), and rotorod test for neurological toxicity (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 to mice as a suspension in 0.5% methylcellulose/water mixture at a dose level 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 administrated orally into 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 are 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 five seconds in 97% (CD_{97}) of animals tested. At the anticipated time of testing the pentylenetetrazole is administrated subcutaneously.

Neurological toxicity (NT)

The neurological toxicity test (NT) induced by a compound was detected in mice using standardized rotorod test [21]. 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

The quantitative determination of the median effective dose (ED_{50}) and the median neurotoxic dose (TD_{50}) values were performed at previously estimated time of peak effect after oral 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 [22].

The authors wish to thank Dr James Stables for providing them with pharmacological data through the Antiepileptic Drug Development Program (Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Rockville, MD, USA).

We are pleased to acknowledge the generous financial support of this work by the grant of the Minister Committee for Scientific Research, Poland (Grant No N405 298536).

The authors have declared no conflict of interest.

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