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Synthesis and anticonvulsant activity of new *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl- and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-diones

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ABSTRACT

Synthesis, physicochemical and anticonvulsant properties of new *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl- and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-diones have been described. Initial anticonvulsant screening was performed using intraperitoneal (ip.) maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure tests. The neurotoxicity was determined applying the rotarod test. The in vivo results in mice showed that all compounds were effective especially in the MES screen. The quantitative evaluation after oral administration in rats showed that the most active was 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**1**) with ED_{50} values of 5.76 mg/kg (MES) and 57.31 mg/kg (*sc*PTZ). This molecule was more potent than phenytoin and ethosuximide which were used as reference antiepileptic drugs. Additionally compound **1** with ED_{50} of 26.06 mg/kg in psychomotor seizure test (6-Hz) in mice showed comparable activity to new generation anticonvulsant – levetiracetam.

1. Introduction

During the last two decades several new antiepileptic drugs (AEDs) have been marked but still about one third of patients with epilepsy do not respond for the applied treatment. Furthermore many of those medications cause serious side effects from which more common are ataxia, nausea, mental dulling, and hepatotoxicity.[1–3] The currently used AEDs can be classified into four categories on the basis of the main molecular mechanisms of action, as follow: (i) modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, (ii) enhancement of GABA-mediated inhibition or other effect on the GABA system, (iii) inhibition of synaptic excitation mediated by ionotropic glutamate receptors.[4]

Conceptually, there are two different methods of obtaining new anticonvulsants namely knowledge-based approaches and screening approaches. Knowledge-based approaches rely 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. Serendipitous approaches involve screening of either diverse or focus compound libraries.[5]

It is well documented that the important core fragment of anticonvulsants is defined by nitrogen heteroatomic system, usually a cyclic imide, at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system.[6–8] This common template is present in the structures of two old, however, well-established AEDs such as ethosuximide and phenytoin as well as among the newest drugs, for example, levetiracetam, brivaracetam, or seletracetam.[9–11] Taking into consideration the above our researches are focused on systematic structural modifications in a group of pyrrolidine-2,5-dione and imidazolidine-2,4-dione derivatives.[12–17] The structure–activity relationships (SARs) studies performed among these molecules showed higher activity for differently substituted imidazolidine-2,4-diones in comparison to respective pyrrolidine-2,5-dione analogs.[18] Therefore in our recent studies we have demonstrated the potent anticonvulsant activity among the *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl-imidazolidine-2,4-diones.[19] Several compounds showed activity in the MES test that was superior to phenytoin, used as a model substance (Fig. 1).

Following these findings, as part of our efforts to design new anticonvulsant agents in the present studies we have synthesized a new series of *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl- (**1**) and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-diones (**2**). These molecules have been designed as analogs of compounds **A** and **B** (Fig. 1) in which an amine function have been modified. The main goal was to obtain compounds active both in maximal electroshock seizure (MES) and the subcutaneous pentylenetetrazole (*sc*PTZ) screens. As basic fragments we have introduced different piperazines with alkyl, alkylene, ketone,

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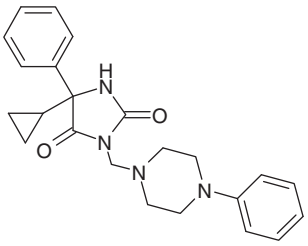
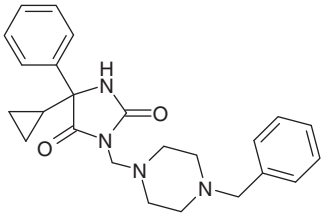


A

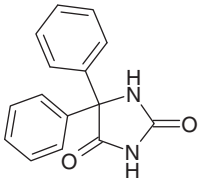
ED_{50} = 5.29 mg/kg (MES, rats, *p.o.*)



B

ED_{50} = 13.11 mg/kg (MES, rats, *p.o.*)

ED_{50} = 17.58 mg/kg (*sc*PTZ, rats, *p.o.*)



Phenytoin

ED_{50} = 28.10 mg/kg (MES, rats, *p.o.*)

Figure 1. Structures of model compounds.

or ester moieties at position-4. Finally, the piperazine fragment has been changed into tetrahydroisoquinoline or morpholine.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **1–16** was accomplished as shown in Scheme 1. The starting 5-cyclopropyl-5-phenyl- (**1**) and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**2**) were obtained from the appropriately substituted ketones by means of the Bücherer–Berg reaction with modifications described by Goodson et al.[20] In the next step the aminoalkylation of the acidic proton (N^3H) of **1** and **2** carried out in the present of formaldehyde and appropriate amines enabled to obtain final compounds **3**–**16**. The following secondary amines were used: 1-(2-phenethyl)-piperazine (**3**, **10**), 1-(3-phenylprop-2-en-1-yl)-piperazine (**4**), 1-(1-phenethyl)-piperazine (**5**, **11**), phenyl-(piperazin-1-yl)-methanone (**6**), benzyl piperazine-1-carboxylate (**7**, **12**), ethyl piperazine-1-carboxylate (**8**), 1-(2-methoxyethyl)-piperazine (**9**), tetrahydro-isoquinoline (**13**, **15**) and morpholine (**14**, **16**). The reaction was carried out in ethanol at a room temperature for ca. 12 h. The crude products were crystallized from ethanol giving the final compound in yields ranging from 46% to 85%. The structures of compounds synthesized were confirmed by both spectral and elemental analysis. The detailed physical and analytical data are listed in the experimental section.

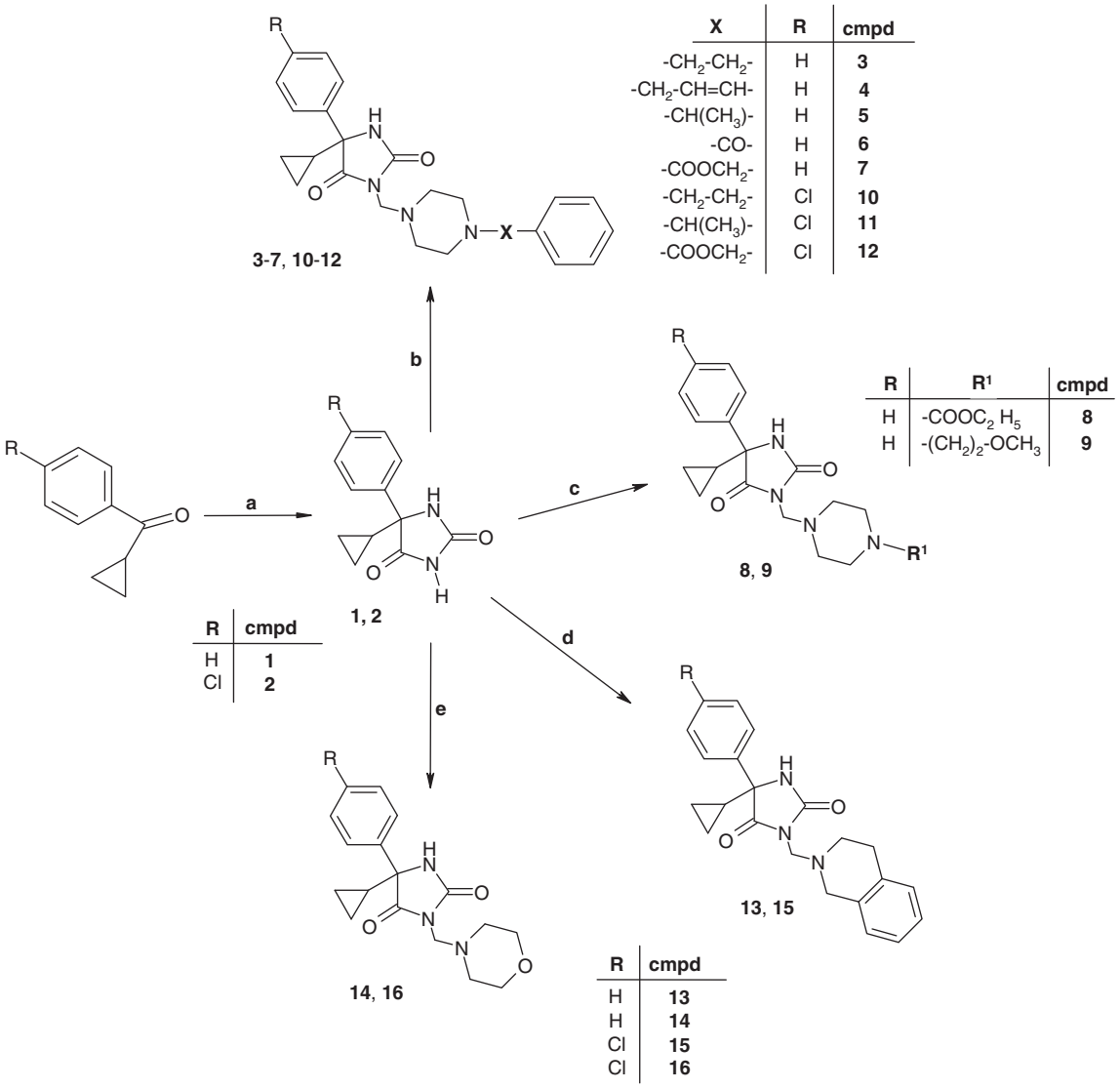
2.2. Anticonvulsant activity

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 6-Hz model. From these tests the MES and *sc*PTZ screens are recognized as the 'gold standards' in the early stages of testing.[21] Furthermore, the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests are claimed to detect compounds affording protection against generalized tonic-clonic seizures and generalized absence seizures, respectively.[22]

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

The most active in the mice ip. MES screen were **1**, **3**, and **14** that showed protection at dose of 30 mg/kg. It was comparable with phenytoin used as reference drug preventing electrically induced seizures. The lower anti-MES activity was observed for **4**–**9**, **12**, **16** (100 mg), and **2**, **10**, **11**, **13**, and **15** (300 mg/kg). Second, routinely used convulsive screen is *sc*PTZ test which identifies substances elevating seizure threshold. The strongest anti-*sc*PTZ activity showed unsubstituted 5-cyclopropyl-5-phenyl- and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-diones (**1**, **2**) that protected mice at a dose of 30 mg/kg. The other molecules were less active, namely 100 mg/kg (**4**, **6**, **8**–**10**, **14**, and **16**) or 300 mg/kg (**3**, **5**, **7**, and **13**). Many of these compounds revealed activity equivalent to ethosuximide used as a model anticonvulsant for this type of seizures. Furthermore they were active in both time intervals (0.5 and 4 h) that indicates quick onset and long duration of anticonvulsant action. In the neurotoxicity screen (NT), compounds **5**–**7** and **15** did not show neurotoxicity in the maximum dose administered – 300 mg/kg. Compounds **1**–**4**, **8**, **11**, **12**, and **16** exhibited motor impairment at the dose of 300 mg/kg. The other derivatives revealed neurotoxicity at doses of 30 mg/kg (**9**, **10**) or 100 mg/kg (**13**, **14**). Molecules neurotoxic at dose of 30 mg/kg have been excluded from further testing.

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. Therefore on the basis of the data obtained in ip. screen in mice and according to the Antiepileptic Drug Development (ADD) Program disposition, seven compounds were selected and examined for their anticonvulsant activity in the MES and NT screens after p.o. administration into rats at a dose of 30 mg/kg (Table 2). The highest activity was observed for **1** that protected 100% of animals in all time intervals that means quick onset an long duration of anticonvulsant action. These molecule was more potent than phenytoin,



Scheme 1. Synthetic pathways of compounds **1**–**16**. Reagents and reaction conditions: (a) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol; (b and c) 4-substituted piperazine derivatives, formaldehyde, 96% ethyl alcohol, 12 h room temperature; (d) tetrahydroisoquinoline, formaldehyde, 96% ethyl alcohol, 12 h room temperature; (e) morpholine, formaldehyde, 96% ethyl alcohol, 12 h room temperature.

used as a model substance effective in electrically induced seizures. The other molecules **3**, **5**, **6**, **7**, **13**, and **14** protected from 25% to 100% of rats. As a continuation of pharmacological studies four molecules **1**, **2**, **14**, and **16** active in pentylenetetrazole seizures in mice were tested in *sc*PTZ screen after p.o. administration into rats. Initially a dose of 50 mg/kg was employed and the anticonvulsant activity was assessed at five time points (Table 3). In this test, the most effective were compounds **1**, **2**, and **16** that showed equable profile of activity and protected up to 75% of tested animals. These molecules were more potent in comparison with ethosuximide which is known as a model anticonvulsant for pentylenetetrazole seizures.

From the whole series six compounds have been chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative evaluation of the median effective dose (ED_{50}) in the MES and *sc*PTZ tests as well as toxic dose (TD_{50}) were performed at previously estimated time of peak effect (TPE) after oral administration into rats. Results of the quantitative tests along with the data for the standard drugs phenytoin and ethosuximide are shown in Table 4.

The analysis of quantitative p.o. data revealed that all compounds tested showed higher activity than phenytoin in the electrically induced seizures. Moreover **1** and **2** were more potent than ethosuximide in the *sc*PTZ screen. These data confirmed potential effectiveness of **1** in treating both generalized tonic–clonic epilepsy and absence seizures. Unfortunately compound **1** displayed higher neurotoxicity as well as lower protection index in comparison to both phenytoin and ethosuximide. The inhibition of electrically induced seizures that is characteristic for phenytoin and phenytoin-like drugs may indicate the influence of compounds on voltage-depended Na^+ channels as the most plausible mechanism of anticonvulsant action.

According to the Antiepileptic Drug Development (ADD) Program disposition compounds **1**, **2**, and **16** were chosen for the evaluation of anticonvulsant activity in the 6-Hz test. The 6-Hz screen has been validated recently as a model of therapy-resistant epilepsy. It was not used widely because of its lack clinical validity since the hydantoins such as phenytoin failed to show protective activity. Nevertheless, the clinically effective antiepileptic drug levetiracetam, which is not active in the conventional MES and

Table 1
Anticonvulsant activity after intraperitoneal administration to mice (**1**–**16**)

Cmpd	R	X	R^1	log*P*[a]	MES[b]		*sc*PTZ[c]		NT[d]	
					0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1	H	–	–	1.66	30	30	30	100	300^{14}	300^{14}
2	Cl	–	–	2.63	–	300	300	30	–	300
3	H	$-CH_2-CH_2-$	–	2.98	–	30	–	300^{25}	–	300
4	H	$-CH_2-CH{=}CH-$	–	3.30	100	100	100	300	300^{14}	300^{13}
5	H	$-CH(CH_3)-$	–	3.19	300	100	–	300^{25}	–	–
6	H	CO	–	2.17	100	100	100^{25}	300^{25}	–	–
7	H	$-COOCH_2-$	–	2.92	300	100	300^{25}	300^{25}	–	–
8	H	–	$-COOC_2H_5$	1.76	100	100	100	300	$300^{33,14}$	300^{14}
9	H	–	$-(CH_2)_2OCH_3$	1.46	100	100	100^{25}	100^{25}	30	30
10	Cl	$-CH_2-CH_2-$	–	3.97	300	300	300^{25}	100^{25}	30	30
11	Cl	$-CH(CH_3)-$	–	3.90	300	300	–	–	300^{14}	–
12	Cl	$-COOCH_2-$	–	3.65	–	100	–	–	300	300
13	H	–	–	3.02	300	300	–	300^{25}	100	–
14	H	–	–	1.33	100	30	100	300	100^{14}	300^{13}
15	Cl	–	–	3.71	–	300	–	–	–	–
16	Cl	–	–	2.00	100	100	100	300	300^{14}	300^{13}
I[e]					–	100	300	–	–	–
II[e]					–	100	–	–	–	–
III[e]					–	100	–	–	–	300^{14}
IV[e]					–	300	–	–	–	–
PHT[f]					30	30	–	–	100	100
ETX[g]					–	–	100	300	–	–

Doses of 30, 100, and 300 mg/kg were administrated intraperitoneally in mice. The figures in the table 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).
Response comments: [13]loss of righting reflex, [14]unable to grasp rotorod, [25]myoclonic jerks, [33]tremors.
[a] Log *P* calculated by use of Pallas 3.2.1.4 program.
[b] Maximal electroshock test.
[c] Subcutaneous pentylenetetrazole test.
[d] Neurotoxicity screening – rotorod test.
[e] Data from Ref. 19: **I**-3-[(4-Phenylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione, **II**-3-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione, **III**-3-[(4-Phenyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione, **IV**-3-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione.
[f] **PHT**-phenytoin, reference drug, data from Ref. 29.
[g] **ETX**-ethosuximide, reference drug, data from Ref. 29.

Table 2
Anticonvulsant activity (MES test) of selected compounds administrated orally to rats

Cmpd	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
1	4	4	4	4	4	0	0	0	0	0
3	0	0	3	3	4	0	0	0	0	0
5	2	1	2	3	3	0	0	0	0	0
6	0	0	3	3	3	0	0	0	0	0
7	0	0	1	1	4	0	0	0	0	0
13	0	0	2	3	4	0	0	0	0	0
14	2	2	2	3	2	0	0	0	0	0
PHT[c]	1	4	3	3	3	0	0	0	0	0

[a] The data indicate the number of rats of four that were protected at a dose of 30 mg/kg.
[b] Neurotoxicity screening – 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 Ref. 30.

Table 3
Anticonvulsant activity (*sc*PTZ test) of selected compounds administrated orally to rats

Cmpd	*sc*PTZ[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
1	0	2	2	3	2	0	0	0	0	0
2	0	2	1	2	3	0	0	0	0	0
14	0	0	0	4	1	0	0	0	0	0
16	0	2	3	2	2	0	0	0	0	0
ETX[c]	0	2	1	1	0	0	0	0	0	0

[a] The data indicate the number of rats of four that were protected at a dose of 50 mg/kg.
[b] Neurotoxicity screening – rotorod test. The data in indicate the number of rats of four in which neurotoxicity was observed at a dose of 50 mg/kg.
[c] **ETX**-ethosuximide, reference drug, data from Ref. 29.

*sc*PTZ tests, 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.[23] The results obtained are shown in Table 5. As can be seen from mice ip. data, the most active was compounds **1** that showed maximal 100% protection at time points 0.5, 1, and 2 h. The other molecules were less active and protected up to 75% of mice. The quantitative studies (Table 6) performed for 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**1**) revealed comparable ED_{50} value with result observed for levetiracetam that was used as model anticonvulsant. It should be noticed that levetiracetam is one of the newest and the most effective antiepileptic drugs marked during last years.

2.3. Structure–activity relationships

The results of preliminary anticonvulsant screening in mice enable to draw some general conclusions about the relations between structure and activity. From the whole series the most active was unsubstituted 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**1**), therefore the presence of the acidic proton (N^3H) seems to be important, however, not crucial for anticonvulsant activity. In general, introduction of chloro atom at *para* position of the 5-phenyl ring (**2**, **10**–**12**, **15**, and **16**) decreased activity in comparison to unsubstituted compounds (**1**, **3**–**9**, **13**, and **14**). Further analysis showed that compounds with dihydro-1*H*-isoquinoline moiety (**13**, **15**) revealed lower protection in comparison to piperazine (**3**–**12**) or morpholine (**14**, **16**) derivatives. Except of **11** and **12**

Table 4
Quantification studies of selected compounds in rats after oral administration

Cmpd	TPE (h)[a]	MES ED_{50}[b] (mg/kg)	*sc*PTZ ED_{50}[b] (mg/kg)	NT TD_{50}[c] (mg/kg)	PI[d]
1	2	5.76 (3.81–7.50)[e]	57.31 (38.61–82.88)	77.57 (53.36–110.57)	13.47 (MES) 1.35 (*sc*PTZ)
2	2	ND	84.98 (44.87–336.42)	>135	>1.60 (MES)
3	4	17.06 (10.13–28.38)	ND	>100	>5.86 (MES)
5	2	10.92 (5.91–19.16)	<100	>200	>18.30 (MES)
7	6	10.03 (3.88–20.25)	ND	>60	>5.98 (MES)
14	4	13.90 (8.72–24.90)	ND	<150	<10.79 (MES)
PTH[f]	1	28.1 (27.7–35.20)	>500	>1000	>35.58 (MES)
ETX[f]	2	>500	167.00 (116.00–237.00)	>500	>3.0 (*sc*PTZ)

ND – not determined.
[a] Time to peak effect.
[b] ED50 – median effective dose required to assure anticonvulsant protection in 50% animals.
[c] TD_{50} – median toxic dose eliciting minimal neurological toxicity in 50% animals.
[d] PI – protective index (TD_{50}/ED_{50}).
[e] 95% Confidence limits given in parentheses.
[f] Reference drugs, data for phenytoin (**PHT**) and ethosuximide (**ETX**), Ref. 31.

Table 5
Anticonvulsant activity – psychomotor seizure test (6-Hz, current 32 mA)

Cmpd	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
1	1	4	4	4	2
2	0	0	2	2	1
16	0	2	3	2	2

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

Table 6
Quantification data – psychomotor seizure test (6 Hz, current 32 mA) after ip. injection into mice

Cmpd	TPE (h)[a]	ED_{50}[b] (mg/kg)	TD_{50}[c] (mg/kg)	PI[d] (TD_{50}/ED_{50})
1	2	26.06 (18.80–26.16)	ND	ND
Levetiracetam[e]	1	19.40 (9.90–36.0)	>500	>26

ND – not determined.
[a] Time to peak effect.
[b] ED_{50} – median effective dose required to assure anticonvulsant protection in 50% animals.
[c] TD_{50} median toxic dose eliciting minimal neurological toxicity in 50% animals.
[d] PI – protective index (TD_{50}/ED_{50}).

the introduction of alkyl, alkylene, ketone or ester linkers between piperazine and phenyl ring extended anticonvulsant protection to pentylenetetrazole seizures as well as made compounds active in both time intervals (0.5 and 4 h in mice). It should be noticed as described previously,[19] the 4-phenylpiperazine derivatives were active exclusively in maximal electroshock (MES) test and showed activity only at 4 h after ip. administration in mice (the data for selected compounds **I–IV** from Ref. 19 are shown in Table 1).

3. Conclusion

The library of sixteen new *N*-Mannich bases derived from 5-cyclopropyl-5-phenyl- and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione derivatives has been synthesized and tested for anticonvulsant activity. The results of anticonvulsant screening revealed that some of derivatives were effective in the MES test. The quantitative studies in rats after oral administration showed that five compounds were more potent than phenytoin in the maximal electroshock test. The highest activity was observed for 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione that showed additionally superior or comparable activity to other model anticonvulsants, namely ethosuximide and levetiracetam.

4. Experimental section

4.1. Chemistry

All chemicals and solvents were obtained from Merck (Darmstadt, Germany) and were used without purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and homogeneity of the compounds were assessed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany), using the developing system consisted of S_1: methanol: 25% ammoniac (10:1.5, v/v). Spots were detected by their absorption under UV light ($\lambda = 254$ nm) and by visualization with 0.05 mol I_2 in 10% HCl. The structures were confirmed by both spectral (^{1}H NMR, LC/MS) and elemental analysis. Elemental analysis for C, H, and N were carried out by a micro method using the elemental Vario EI III elemental analyzer (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values. ^{1}H NMR spectra were obtained in a Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, with TMS as an internal standard. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), m (multiplet). The liquid chromatography/mass spectrometry (LC/MS) spectra for chosen compounds were obtained on Applied Biosystem/MDS-SCIEX API 2000 with Agilent HPLCs.

The synthesis, physicochemical and spectral data of 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (**1**) and 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (**2**) were described elsewhere.[19]

4.1.1. General procedure for preparation of compounds 3–16

To a mixture of 5-cyclopropyl-5-phenyl- (0.01 mol; **1**) or 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (0.01 mol; **2**), 40% solution of formaldehyde (0.01 mol) in 96% ethanol (20 mL), corresponding 4-substituted piperazines (0.01 mol), tetrahydroisoquinoline (THIQ) or morpholine dissolved in 96% ethanol (10 mL) were added. The mixture was left 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.

4.1.1.1. 3-[(4-Phenethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (3). White powdery crystals. TLC: R_f = 0.59 (S_1), yield: 66%, mp 86–88 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32–0.38 (m, 1H, cyclopropane), 0.53–0.59 (m, 1H, cyclopropane), 0.64–0.75 (m, 2H, cyclopropane), 1.58–1.68 (m, 1H, cyclopropane), 2.40–2.82 (m, 10H, 8H, piperazine, 2H, CH_2), 3.65–3.74 (m, 2H, CH_2), 4.46 (s, 2H, CH_2), 6.00 (s, 1H, N_1H), 7.15–7.26 (m, 5H, ArH), 7.36–7.40 (m, 3H, ArH), 7.49–7.52 (m, 2H, ArH). Anal. Calcd for $C_{25}H_{30}N_4O_2$: C, 71.74; H, 7.22; N, 13.39. Found: C, 71.00; H, 7.33; N, 12.78.

4.1.1.2. 3-[{4-(3-Phenylallyl)-piperazin-1-yl}-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (4). White powdery crystals. TLC: R_f = 0.84 (S_1), yield: 46%, mp 138–140 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.30-0.38 (m, 1H, cyclopropane), 0.52–0.73 (m, 3H, cyclopropane), 1.64–1.73 (m, 1H, cyclopropane), 2.48 (t, 4H, piperazine, J = 4.75 Hz), 2.68 (t, 4H, piperazine, J = 4.80 Hz), 3.12 (d, 2H, CH_2, J = 7.00 Hz), 4.45 (s, 2H, CH_2), 5.06 (s, 1H, N_1H), 6.17–6.27 (m, 1H, CH), 6.52 (d, 1H, CH, J = 16.00 Hz), 7.18–7.42 (m, 8H, ArH), 7.55–7.57 (m, 2H, ArH). Anal. Calcd for $C_{26}H_{30}N_4O_2$: C, 72.35; H, 7.00; N, 12.98. Found: C, 71.35; H, 6.58; N, 12.92. LC/MS: m/z calcd for $C_{26}H_{30}N_4O_2$ [M+H]$^+$: 431.24, found: 431.30.

4.1.1.3. 3-[4-(1-Phenyl-ethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (5). White powdery crystals. TLC: R_f = 0.58 (S_1), yield: 80%, mp 73–75 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32-0.38 (m, 1H, cyclopropane), 0.48–0.72 (m, 2H, cyclopropane), 1.32 (s, 3H, –CH_3), 1.60–1.75 (m, 2H, cyclopropane), 2.40–2.59 (m, 8H, piperazine), 3.66–3.72 (m, 1H, CH), 4.46 (s, 2H, CH_2), 6.05 (s, 1H, N_1H), 7.12–7.26 (m, 5H, ArH), 7.34–7.42 (m, 3H, ArH), 7.53–7.57 (m, 2H, ArH). Anal. Calcd for $C_{25}H_{30}N_4O_2$: C, 71.74; H, 7.22; N, 13.39. Found: C, 71.54; H, 7.20; N, 12.68.

4.1.1.4. 3-[(4-Benzoyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (6). White powdery crystals. TLC: R_f = 0.80 (S_1), yield: 56%, mp 142–144 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32–0.42 (m, 2H, cyclopropane), 0.48–0.57 (m, 1H, cyclopropane), 0.63–0.68 (m, 1H, cyclopropane), 1.23–1.32 (m, 1H, cyclopropane), 2.63–2.72 (m, 4H, piperazine), 3.46–3.74 (m, 2H, piperazine), 3.75–3.84 (m, 2H, piperazine), 4.53 (s, 2H, CH_2), 6.00 (s, 1H, N_1H), 7.21–7.36 (m, 5H, ArH), 7.40–7.49 (m, 3H, ArH), 7.54–7.57 (m, 2H, ArH). Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 68.88; H, 6.26; N, 13.39. Found: C, 68.83; H, 6.15; N, 12.97. LC/MS: m/z calcd for $C_{24}H_{26}N_4O_2$ [M+H]$^+$: 419.20, found: 419.50.

4.1.1.5. 3-[(4-Benzyloxycarbonyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (7). White powdery crystals. TLC: R_f = 0.72 (S_1), yield: 65%, mp 67–69 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.34–0.38 (m, 1H, cyclopropane), 0.42–0.56 (m, 1H, cyclopropane), 0.60–0.76 (m, 2H, cyclopropane), 1.61–1.70 (m, 1H, cyclopropane), 2.58 (t, 4H, piperazine, J = 4.74 Hz), 3.48 (t, 4H, piperazine, J = 4.80 Hz), 4.49 (s, 2H, CH_2), 5.10 (s, 2H, COOCH_2) 6.48 (br s, 1H, N_1H), 7.29–7.34 (m, 5H, ArH), 7.40–7.44 (m, 3H, ArH) 7.48–7.52 (m, 2H, Ph). Anal. Calcd for $C_{25}H_{28}N_4O_4$: C, 66.95; H, 6.29; N, 12.49. Found: C, 66.38; H, 6.54; N, 12.13.

4.1.1.6. 3-[(4-Ethoxycarbonyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (8). White powdery crystals. TLC: R_f = 0.77 (S_1), yield: 53%, mp 79–81 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32–0.39 (m, 1H, cyclopropane), 0.43–0.59 (m, 1H, cyclopropane), 0.61–0.74 (m, 2H, cyclopropane), 1.13 (s, 3H, CH_3), 1.65–1.75 (m, 1H, cyclopropane), 2.58 (t, 4H, piperazine, J = 5.00 Hz), 3.42 (t, 4H, piperazine, J = 5.00 Hz), 4.09 (q, 2H, CH_2, J = 7.00 Hz), 4.48 (s, 2H, CH_2), 6.54 (s, 1H, N_1H), 7.32–7.43 (m, 3H, ArH), 7.54–7.58 (m, 2H, ArH). Anal. Calcd for $C_{20}H_{26}N_4O_4$: C, 62.15; H, 6.78; N, 14.49. Found: C, 61.83; H, 6.68; N, 14.34.

4.1.1.7. 3-[4-(2-Methoxyethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (9). White powdery crystals. TLC: R_f = 0.68 (S_1), yield: 83%, mp 97–103 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.31–0.38 (m, 1H, cyclopropane), 0.54–0.59 (m, 1H, cyclopropane), 0.63–0.72 (m, 2H, cyclopropane), 1.64–1.73 (m, 1H, cyclopropane), 2.42–2.57 (m, 6H, 4H, piperazine, 2H, CH_2), 2.69 (t, 4H, piperazine, J = 4.90 Hz), 3.31 (s, 3H, CH_3), 3.50 (s, 2H, CH_2), 4.49 (s, 2H, CH_2), 5.80 (s, 1H, N_1H), 7.32–7.58 (m, 5H, ArH). Anal. Calcd for $C_{20}H_{28}N_4O_3$: C, 64.49; H, 7.58; N, 15.04. Found: C, 64.15; H, 7.63; N, 14.93. LC/MS: m/z calcd for $C_{20}H_{28}N_4O_3$ [M+H]$^+$: 473.22, found: 473.50.

4.1.1.8. 3-[(4-Phenethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (10). White powdery crystals. TLC: R_f = 0.59 (S_1), yield: 83%, mp 84–87 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.31–0.38 (m, 1H, cyclopropane), 0.54–0.62 (m, 1H, cyclopropane), 0.65–0.76 (m, 2H, cyclopropane), 1.60–1.69 (m, 1H, cyclopropane), 2.17–2.76 (m, 10H, 8H piperazine, 2H, CH_2), 3.67–3.76 (m, 2H, CH_2), 4.50 (s, 2H, CH_2), 6.00 (s, 1H, N_1H), 7.17–7.30 (m, 5H, ArH), 7.39 (d, 2H, ArH, J = 4.60 Hz) 7.55 (d, 2H, ArH, J = 4.60 Hz). Anal. Calcd for $C_{25}H_{29}ClN_4O_2$: C, 66.28; H, 6.45; N, 12.37. Found: C, 66.54; H, 6.70; N, 12.46. LC/MS: m/z calcd for $C_{25}H_{29}ClN_4O_2$ [M+H]$^+$: 453.20, found: 453.50.

4.1.1.9. 3-[4-(1-Phenyl-ethyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (11). White powdery crystals. TLC: R_f = 0.73 (S_1), yield: 67%, mp 73–78 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32–1.20 (m, 5H, cyclopropane), 1.32 (d, 3H, CH_3), 2.38–2.60 (m, 8H, piperazine), 3.67–3.73 (m, 1H, CH), 4.41 (s, 2H, CH_2), 6.01 (s, 1H, N_1H), 7.24–7.39 (m, 5H, ArH), 7.40 (d, 2H, ArH, J = 2.20 Hz), 7.52 (d, 2H, ArH, J = 2.20 Hz). Anal. Calcd for $C_{25}H_{29}ClN_4O_2$: C, 66.28; H, 6.45; N, 12.37. Found: C, 66.07; H, 6.71; N, 11.96.

4.1.1.10. 3-[4-(Benzyloxycarbonyl-piperazin-1-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (12). White powdery crystals. TLC: R_f = 0.55 (S_1), yield: 75%, mp 121–123 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.35–0.37 (m, 1H, cyclopropane), 0.50–0.62 (m, 1H, cyclopropane), 0.65–0.69 (m, 2H, cyclopropane), 1.61–1.64 (m, 1H, cyclopropane), 2.58 (t, 4H, piperazine, J = 4.90 Hz), 3.48 (t, 4H, piperazine, J = 5.05 Hz), 4.50 (s, 2H, CH_2), 5.10 (s, 2H, COOCH_2), 6.48–6.59 (br s, 1H, N_1H), 7.32–7.37 (m, 7H, ArH), 7.49–7.52 (d, 2H, ArH). Anal. Calcd for $C_{25}H_{27}ClN_4O_4$: C, 62.17; H, 5.63; N, 11.60. Found: C, 62.45; H, 5.80; N, 11.37.

4.1.1.11. 3-[(3,4-Dihydro-1*H*-isoquinolin-2-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (13). White powdery crystals. TLC: R_f = 0. 64 (S_1), yield: 85%, mp 171–174 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.34–0.38 (m, 1H, cyclopropane), 0.57–0.83 (m, 3H, cyclopropane), 1.77–1.78 (m, 1H, cyclopropane), 2.95–3.00 (t, 4H, isoquinoline J = 4.13 Hz), 3.87 (s, 2H, isoquinoline), 4.78 (s, 2H, CH_2), 5.59 (s, 1H, N_1H), 7.06–7.19 (m, 4H, ArH), 7.39–7.48 (m, 3H, ArH), 7.60–7.64 (m, 2H, ArH). Anal. Calcd for $C_{22}H_{23}N_3O_2$: C, 71.10; H, 6.25; N, 11.73. Found: C, 71.98; H, 6.13; N, 11.72.

4.1.1.12. 3-[(Morpholin-4-yl)-methyl]-5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione (14). White powdery crystals. TLC: R_f = 0.38 (S_1), yield: 70%, mp 110–112 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.35–0.43 (m, 1H, cyclopropane), 0.50–0.76 (m, 3H, cyclopropane), 1.68–1.77 (m, 1H, cyclopropane), 2.59–2.61 (t, 4H, morpholine, J = 3.80 Hz), 3.63–3.66 (t, 4H, morpholine, J = 3.80 Hz), 4.46 (s, 2H, CH_2), 6.73 (s, 1H, N_1H), 7.26–7.59 (m, 5H, ArH). Anal.

Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.46; H, 6.42; N, 13.30. LC/MS: *m/z* calcd for $C_{17}H_{21}N_3O_3$ $[M+H]^+$: 316.16, found: 316.40.

4.1.1.13. 3-[(3,4-Dihydro-1*H*-isoquinolin-2-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (15). White powdery crystals. TLC: $R_f = 0.67$ (S_1), yield: 74%, mp 166–168 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.32–0.36 (m, 1H, cyclopropane), 0.53–0.72 (m, 3H, cyclopropane), 1.65–1.68 (m, 1H, cyclopropane), 2.90–2.93 (m, 4H, isoquinoline), 3.80 (s, 2H, isoquinoline), 4.69 (s, 2H, C*H*$_2$), 6.08–6.12 (br s, 1H, N$_1$H), 7.09–7.12 (m, 4H, ArH), 7.37–7.38 (d, 2H, ArH, $J = 2.05$), 7.50–7.51 (d, $J = 2.05$, 2H, ArH). Anal. Calcd for $C_{22}H_{22}ClN_3O_2$: C, 66.75; H, 5.60; N, 10.61. Found: C, 66.61; H, 5.76; N, 10.65. LC/MS: *m/z* calcd for $C_{22}H_{22}ClN_3O_2$ $[M+H]^+$: 369.14, found: 369.60.

4.1.1.14. 3-[(Morpholin-4-yl)-methyl]-5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione (16). White powdery crystals. TLC: $R_f = 0.39$ (S_1), yield: 67%, mp 75–79 °C. ^{1}H NMR (300 MHz) $CDCl_3$: δ 0.33–0.39 (m, 1H, cyclopropane), 0.62–0.73 (m, 2H, cyclopropane), 1.63–1.72 (m, 2H, cyclopropane), 2.54–2.64 (m, 4H, morpholine), 3.63–3.66 (t, 4H, morpholine, $J = 4.60$ Hz), 4.46 (s, 2H, C*H*$_2$), 6.57 (s, 1H, N$_1$H), 7.36–7.39 (m, 2H, ArH), 7.49–7.54 (m, 2H, ArH). Anal. Calcd for $C_{17}H_{20}ClN_3O_3$: C, 58.37; H, 5.76; N, 12.01. Found: C, 58.19; H, 5.92; N, 11.57.

4.2. Pharmacology

The initial anticonvulsant evaluation was performed 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.[24,25]

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 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 are employed. Phase I studies in mice involved two convulsant tests: maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole seizure test (*sc*PTZ) and rotarod test for neurological toxicity (NT).

4.2.1. 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 rat at 60 Hz) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

4.2.2. Subcutaneous pentylenetetrazole seizure test (*sc*PTZ)

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 5 s in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously.

All the compounds were injected intraperitoneally into mice at the dose levels of 30, 100, and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h after administration. The results are presented in Table 1.

Selected derivatives were administrated orally into rats using four animals at a fixed dose of 30 mg/kg (MES test) and 50 mg/kg (*sc*PTZ test) (Phase VIa). 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 are shown in Tables 2 and 3.

4.2.3. Neurological toxicity (NT)

Neurological toxicity induced by a compound was detected in mice using standardized rotorod test.[26] Untreated control mice or rats, when placed on the rod, can maintained their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of the animal to maintain equilibrium for a given time (1 min).

4.2.4. Quantification studies

The quantitative determination of ED_{50} and TD_{50} values was 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 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 results are shown in Table 4.

4.2.5. The 6-Hz model

This screen was carried out according to the protocol originally described by Brown et al.[27] and more recently by Barton et al.[23] and Kaminski et al.[28] It is an alternative electroshock paradigm that uses low-frequency (6 Hz), long-duration (3 s) electrical stimulation. Corneal stimulation (0.2 ms-duration monopolar rectangular pulses at 6 Hz for 3 s) was delivered by a constant-current device. During the stimulation, mice were manually restrained and released into the observation cage immediately after the current application. The seizures manifest in 'stunned' posture associated with rearing, forelimb, automatic movements and clonus, twitching of the vibrissae and Straub-tail. The duration of the seizure activity ranges from 60 to 120 s in untreated animals. At the end of the seizure, animals resume their normal exploratory behavior. The experimental end point is protection against the seizure. The animal is considered to be protected if it resumes its normal exploratory behavior within 10 s from the stimulation[28] (Table 5).

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