Anticonvulsant properties of N-(4-methylpiperazin-1-yl)- and N-[3-(4-methyl-piperazin-1-yl)-propyl] derivatives of 3-aryl- and 3-spirocycloalkyl-pyrrolidine-2,5-dione

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Abstract:

Two series of N-(4-methylpiperazin-1-yl)- and N-[3-(4-methylpiperazin-1-yl)-propyl]-3-aryl- (**3–10**) and 3-spirocycloalkyl-pyrrolidine-2,5-dione (**11–14**) derivatives were synthesized and tested for anticonvulsant activity in the maximum electroshock (MES) seizure and pentetrazole (*sc* PTZ) seizure threshold tests. Compounds **3–10** with an aromatic ring at position-3 of pyrrolidine-2,5-dione exhibited anticonvulsant activity in the MES test. For that series of compounds, ED_{50} values were determined. The most potent in the series were derivatives **5, 6** and **9, 10** with a chlorine atom at position-3 or 4 of the aromatic ring. Those compounds exhibited strong anticonvulsant activity, and their ED_{50} values ranged from 29 to 48 mg/kg. Introduction of the spirocycloalkyl ring into the position-3 of pyrrolidine-2,5-dione (**11–14**) made those compounds inactive.

Key words:
anticonvulsant activity, 4-methylpiperazine derivatives, 3-arylpyrrolidine-2,5-diones, 3-spirocycloalkylpyrrolidine-2,5-diones

Introduction

Epilepsy is one of the most frequent neurological disorders characterized by spontaneous recurrent seizures arising from excessive electrical activity in a portion of the brain. Clinically available antiepileptic drugs exert satisfactory control in 60%–70% of patients, however, many of these drugs also produce undesirable side-effects, such as drowsiness, mental dullness, ataxia, hepatotoxicity, megaloblastic anemia. Therefore, there is a growing demand for new antiepileptic drugs with novel therapeutic targets, enhanced efficacy and minimal side-effects [2].

At present, the majority of currently available anti-seizure drugs fall into one of the four pharmacological classes which can be characterized as follow: (i) the use-dependent blockade of voltage-sensitive sodium channels (e.g. phenytoin, carbamazepine, zonisamide, lamotrigine); (ii) the enhancement of the inhibitory activity of GABAergic neurotransmission (e.g. vigabatrine, tiagabine, gabapentin); (iii) the interaction
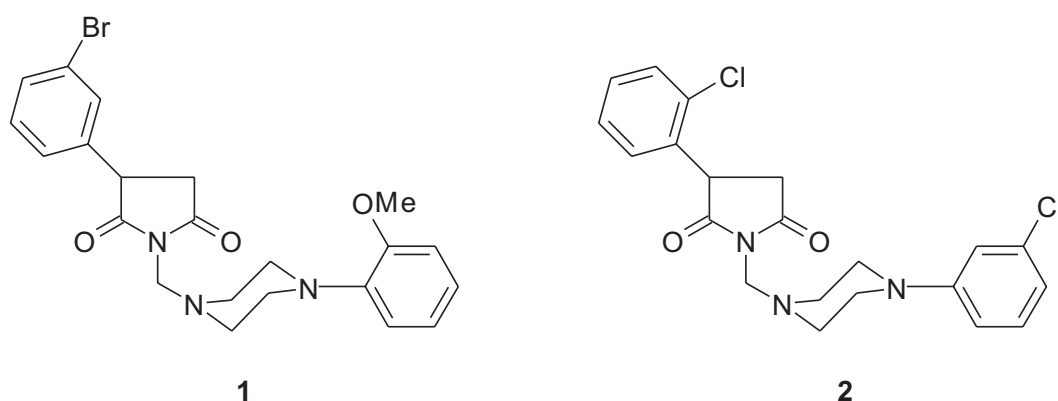
Fig. 1. Chemical structure of compounds with anti-MES activity

with voltage-operated calcium channels (e.g. ethosuximide, levetiracetam) [9]; (iiii) the final class includes drugs that bind to excitatory amino acid receptors (e.g. harkoseride, losigamone, remacemide) [4, 12, 18, 19].

It is well known that numerous derivatives with anticonvulsant activity contain 5-, 6-membered heterocyclic rings, one or two carbonyl groups, and an aromatic system [6, 7, 24]. Following these findings, our attention has focused on a group of 3-substituted pyrrolidine-2,5-diones with different substituents at the nitrogen atom [14–17, 27]. We recently reported [15] that the ED_{50} values for N-[4-(3-chlorophenyl)-piperazin-1-yl-methyl]-3-(2-chlorophenyl)- pyrrolidine-2,5-dione (**2**) and N-[4-(2-methoxyphenyl)-piperazin-1-yl-methyl]-3-(3-bromophenyl)-pyrrolidine-2,5-dione (**1**) (Fig. 1) in the maximal electroshock (MES)-induced seizure test were 14.20 and 33.60 mg/kg, respectively.

To continue our work we replaced the 4-aryl-piperazine moiety with 4-methyl-piperazine and, at the same time, we changed the length of the alkyl spacer between piperazine and the imide nitrogen atom. Additionally, in some of the obtained compounds (**11–14**), we replaced the aryl moiety at position-3 of pyrrolidine-2,5-dione with a cyclopentyl or cyclohexyl ring connected to the heterocycle with a spiro carbon atom. The great number of compounds with potent anticonvulsant activity, containing 3-spirocycloalkylsuccinimide moiety were reported by Scott et al. [1, 20, 23], hence, we expected that the above-described modification could further improve the activity of these compounds.

Materials and Methods

CHEMICAL PART

The synthesis of compounds **4, 8, 11–14** is presented in Figure 2. The starting 2-(2-chlorophenyl)-succinic acids or 1-cyclopentane-, 1-cyclohexane-1-carboxy-1-acetic acids were prepared according to the described procedure [13, 20]. The obtained acids were cyclized to N-substituted pyrrolidine-2,5-diones (**3–14**) by heating them at ca. 180–200°C for 1.5 h with 1-amino-4-methylpiperazine (**3–6, 11, 13**) or 1-(3-aminopropyl)-4-methylpiperazine (**7–10, 12, 14**). The

Tab. 1. Physicochemical data of the new compounds

Comp.	M.p.(°C)	Yield (%) cryst. solvent	Molecular formula[a] Molecular weight	R_f
4	248–251	58 ethanol	$C_{15}H_{18}O_2N_3Cl$ x HCl 344.25	A 0.28 B 0.91
8	236–238	60 ethanol	$C_{18}H_{24}O_2N_3Cl$ x 2HCl 422.78	A 0.11 B 0.67
11	265–267	62 ethanol	$C_{13}H_{21}O_2N_3$ x HCl 287.80	A 0.31 B 0.97
12	262–264	68 ethanol	$C_{16}H_{27}O_2N_3$ x 2HCl 366.34	A 0.49 B 0.89
13	250–252	53 ethanol	$C_{14}H_{23}O_2N_3$ x HCl 301.80	A 0.28 B 0.94
14	257–259	65 ethanol	$C_{17}H_{29}O_2N_3$ x 2HCl 380.37	A 0.44 B 0.87

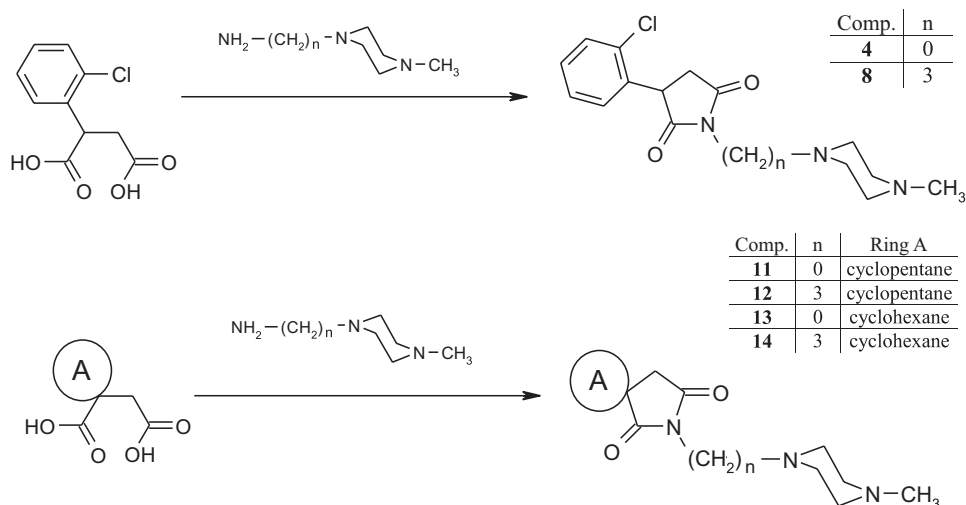[a] calculated from an elemental analysis

Fig. 2. Method of preparation of compounds 4, 8 and 11–14

physicochemical properties of the new compounds are presented in Table 1. It should be noted that the synthesis and physicochemical data of compounds **3, 5–7, 9–10** had been described by us previously [26], but none of them were tested for their anticonvulsant activity. Their structures are presented in Table 2. Products **4, 8** and **11–14** are the new ones (Fig. 2).

The purity of the compounds was checked by a thin-layer chromatography performed on Merck silica gel GF_{254} aluminium sheets, using the developing systems: A) butanol : acetic acid : water (5:4:1); B) acetone : isopropanol : 25% ammonia (9:11:2). Spots were detected by their absorption under UV light, and visualization was carried out with 0.05 M I_2 in a 10% HCl. The elemental analyses (C, H, N) of the hydrochloride salts were within ± 0.4% of the theoretical values.

Melting points (m.p.) were determined with electrothermal digital m.p. apparatus and are uncorrected. ^{1}H-NMR spectra were determined with a Varian Mercury spectrometer (300 MHz), in a CHCl$_3$-d$_1$ solution with TMS as an internal standard. Chemical shifts are shown as δ (ppm), and the coupling constants J are given in hertz (Hz).

General procedure for preparation of compounds 4, 8, 11–14

To a suspension of 2-(2-chlorophenyl)-succinic acid, 1-carboxy-1-cyclopentane- or 1-carboxy-1-cyclohexa-ne-1-acetic acids (0.02 mol) in 20 ml of water, 1-amino-4-methylpiperazine or 1-(3-aminopropyl)-4-methylpiperazine (0.02 mol) were gradually added. The mixture was heated in an oil bath, and water was simultaneously distilled. After the complete removal of water, the temperature of the reaction mixture rose up to 190–200°C, and was maintained at that temperature for 1.5 h. The crude oil products were converted to hydrochloride salts. The hydrochlorides of **4, 8** and **11–14** were prepared by introducing dry hydrogen chloride into the alcoholic solution of the corresponding compounds. The precipitated salts were crystallized from ethanol.

PHARMACOLOGICAL PART

Preliminary anticonvulsant assays

Preliminary anticonvulsant assays for all the compounds (**3–14**) were provided by the Antiepileptic Drug Development (ADD) Program using the testing procedures described elsewhere [8, 10]. Phase I studies of the investigated compounds involved three tests: MES, sc PTZ and a rotarod test for neurological toxicity (TOX). Phase I involved *ip* administration of a compound as a suspension in a 0.5% methylcellulose, and was a qualitative assay involving a small number of mice (1–3) at dose levels of 30, 100 and 300 mg/kg.

Quantitative anticonvulsant assays

Male Albino-Swiss mice (25–28 g) were used throughout the experiment after at least one-week acclimatization. The animals were housed under standard laboratory conditions (an ambient temperature of $20° \pm 1°C$, natural light-dark cycle). Tap water and chow pellets were freely available before the experiment. All the experiments were conducted between 10.00 a.m. and 2.00 p.m. (December-January). Each experimental group consisted of 10 animals. The experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

The tested compounds and valproate magnesium (ICN Polfa SA, Rzeszów, Poland) were administered *ip* as a suspensions in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in a volume of 10 ml/kg. The control groups were injected *ip* appropriate volumes of the solvent.

Seizures were evoked in mice with an electric current (50 Hz, 50 mA, duration of impulse 0.2 s, ear-clips electrodes) 30 min after administration of the investigated compounds, according to the method of Swinyard et al. [22]. The number of mice reacting by the tonic extention of hind limbs was recorded. ED_{50} values, i.e. doses protecting 50% of mice against the MES, were determined on the basis of effect of at least four doses for each compound. The ED_{50} values with 95% confidence limits were calculated according to the log-probit method of Litchfield and Wilcoxon [5, 11, 25].

Results and Discussion

For compounds **3–14** preliminary anticonvulsant activity and neurotoxicity data were obtained in a small number of animals (Tab. 3). Compounds with 3-aryl substituents (**3–10**) showed anticonvulsant activity in the Phase I screening project in the MES test only. The most potent anticonvulsant compounds **5, 6, 9** and **10** were active at doses of 30, 100 and 300 mg/kg (1/1, 3/3 and 1/1 of the animals protected, respectively) 30 min after administration, and were classified to class I according to ASP Program. Compounds **5** and **10** were also active at doses of 100 and 300 mg/kg 4 h after administration (1/3, 1/1 of the animals protected). Derivatives **6** and **9** exhibited activity only at

Tab. 2. Structures of the investigated compounds and their ED_{50} values in the electroshock seizure test in mice
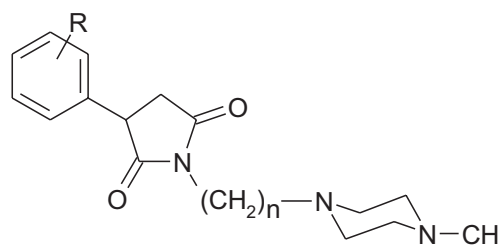
Comp.	R	n	ED_{50}, mg/kg, *ip**
3	H	0	125 (83.3–187.5)
4	2-Cl	0	115 (92.0–143.8)
5	3-Cl	0	29 (25.7–32.8)
6	4-Cl	0	41 (32.3–52.1)
7	H	3	78 (58.9–103.0)
8	2-Cl	3	120 (101.7–141.6)
9	3-Cl	3	41 (29.3–69.7)
10	4-Cl	3	48 (34.8–66.2)
Valproate magnesium			211 (168.8–263.8)

* The tested compounds were administrated *ip* 30 min before the test, 95% confidence limits are given in parentheses

a dose of 300 mg/kg (1/1 of the animals protected). In the TOX test, the latter derivatives were toxic at a dose of 100 mg/kg. At a dose of 300 mg/kg, the mice were unable to grasp the rotarod; additionally, at the same dose, compounds **9** and **10** caused death of animals.

3-Phenyl- (**3, 7**) and 3-(2-chlorophenyl)- (**4, 8**) derivatives exhibited activity at doses of 100 mg/kg (1/3, 1/3, 3/3, and 1/3 of the animals protected after 30 min), but none of them was active at a dose of 30 mg/kg. In the TOX screening, compounds **4, 7, 8** at a dose of 300 mg/kg disturbed the motor coordination of mice. Additionally, 30 min after administration, compounds **4** and **8** at a dose of 300 mg/kg caused death of the animals. In contrast, the compounds with the cycloalkyl moiety connected to the position-3 of pyrrolidine-2,5-dione ring (**11–14**) at doses up to 300 mg/kg, were inactive in the used tests.

ED_{50} values of compounds **3–10,** active in the preliminary anticonvulsant tests, were assessed in the

Comp	Dose mg/kg	MES[a]		Toxicity[b]		ASP[c] class
		0.5 h	4 h	0.5 h	4 h	
3	30	0/1	0/1	0/4	0/2	1
	100	1/3	0/3	0/8	0/4	
	300	1/3	0/1	1/4	0/2	
4	30	0/1	0/1	0/4	0/2	1
	100	1/3	0/1	1/8	0/4	
	300	0/3	0/1	4/4[1,2]	0/1	
5	30	1/1	0/1	0/4	0/2	1
	100	3/3	1/3	1/8	0/4	
	300	1/1	1/1	4/4[2]	1/2	
6	30	1/1	0/1	0/4	0/2	1
	100	3/3	0/3	3/8	0/4	
	300	1/1	1/1	4/4[2]	0/2	
7	30	0/1	0/1	0/4	0/2	1
	100	3/3	0/3	1/8	0/4	
	300	1/1	1/1	4/4[2]	2/2	
8	30	0/1	0/1	0/4	0/2	1
	100	1/3	0/3	1/8	0/4	
	300	1/1	0/1	4/4[1,2]	–	
9	30	1/1	0/1	0/4	0/2	1
	100	3/3	0/3	0/8	0/4	
	300	1/1	1/1	4/4[1,2]	–	
10	30	1/1	0/1	0/4	0/2	1
	100	1/3	1/3	0/8	0/4	
	300	1/1	1/1	4/4[1,2]	–	
11	30	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/8	0/4	
	300	0/1	0/1	0/4	0/2	
12	30	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/8	0/4	
	300	0/1	0/1	1/4[1]	0/2	
13	30	0/1	0/1	0/4	0/2	3
	100	0/3	0/1	0/8	/4	
	300	0/1	0/1	3/4[1]	0/2	
14	30	0/1	0/1	0/4	0/2	3
	100	0/3	0/1	0/8	0/4	
	300	0/1	0/1	1/4[1]	0/2	

[a] MES test (number of animals protected/number of animals tested) [b] Rotarod toxicity (number of animals affected by toxicity of a compound/number of animals tested). [c] The classification is as follows: 1 – anticonvulsant activity at doses of 100 mg/kg or lower; 2 – anticonvulsant activity at doses higher than 100 mg/kg; 3 – compound inactive at a dose of 300 mg/kg. Response comments: [1] death; [2] unable to grasp the rotarod

MES in mice (Tab. 2). The obtained results showed that 1-[4-methylpiperazin-1-yl or propyl]-3-arylpyrrolidine-2,5-dione derivatives **3**–**10** inhibited the hind limb extension produced by MES seizure (ED$_{50}$ = 29–125 mg/kg). In the used test, compounds **3**–**10** were more potent than valproate magnesium, a standard antiepileptic drug (ED$_{50}$ = 211 mg/kg). The most active was 3-(3-chlorophenyl) derivative of pyrrolidine-2,5dione (**5**), which was directly connected to the 4-methyl-piperazin-1-yl moiety at the imide nitrogen atom (**5**) (ED$_{50}$ = 29 mg/kg).

As can be seen from the present study, modification of the phenyl part in the investigated compounds may have some influence on their anticonvulsant activity. In fact, introduction of the chlorine atom into position-3 (**5** and **9**) or -4 (**6** and **10**) yields compounds with the highest activity (ED$_{50}$ = 29–48 mg/kg), whereas 3-phenyl (**3**, **7**) and 2-chlorophenyl (**4**, **8**) derivatives should be regarded as slightly weaker anticonvulsants (ED$_{50}$ = 78–125 mg/kg).

In our series of compounds, the observed activity was independent of the length of the alkyl side chain. Additionally, when we compared our compounds with the previously obtained **1** and **2,** we can assume that the removal of the phenyl ring connected to piperazine was only inconsiderably involved in that activity.

Surprisingly enough, none of the 3-spirocycloalkyl derivatives exhibited any significant activity, though, as has been mentioned above, there are a lot of active compounds containing this substituent. A simple comparison of the structure of our non-active compounds with that of active 3-spirocycloalkyl-succinimide derivatives described in the literature [1, 3, 23], leads to the conclusion that the presence of at least one phenyl ring in the molecule is essential for the anticonvulsant activity. Indeed, all the active 3-spiro-cycloalkylsuccinimides described by Scott et al. [20, 21] contain a phenyl ring on the side chain, while our compounds with low activity lack it. This conclusion can also be supported by our present observation that the substituents in this crucial phenyl ring are of great importance to anticonvulsant activity.

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