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Synthesis, and anticonvulsant activity of new amides derived from 3-methyl- or 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acids

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Abstract

This paper describes the synthesis of the library of 22 new 3-methyl- and 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetamides as potential anticonvulsant agents. The maximal electroshock (MES) and the subcutaneous pentylenetetrazole (*sc*PTZ) seizure models were used for screening all the compounds. The 6 Hz model of pharmacoresistant limbic seizures was applied for studying selected derivatives. Six amides were chosen for pharmacological characterization of their antinociceptive activity in the formalin model of tonic pain as well as local anesthetic activity was assessed in mice. The pharmacological data indicate on the broad spectra of activity across the preclinical seizure models. Compounds **10** (ED_{50} = 32.08 mg/kg, MES test) and **9** (ED_{50} = 40.34 mg/kg *sc*PTZ test) demonstrated the highest potency. These compounds displayed considerably better safety profiles than clinically relevant antiepileptic drugs phenytoin, ethosuximide, or valproic acid. Several molecules showed antinociceptive and local anesthetic properties. The *in vitro* radioligand binding studies demonstrated that the influence on the sodium and calcium channels may be one of the essential mechanisms of action.

Keywords: Succinimides; Anticonvulsant activity; Antinociceptive properties; Anesthetic activity; Ion channels

1. Introduction

The past decades have demonstrated many attempts to identify the structural features of compounds crucial for anticonvulsant activity. It is well documented that important core fragment of anticonvulsants is defined by nitrogen heteroatomic system, usually an imide, with at least one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system.[1–3] This common template is present in the structures of old-generation, however, well-established antiepileptic drugs (AEDs) such as ethosuximide and phenytoin as well as levetiracetam, brivaracetam, or seletracetam, e.g., among the newest drugs (Figure 1). In the previous studies, we have found the pyrrolidine-2,5-diones differently substituted at position-1 and -3 as targets for new AEDs. Many of these compounds were effective in the MES and *sc*PTZ tests that are still recognized as the "gold standard" in the early stages of testing new drug candidates.[4–8]

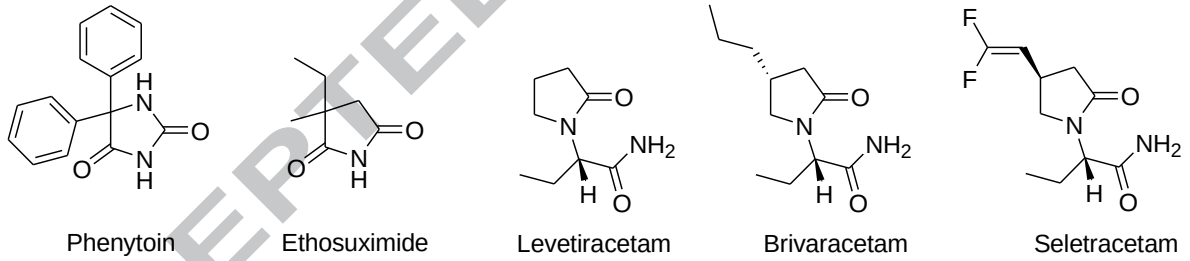
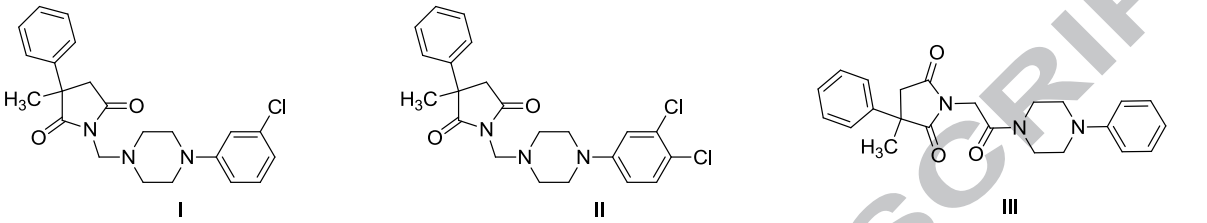


Figure 1. Structures of ADEs with five-member heterocyclic ring.

Recent studies on the structure-activity relationship (SAR) demonstrated the potent anticonvulsant activity of the *N*-(4-arylpiperazin-1-yl)-methyl-3,3-disubstituted-pyrrolidine-2,5-dio-nes among which the most active were 1-[{4-(3-chlorophenyl)-piperazin-1-yl}methyl]-3-methyl-3-phenyl-pyrrolidine-2,5-dione (**I**) and its 3,4-dichloro analog (**II**), showing protection exclusively in the MES test.[9] Further SAR analysis revealed that exchange

of the methylene linker between imide and amine function into acetamide moiety yielded compounds active in both MES and PTZ seizure models or predominantly in the PTZ test – compound **III** (Figure 2).[10]



I: ED_{50}=41.1 mg/kg, MES test, mice *i.p.*; TD_{50}=496.4 mg/kg, rotarod test, mice *i.p.*

II: ED_{50}=37.3 mg/kg, MES test, mice *i.p.*; TD_{50}>500 mg/kg, rotarod test, mice *i.p.*

III: ED_{50}=40.9 mg/kg, PTZ test, mice *i.p.*; TD_{50}>500 mg/kg, rotarod test, mice *i.p.*

Figure 2. Anticonvulsants obtained in the previous studies.

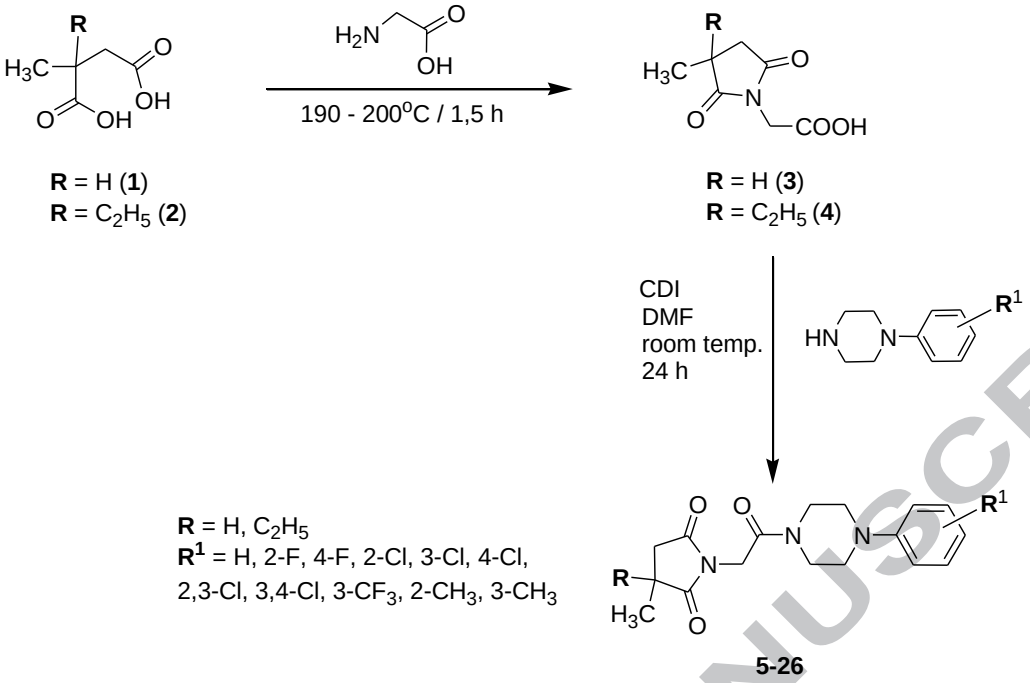
Considering the aforementioned results, as part of our efforts to design new anticonvulsant agents, in the present study, we have synthesized a new series of analogs in which we introduced one methyl or ethyl and methyl substituents at position-3 of imide ring. The proposed structural modifications enable to assess the influence of alkyl groups on anticonvulsant properties in this series of derivatives. With the aim of ensuring the reliable SAR discussion as an amine function, variously substituted 4-arylpiperazines have been introduced.

Note that several AEDs such as pregabalin, gabapentin, phenytoin, lacosamide, and topiramate are clinically effective for the treatment of certain kinds of pain (neuropathic pain, migraine prophylaxis).[11] Using different models of pain, studies performed by various groups found that AEDs can inhibit sensitized signaling associated with allodynia and hyperalgesia.[12–14] Moreover, local anesthetics, which act by blocking nerve conduction by altering the function of voltage-gated sodium channels, are used as adjuvant analgesics for the treatment of neuropathic pain.[15] Therefore, selected compounds were examined toward their antinociceptive as well as local anesthetic activity.

2. Results and discussion

2.1. Chemistry

The final compounds **5**–**26** were synthesized according to Scheme 1. The starting material 3-methylsuccinic acid (**1**) was commercially purchased from Merck (Darmstadt, Germany) whereas 3-ethyl-3-methyl-succinic acid (**2**) was prepared as previously reported.[16] In the next step, the condensation reaction of **1** or **2** with aminoacetic acid yielded in 3-methyl- or 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acids (**3**, **4**). These intermediates were converted to final compounds **5**–**26** in the coupling reaction with equimolar amounts of appropriate 4-phenylpiperazines in the presence of carbonyldiimidazole (CDI). The reaction was carried out at room temperature in dry DMF for 24 h. The crude products were crystalized from 2-propanol. Their purity and homogeneity were assessed by TLC and gradient HPLC chromatography. The structures of compounds synthesized were confirmed by both spectral (^{1}H NMR, LC-MS) and elemental analyses. The detailed physical and analytical data are listed in the experimental section.



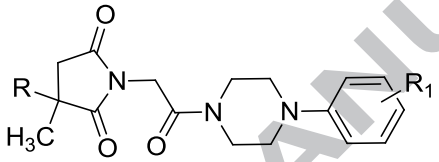
Scheme 1. Synthetic protocol of the target compounds **5**–**26**.

2.2. Anticonvulsant activity

The preclinical development of new chemical agents for the treatment of epilepsy is based on the use of predictable animal seizure models, which correspond to different types of human epilepsies. Such models can be categorized into two main categories: 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). For practical reasons, screening is often carried out using acute seizure models, although various types of kindling models, a class of chronic model, are commonly included in the battery of tests to which early stage compounds are subjected. Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, the MES model and the *sc*PTZ model are the most frequently used in the early stages of testing. The MES test is the mechanism-independent animal seizure model that enables the identification of compounds preventing seizure spread. This test uses an electrical stimulus to produce generalized tonic-clonic seizures, and thus is thought to be an

experimental model of tonic-clonic epilepsy and of partial convulsions with or without secondary generalization in humans. The *sc*PTZ test uses chemically induced clonic seizures and is proposed to identify the agents raising the seizure threshold. This test is related to human generalized absence seizures.[17–19] Therefore, all the final substances were screened in the MES and *sc*PTZ tests. Furthermore, in addition to the primary anticonvulsant screening, the acute neurological toxicity (NT) was determined in mice by the rotarod test (Table 1).

Table 1. Anticonvulsant activity MES, *sc*PTZ, and rotarod test in mice *i.p.*



Cmpd	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
5*	H	H	300	-	300	300	300	-
6*	H	2-F	300	300	-	-	300	-
7*	H	4-F	300	100	-	-	-	-
8*	H	2-Cl	300	-	300	-	300	-
9	H	3-Cl	100	300	100	-	-	-
10*	H	4-Cl	100	30	-	-	-	300
11	H	2,3-Cl	-	-	100	100	-	-
12	H	3,4-Cl	300	100	100	100	-	-
13*	H	3-CF_3	100	100	300	-	300	300
14	H	2-CH_3	300	-	100	-	300	-
15	H	3-CH_3	300	-	100	-	300	-
16	C_2H_5	H	-	-	-	-	-	-
17	C_2H_5	2-F	-	-	-	-	-	-
18	C_2H_5	4-F	100	-	100	-	300	-
19	C_2H_5	2-Cl	100	-	-	-	300	-
20	C_2H_5	3-Cl	-	-	-	-	-	-
21	C_2H_5	4-Cl	-	-	100	-	300	-

22	C_2H_5	2,3-Cl	-	-	-	-	-	-
23	C_2H_5	3,4-Cl	-	-	-	-	-	-
24	C_2H_5	3-CF_3	100	100	-	-	300	-
25	C_2H_5	2-CH_3	100	-	100	-	300	-
26	C_2H_5	3-CH_3	-	-	-	-	-	-
ETX[e]	-	-	-	-	100	300	-	-
PHT[e]	-	-	30	30	-	-	100	100
VPA[e]	-	-	300	-	300	-	-	-

Doses of 100, and 300 mg/kg were administered intraperitoneally (*i.p.*). The data indicate the minimum dose whereby anticonvulsant activity was demonstrated. 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 - rotorod test.
[d] Reference drugs, data for ethosuximide (ETX), phenytoin (PHT), and valproic acid (VPA) from ref.[20]

The preliminary pharmacological data revealed that sixteen compounds (**5**–**15**, **18**, **19**, **21**, **24**, and **25**) showed protection in half or more of the animals tested in the MES or/and *sc*PTZ tests. As Table 1 shows, nine molecules displayed a broad pharmacological activity in both MES and PTZ seizures. In the MES test, compound **10** was most effective, displaying anticonvulsant activity at a dose of 30 mg/kg at 4 h and 100 mg/kg at 0.5 h. The other derivatives provided anti-MES protection at a dose of 100 mg/kg (**7**, **9**, **12**, **13**, **18**, **19**, **24**, and **25**) or 300 mg/kg (**5**, **6**, **8**, **14**, and **15**). Compounds **5**, **8**, **9**, **11**–**15**, **18**, **21**, and **25** were found to be active in the *sc*PTZ test, which identify substances elevating seizure threshold. Among these molecules, **9**, **11**, **12**, **14**, **15**, **18**, **21**, and **25** showed protection at a dose of 100 mg/kg at time point of 0.5 h (**9**, **14**, **15**, **18**, **21**, and **25**) or in both time intervals (**11** and **12**). It should be stressed that **11** and **12** were more potent than ethosuximide, which is known to be a model AED in PTZ test. The other derivatives (**5**, **8**, **13**) exhibited anti-PTZ protection at a dose of 300 mg/kg. In the rotarod test for acute neurological toxicity, **7**, **9**, **11**, **12**, **16**, **17**, **20**, **22**, **23**, and **26** did not show neurotoxicity in the maximum dose administered (300 mg/kg). Among these molecules, **16**, **17**, **20**, **22**, **23**, and **26** were also inactive in the seizure tests. The rest of

the compounds revealed neurotoxicity at the highest dose of 300 mg/kg in different pretreatment times. As a result, compounds **7**, **9**, **11**, and **12** emerged as anticonvulsants without neurotoxic properties.

Looking for new anticonvulsants with a wide spectrum of activity in the preclinical studies, six chemically and biologically diversified compounds **7**, **8**, **18**, **19**, **21**, and **24** were studied in the psychomotor 6 Hz model of human pharmacoresistant limbic seizures. The results are shown in Table 2.

Table 2. Anticonvulsant evaluation of compounds in the 6-Hz test (current 32 mA) following intraperitoneal (*i.p.*) administration in mice.

Cmpd	Pretreatment time[a]			
	0.25 h	0.5 h	1 h	2 h
7	2/4	3/4	3/4	1/4
8	4/4	2/4	2/4	0/4
18	3/4	3/4	1/4	0/4
19	3/4	4/4	2/4	1/4
21	2/4	2/4	1/4	1/4
24	2/4	3/4	2/4	1/4

Dose of 100 mg/kg was administrated *i.p.* in mice.
[a] The data indicate: number of mice protected /number of mice tested.

As is shown in Table 2, the most potent anticonvulsant properties were observed for **8** and **19**, which displayed two points of 100% protection at 0.25 and 0.5 h, respectively. High efficacy with two peaks of 75% protection revealed **7** and **18**.

In the next step of the pharmacological investigations, for the most potent compounds in the screening studies (**7**, **9**–**12**, **18**, **19**, and **24**), the median effective (ED_{50} MES or/and PTZ test) and toxic (TD_{50} rotarod test) doses were determined after intraperitoneal (*i.p.*) injection in mice. The quantitative evaluation was performed at previously estimated time of

peak effect (TPE). Results of the quantitative tests along with the data for the standard drugs such as phenytoin, ethosuximide, and valproic acid are shown in Table 3.

Table 3. Quantitative data following *i.p.* administration in mice.

Cmpd	TPE (h)[a]	ED_{50} MES (mg/kg)[b]	ED_{50} *sc*PTZ (mg/kg)[b]	TD_{50} Rotarod (mg/kg)[b]	PI[c]
7*	1.0	87.36 (46.82–197.10)	-	>300	>3.43 (MES)
9	1.0	82.74 (54.53–125.52)	40.34 (29.33–55.51)	323.34 (237.66–439.93)	3.91(MES) 8.0 (*sc*PTZ)
10*	4.0	32.08 (26.7–36.1)	>250	>500	>15.59 (MES) >2.0 (*sc*PTZ)
11	1.0	–	74.65 (42.28–131.80)	343.28 (311.62–378.16)	4.60 (*sc*PTZ)
12	1.0	75.60 (53.84–106.16)	54.13 (37.41–78.30)	263.21 (188.52–367.79)	3.48 (MES) 4.86 (*sc*PTZ)
18	0.5	69.05 (63.54–75.02)	129.50 (114.21–146.84)	190.62 (138.42–262.50)	2.76 (MES) 1.47 (*sc*PTZ)
19	0.5	122.04 (100.26–148.56)	–	131.66 (94.26–183.89)	1.08 (MES)
24	0.5	70.08 (64.77–75.82)	–	284.56 (242.67– 333.68)	4.06 (MES)
PHT[d]	1.0	6.65 (5.42–8.16)	-	56.9 (48.5–66.7)	8.56 (MES)
ETX[d]	0.25	-	140.40 (115.81–170.21)	318.01 (295.80–341.89)	2.26 (*sc*PTZ)
VPA[d]	0.5	252.74 (220.1–290.22)	239.45 (209.18–274.10)	430.77 (407.92–454.90)	1.70 (MES) 1.80 (*sc*PTZ)

[a] Time to peak effect.
[b] Results are represented as mean ± SEM at 95% confidence limit.
[c] Protection index (TD_{50}/ED_{50}).
[d] Model AEDs: ethosuximide (ETX), Phenytoin (PHT), Valproic acid (VPA) tested in the same condition.

The analysis of quantitative data revealed that excluding compound **10**, all other derivatives (**9**, **11**, **12**, and **18**) were more potent than ethosuximide in the *sc*PTZ test. In the MES model,

none of the compounds tested was more active than phenytoin used as the reference antiepileptic drug for MES seizures; however, all the substances were more effective compared to valproic acid. In the MES test, the most potent was **10** with ED_{50} value of 32.1 mg/kg, TD_{50} > 500 mg/kg that resulted in favorable protection index (TD_{50}/ED_{50}) > 15.6. In the *sc*PTZ test, the best ED_{50} values revealed **9**, namely, 40.3 mg/kg, TD_{50} = 323.3 mg/ kg, and PI = 8.0.

Given high activity in the 6-Hz test, compounds **8**, **19**, **21**, and **24** were selected for the quantification of pharmacological parameters in the aforementioned seizure model. As it is shown in Table 4, two compounds **19** and **24** were more effective than valproic acid, nevertheless less active compared to levetiracetam.

Table 4. Quantitative anticonvulsant data test 6–Hz in mice dosed *i.p.*

Cmpd	TPE (h)[a]	ED_{50} 6-Hz (mg/kg)[b]	TD_{50} Rotarod (mg/kg)[b]	PI[c]
8	0.25	151.82 (114.07–235.25)	>500	>3.29
19	0.5	73.26 (57.41–93.48)	131.66 (94.26–183.89)	1.80
21	0.5	128.22 (94.76–173.49)	195.74 (164.56–232.84)	1.53
24	0.5	90.26 (66.48–122.54)	284.56 (242.67–333.68)	3.15
VPA[d]	0.5	130.64 (117.61–145.19)	430.77 (407.92–454.90)	3.30
LEV[d]	1	15.73 (10.45–23.69)	>500	>31

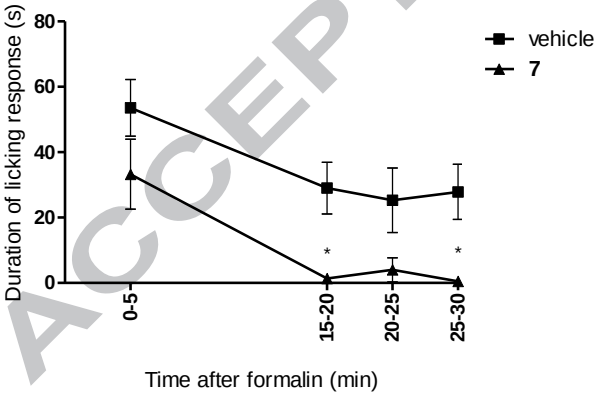
[a] Time to peak effect.
[b] Results are represented as mean ± SEM at 95% confidence limit.
[c] Protection index (TD_{50}/ED_{50}).
[d] Model AEDs: Valproic acid (VPA), Levetiracetam (LEV) tested in the same condition.

Based on the aforementioned pharmacological data, several SAR conclusions can be deduced. In general, visibly higher activity was observed for 3-methyl-pyrrolidine-2,5-diones in comparison to their 3-ethyl-3-methyl- analogs. The most active were compounds with electronegative fluorine and chlorine atoms or trifluoromethyl group at the 4-arylpiperazine fragment. It was in agreement with our previous studies that have demonstrated that the presence of electron-attracting substituents increases anticonvulsant activity. The replacement of the above substituents by the electron-donating group decreased the anticonvulsant activity.

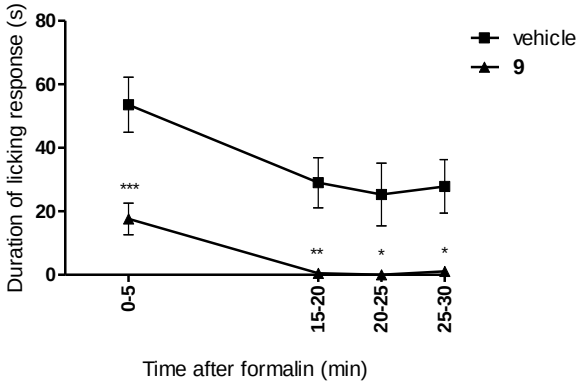
2.3. Antinociceptive activity in the formalin hind paw test

Because of anticonvulsant activity of compounds **7**, **9**, **10**, **12**, **18**, and **24** in electrically evoked seizures, their analgesic properties were investigated at doses equal to the respective ED_{50} values obtained in MES test. A significant antinociceptive activity was observed for each of the substance tested (Figure 3A–F).

A



B



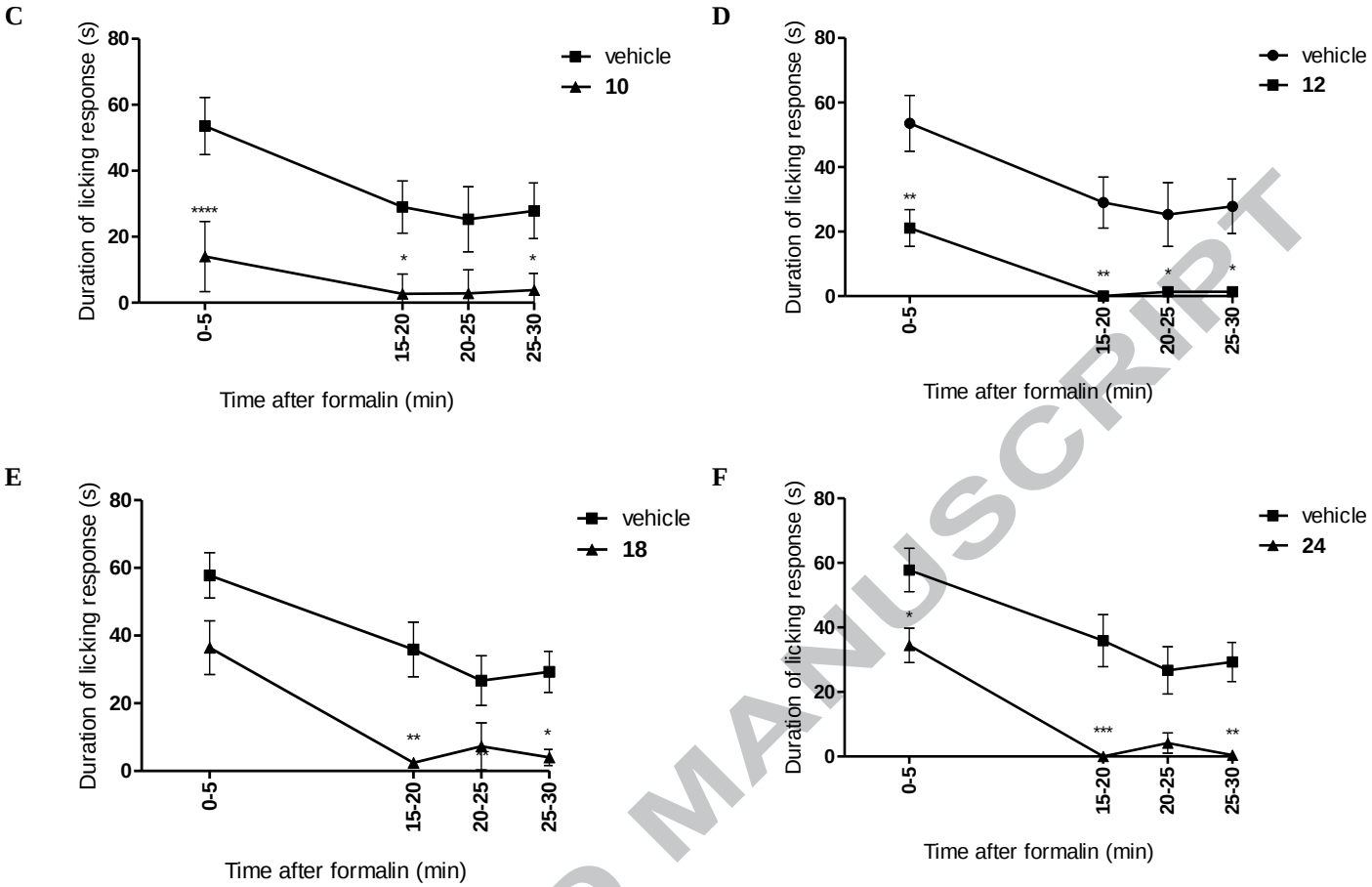


Figure 3. A–F. Time course of the antinociceptive activity of compounds **7**, **9**, **10**, **12**, **18**, and **24** in the formalin test. Statistical analysis of the result was conducted using two-way repeated measures ANOVA, followed by Bonferroni's multiple comparison. Results compared to vehicle-treated mice at the same time points: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

In the first (neurogenic) phase, four compounds reduced the duration of the licking response up to 76%. In this phase, derivative **10**, which was tested at the dose of 32.08 mg/kg, had the highest antinociceptive efficacy (76%, $p < 0.0001$). Compounds **9**, **12**, and **24**, tested at the doses of 82.74 mg/kg, 75.60 mg/kg, and 70.08 mg/kg, respectively, reduced the duration of licking response by 67% ($p < 0.001$), 61% ($p < 0.01$), and 40% ($p < 0.05$), respectively. Compounds **7** and **18** did not attenuate the nocifensive response in statistically significant manner. In the second (late) phase of the formalin test, a strong antinociceptive effect was observed for all compounds because they significantly reduced the duration of the licking response. The evaluation of the time course of the antinociceptive activity at selected time

intervals showed that between 15 and 20 min of the observation, all compounds diminished the pain responses **7** (95%, $p < 0.05$), **9** (98%, $p < 0.01$), **10** (91%, $p < 0.05$), **12** (100%, $p < 0.01$), **18** (93%, $p < 0.01$), and **24** (100%, $p < 0.001$). Next, between 20 and 25 min of the assay, a statistically significant reduction of the licking response was observed for derivatives **9** and **12**. Their analgesic activity ranged from 95% (**12**) to 100% (**9**). Between 25 and 30 min of the test, all the molecules decreased the pain reaction significantly: **7** (98%, $p < 0.05$), **9** (96%, $p < 0.05$), **10** (86%, $p < 0.05$), **12** (95%, $p < 0.05$), **18** (92%, $p < 0.05$), and **24** (99%, $p < 0.01$) (Figure 1A–F). In the previous study carried out in the same experimental condition morphine used as a reference drug significantly reduced the duration of the licking response in both phases of the formalin test.[21,22] Moreover, new anticonvulsant drug – lacosamide tested previously in our laboratory displayed antinociceptive activity in this test of tonic pain.[23]

2.4. Local anesthetic activity

The effect of selected derivatives **7**, **9**, **10**, **12**, **18**, and **24** on local anesthesia was examined using the tail immersion test. In this test, compounds **10** and **18** at a concentration of 2% significantly prolonged the animals' reaction time to a heat stimulus by 124% and 61%, respectively. Phenytoin, a well-known sodium channel blocker, in the same concentration prolonged the time reaction by 84%. Furthermore, mepivacaine, used as reference drug was highly active in this test. The results obtained are presented in Table 5.

Table 5. Local anesthetic activity in the tail immersion test.

Cmpd	Concentration (%)	Reaction time (s) to painful stimulus (± SEM)	Prolongation of the time reaction (%)
vehicle	0.5 % MC	4.83 ± 0.60	-
7	2	5.41 ± 0.53	12

9	2	5.66 ± 0.60	17
10	1	6.07 ± 1.13	26
10	2	10.80 ± 1.74**	124
12	2	5.53 ± 1.06	14
18	2	7.79 ± 0.95*	61
24	2	7.06 ± 0.76	46
PHT	1	5.72 ± 0.63	18
	2	8.90 ± 0.80***	84
vehicle	0.5 % MC	7.26 ± 2.17	-
Mepivacaine[a]	1	14.13 ± 2.67	94.63
	2	15.73 ± 2.09**	116.67

Each value represents the mean ± SEM obtained from 8 mice. Significant difference compared to the vehicle-treated group: *t*-test for cmpds. **7**, **9**, **12**, **18**, and **24** or one-way analysis of variance ANOVA followed by Dunnett's *post hoc* test (cmpd **10** and phenytoin - PHT): *p < 0.05, **p < 0.01, ***p < 0.001.

[a] Data from the previous study.[23]

2.5. *In vitro* radioligand binding studies

Intensive studies into the physiological and biochemical events occurring during epileptic seizures have provided insight into the molecular mechanisms by which these might be controlled. The fundamental role in establishing and regulating excitability of CNS neurons as well as suppression of seizures is ascribed to voltage-gated sodium channels (VGSCs) and voltage-dependent calcium channels (VDCC).[24] Thus, the brain ion channels are the molecular targets of a number of chemically diverse antiepileptic drugs from which the most important are phenytoin, lamotrigine, carbamazepine, and oxcarbazepine that are known to be a sodium channel blockers.[25–27] It should be stressed that such mode of action is characteristic for compounds active in the MES test. Considering the activity of compounds synthesized in the electrically induced convulsions as well as in pentylenetetrazole test for six molecules **7**, **9**, **10**, **12**, **18**, and **24** with various activity in the preclinical studies, their binding to Na^+ and Ca^{2+} (L-type) channels was assessed *in vitro*.

Table 6. *In vitro* binding assays (concentration 100 μM).

Cmpd	% Inhibition of control specific binding[a]	
	Voltage-sensitive Na^+ channel (site 2)[b]	Voltage-sensitive L-type Ca^{2+} channel (antagonist site)[c]
7	36.6	13.5
9	28.5	17.2
10	20.9	4.2
12	33.4	**71.0**
18	32.5	30.8
24	**63.5**	**82.4**
Phenytoin[d]	**53.9**	**57.8**

[a] Results showing an inhibition higher than 50% are considered to represent significant effects of the test compounds; results showing an inhibition between 25% and 50% are indicative of moderate effect; results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level.
[b] Compounds were evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]BTX to the voltage-sensitive sodium channel.
[c] Compounds were evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]nitrendipine to the voltage-sensitive Ca^{2+} channel.
[d] Reference drug tested in the same conditions.

As indicated in Table 6, the most effective binder to Na^+ and Ca^{2+} (L-type) channels was **24**, which showed activity only in MES test. High affinity to calcium channel demonstrated also **12**, which was active in both MES and *sc*PTZ tests. It should be highlighted that **12** and **24** were stronger sodium and calcium channel blockers than phenytoin.

Other compounds revealed only a moderate effect on both ion channels. The lack of the correlation between *in vivo* results and binding studies may suggest an additional mechanism of action responsible for anticonvulsant activity beyond the interaction with Na^+ and Ca^{2+} (L-type) channels.

3. Conclusion

In the current study, the series of 26 new compounds was synthesized and tested for anticonvulsant and neurotoxic properties. The obtained results revealed that the majority of

compounds exhibited anticonvulsant activity in the MES test and/or in the PTZ test. Several substances were also active in 6-Hz screen. The most potent compounds were more effective than ethosuximide and valproic acid used as the reference AEDs. Six molecules showed a strong antinociceptive activity and two of them also revealed local anesthetic properties. The *in vitro* binding studies revealed that the mechanism of anticonvulsant action may be partially connected with the influence on voltage-sensitive sodium and L-type calcium channels. The results obtained in the current study proved that compounds based on the pyrrolidine-2,5-dione template may be recognized as candidates for new effective AEDs.

4. Experimental Part

4.1. General

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp:) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and homogeneity of the compounds were confirmed by use of the thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). The TLC was performed on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany). Spots were detected by their absorption under UV light (λ = 254 nm) and by visualization with 0.05 mol I_2 in 10% HCl. The mass spectra were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, USA). The ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 100 mm column was used (Waters, Milford, USA). Preparative column chromatography was performed using silica gel 60 (particle size 0,063-0,200; 70-230 Mesh ATM) purchased from Merck (Darmstadt, Germany). Elemental analysis for C, H, and N were carried out by a micro method using the elemental Vario EI III Elemental analyzer (Hanau, Germany). The results of elemental analyses were within ±0.4%

of the theoretical values. ^{1}H NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$ operating at 300 MHz. Chemical shifts are reported in δ values (ppm) relative to TMS δ = 0 (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet).

4.2. Chemical synthesis

4.2.1. General procedure for the preparation of the 3-methyl- (3) and 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acids (4)

2-Methyl- or 2-ethyl-2-methyl-succinic acids (0.04 mol) were dissolved in 20 ml of water and 2-aminoacetic acid (0.04 mol) was gradually added. The mixtures were heated in a term-regulated sand bath with simultaneous distillation of water. After complete removal of water, the temperature of the reaction mixture raised up to 180°C and was maintained for ca. 1.5 h. The crude products, 3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (**3**) was recrystallized from methanol, while 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (**4**) was purified by column chromatography (S_1: dichloromethane: methanol, 9 : 1, *v/v*) to afford intermediates **4** as yellow oils.

4.2.1.1. 3-Methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (3). White solid; yield: 82%; R_f=0.58 (S_1); mp: 86–88ºC; HPLC: t_R=3.2 min.; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (d, 3H CH_3, *J*=6.9 Hz), 2.41 (d, 1H, H_b imide, *J*=13.5 Hz), 2.95–3.08 (m, 2H, imide), 4.28 (s, 2H, CH_2), 9.32 (brs, 1H, COOH); MS: *m/z* $[M+H]^+$ calcd for $C_7H_9N_1O_4$:172.15, found: 172.12.

4.2.1.2. 3-Ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (4). Yellow oil, yield: 70%; R_f=0.46 (S_1); HPLC: t_R=3.08 min., ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H, CH_3, *J*=7.5

Hz), 1.29 (s, 3H, CH_3), 1.59 (m, 1H, CH_2), 1.73 (m, 1H, CH_2), 2.57 (dd, 2H, imide, *J*=7.4 Hz), 4.22 (s, 2H, CH_2), 7.44 (brs, 1H, COOH); MS: *m/z* $[M+H]^+$ calcd for $C_9H_{13}N_1O_4$: 200.08, found: 200.10.

4.2.2. General procedure for the synthesis of compounds 5–26

The obtained 3-methyl- or 3-ethyl-3-methyl-2,5-dioxo-pyrrolidin-1-yl-acetic acids (**3**, **4**) (0.01 mol) were dissolved in 20 ml of DMF and *N*,*N*-carbonyl-diimidazole (0.01 mol) was added. The mixtures were stirred for 0.5 h at a room temperature. Afterwards, the appropriate 4-arylpiperazine (0.01 mol) was added. After 24 h of stirring the reaction mixtures were left in an ice-cold bath. The products were precipitated out with cold water and were purified by recrystallization from isopropyl alcohol giving the final compounds in yields ranging from 50% to 80%.

4.2.2.1. 3-Methyl-1-[2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-pyrrolidine-2,5-dione (5). White powdery crystals; yield: 58%; R_f=0.71 (S_1); HPLC: t_R=4.56 min.; mp: 131–132ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.2 Hz), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.97–3.07 (m, 2H, imide), 3.17 (t, 2H, piperazine, *J*=5.0 Hz), 3.25 (t, 2H, piperazine, *J*=5.1 Hz), 3.64 (t, 2H, piperazine, *J*=5.0 Hz), 3.76 (t, 2H, piperazine, *J*=5.0 Hz), 4.34 (s, 2H, CH_2), 6.90–6.95 (m, 3H, ArH), 7.26–7.32 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}N_3O_3$: 316.16, found: 316.18.

4.2.2.2. 1-{2-[4-(2-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (6). White powdery crystals; yield: 65%; R_f =0.70 (S_1); HPLC: t_R=4.99 min.; mp: 118–120ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.2 Hz), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.97–3.04 (m, 2H, imide), 3.07 (t, 2H, piperazine, *J*=5.0 Hz), 3.15 (t, 2H,

piperazine, *J*=5.0 Hz), 3.65 (t, 2H, piperazine, *J*=5.0 Hz), 3.77 (t, 2H, piperazine, *J*=5.0 Hz), 4.34 (s, 2H, CH_2), 6.90–7.10 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.77, 34.91, 36.48, 39.48, 42.35, 44.90, 50.13, 116.12, 119.26, 123.27, 124.55, 139.30, 154.09, 157.35, 163.30, 176.13, 180.24; MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_3F_1$: 334.37, found: 334.35.

4.2.2.3. 1-{2-[4-(4-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (7). White powdery crystals; yield: 62%; R_f =0.75 (S_1); HPLC: t_R=4.79 min.; mp: 166–168ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.2 Hz), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.97–3.04 (m, 2H, imide), 3.07 (t, 2H, piperazine, *J*=5.0 Hz), 3.15 (t, 2H, piperazine, *J*=5.0 Hz), 3.65 (t, 2H, piperazine, *J*=5.0 Hz), 3.77 (t, 2H, piperazine, *J*=5.0 Hz), 4.34 (s, 2H, CH_2), 6.86–6.99 (m, 2H, ArH), 7.01–7.27 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_3F_1$: 334.37, found: 334.35.

4.2.2.4. 1-{2-[4-(2-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (8). White powdery crystals; yield: 68%; R_f =0.73 (S_1); HPLC: t_R=5.62 min.; mp: 133–134ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.2 Hz), 2.43 (d, 1H, imide, *J*=13.3 Hz), 2.95–3.07 (m, 4H, 2H imide, 2H piperazine), 3.11 (t, 2H, piperazine, *J*=5.1 Hz), 3.65 (t, 2H, piperazine, *J*=5.0 Hz) 3.78 (t, 2H, piperazine, *J*=4.8 Hz), 4.34 (s, 2H, CH_2), 6.99–7.05 (m, 2H, ArH), 7.21–7.27 (m, 1H, ArH), 7.37–7.40 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.78, 34.92, 36.49, 39.53, 42.54, 45.04, 50.79, 51.20, 120.60, 124.43, 127.73, 128.96, 130.71, 148.45, 163.37, 176.13, 180.25; MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_3Cl_1$: 350.12, found: 350.37.

4.2.2.5. 1-{2-[4-(3-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (9). White powdery crystals; yield 70%; R_f =0.71 (S_1); HPLC: t_R=5.68 min.; mp: 139–

141ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (d, 3H, CH_3, *J*=7.2 Hz), 2.42 (d, 1H, imide, *J*=13.6 Hz), 2.91–3.06 (m, 2H, imide), 3.18 (t, 2H, piperazine, *J*=4.7 Hz), 3.26 (d, 2H, piperazine, *J*=4.6 Hz), 3.62 (d, 2H, piperazine, *J*=5.1 Hz), 3.73 (d, 2H, piperazine, *J*=4.6 Hz), 4.33 (s, 2H, CH_2), 6.76–6.88 (m, 3H, ArH), 7.18 (t, 1H, ArH, *J*=8.33 Hz); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_3Cl_1$:350.12, found: 350.37.

4.2.2.6. 1-{2-[4-(4-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (10). White powdery crystals; yield: 72%; R_f=0.73 (S_1); HPLC: t_R=5.61 min.; mp: 140–142ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (d, 3H, CH_3, *J*=7.2 Hz), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.97–3.06 (m, 2H, imide), 3.13 (t, 2H, piperazine, *J*=5.1 Hz), 3.21 (t, 2H, piperazine, *J*=5.1 Hz), 3.63 (t, 2H, piperazine *J*=5.0 Hz), 3.75 (t, 2H, piperazine, *J*=5.1 Hz), 4.33 (s, 2H, CH_2), 6.82–6.87 (m, 2H, ArH), 7.21–7.25 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}N_3O_3Cl_1$: 350.12, found: 350.37.

4.2.2.7. 1-{2-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (11). White powdery crystals; yield: 69%; R_f=0.73 (S_1); HPLC: t_R=6.29 min.; mp: 148–150ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (d, 3H, CH_3, *J*=6.9 Hz), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.99–3.09 (m, 6H, 2H, imide, 4H piperazine), 3.66 (brs, 2H piperazine), 3.77 (brs, 2H, piperazine), 4.33 (s, 2H CH_2), 6.92 (d, 1H, ArH, *J*=7.2 Hz), 7.14–7.19 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{19}N_3O_3Cl_2$: 384.27, found: 384.34.

4.2.2.8. 1-{2-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (12). White powdery crystals; yield: 77%; R_f=0.70 (S_1); HPLC: t_R=6.31 min; mp: 136–138°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (d, 3H, CH_3, *J*=6.6 Hz), 2.36 (d, 1H, imide, *J*=13.3 Hz), 2.87–2.99 (m, 2H, imide), 3.17 (d, 2H, piperazine, *J*=4.8 Hz), 3.25 (brs, 2H,

piperazine), 3.53 (brs, 2H, piperazine), 3.62 (d, 2H, piperazine, *J*=4.8 Hz), 4.29 (s, 2H, CH_2), 6.92–6.96 (m, 1H, ArH), 7.15 (d, 1H, ArH, *J*=2.5 Hz), 7.40 (d, 1H, ArH, *J*=8.9 Hz); MS: *m/z* $[M+H]^+$ calcd for $C_{17}H_{19}N_3O_3Cl_2$: 384.27, found: 384.34.

4.2.2.9. 1-{2-[4-(3-Trifluoromethylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (13). White powdery crystals; yield: 80%; R_f =0.77 (S_1); HPLC: t_R=6.05 min.; mp: 123–124ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.2 Hz), 2.44 (d, 1H, imide, *J*=13.6 Hz), 2.95–3.07 (m, 2H, imide), 3.23 (t, 2H, piperazine, *J*=5.1 Hz), 3.31 (t, 2H, piperazine, *J*=5.0 Hz), 3.66 (t, 2H, piperazine *J*=5.0 Hz), 3.77 (t, 2H, piperazine, *J*=5.1 Hz), 4.34 (s, 2H, CH_2), 7.06–7.16 (m, 3H, ArH), 7.38 (t, 1H, ArH, *J*=7.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.75, 34.92, 34.94, 36.47, 39.45, 41.96, 44.45, 48.84, 112.91, 116.87, 119.42, 129.76, 139.30, 150.86, 163.43, 176.11, 180.23; MS: *m/z* $[M+H]^+$ calcd for $C_{18}H_{20}N_3O_3F_3$: 383.36, found: 384.40.

4.2.2.10. 1-{2-[4-(2-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (14). White powdery crystals; yield: 79%; R_f=0.78 (S_1); HPLC: t_R=5.67 min; mp: 115–117°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (d, 3H, CH_3, *J*=7.7 Hz), 2.32 (s, 3H, CH_3), 2.43 (d, 1H, imide, *J*=13.6 Hz), 2.89 (t, 2H, imide, *J*=5.0 Hz), 2.95–3.05 (m, 4H, piperazine), 3.62 (t, 2H, piperazine, *J*=4.8 Hz), 3.74 (t, 2H, piperazine, *J*=4.5 Hz), 4.34 (s, 2H, CH_2), 6.98–7.05 (m, 2H, ArH), 7.15–7.21 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.79, 17.78, 34.92, 36.50, 39.56, 42.81, 45.28, 51.64, 119.29, 123.92, 126.74, 131.18, 132.72, 150.65, 163.37, 176.16, 180.28; MS: *m/z* $[M+H]^+$ calcd for $C_{18}H_{23}N_3O_3$: 330.39, found: 330.42.

4.2.2.11. 1-{2-[4-(3-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-methyl-pyrrolidine-2,5-dione (15). White powdery crystals; yield: 75%; R_f=0.71 (S_1); HPLC: t_R=5.10 min.; mp: 126–

128°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (d, 3H, CH_3, *J*=7.2 Hz), 2.32 (s, 3H, CH_3), 2.43 (d, 1H, imide, *J*=13.3 Hz), 2.94–3.06 (m, 2H, imide), 3.16 (t, 2H, piperazine, *J*=5.1 Hz), 3.23 (t, 2H, piperazine, *J*=5.1 Hz), 3.63 (t, 2H, piperazine, *J*=5.1 Hz), 3.74 (t, 2H, piperazine, *J*=5.0 Hz), 4.33 (s, 2H, CH_2), 6.72–6.75 (m, 3H, ArH), 7.15–7.20 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.78, 21.72, 34.91, 36.48, 39.50, 42.21, 44.69, 49.34, 49.52, 113.89, 117.67, 121.64, 129.09, 138.99, 150.83, 163.33, 176.13, 180.25; MS: *m/z* $[M+H]^+$ calcd for $C_{18}H_{23}N_3O_3$: 330.39, found: 330.42.

4.2.2.12. 3-Ethyl-3-methyl-1-[2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-pyrrolidine-2,5-dione (16). White powdery crystals; yield: 65%; R_f=0.84 (S_1); HPLC: t_R=5.65 min.; mp: 134–135°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.5 Hz), 1.36 (s, 3H, CH_3), 1.52–1.60 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.8 Hz), 3.17 (t, 2H, piperazine, *J*=5.1 Hz), 3.24 (t, 2H, piperazine, *J*=4.6 Hz), 3.64 (t, 2H piperazine, *J*=5.2 Hz), 3.74 (t, 2H, piperazine, *J*=5.3 Hz), 4.33 (s, 2H, CH_2), 6.88–6.97 (m, 2H, ArH), 7.26–7.32 (m, 3H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{25}N_3O_3$:344.19, found: 344.12.

4.2.2.13. 1-{2-[4-(2-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (17). White powdery crystals; yield: 55%; R_f =0.83 (S_1); HPLC: t_R=5.90 min.; mp: 118–119°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.4 Hz), 1.36 (s, 3H, CH_3), 1.60–1.84 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.3 Hz), 3.06 (t, 2H, piperazine, *J*=5.1 Hz), 3.14 (t, 2H, piperazine, *J*=4.8 Hz), 3.64 (t, 2H piperazine, *J*=5.4 Hz), 3.75 (t, 2H, piperazine, *J*=5.1 Hz), 4.33 (s, 2H, CH_2), 6.90–7.02 (m, 2H, ArH), 7.03–7.12 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.71, 23.93, 30.80, 39.39, 40.43, 42.33, 44.54, 50.14, 116.11, 119.24, 123.24, 124.55, 139.31, 154.08, 157.34, 163.33, 175.85, 182.52; MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_3F_1$: 362.18, found: 362.13.

4.2.2.14. 1-{2-[4-(4-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (18). White powdery crystals; yield: 50%; R_f =0.73 (S_1); HPLC: t_R=5.78 min.; mp: 122–124°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.4 Hz), 1.36 (s, 3H, CH_3), 1.56–1.84 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.3 Hz), 3.08 (t, 2H, piperazine, *J*=5.2 Hz), 3.15 (t, 2H, piperazine, *J*=5.0 Hz), 3.63 (t, 2H piperazine, *J*=4.9 Hz), 3.75 (t, 2H, piperazine, *J*=5.3 Hz), 4.32 (s, 2H, CH_2), 6.86–6.91 (m, 2H, ArH), 6.96–7.02 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_3F_1$: 362.18, found: 362.13.

4.2.2.15. 1-{2-[4-(2-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (19). White powdery crystals; yield: 55%; R_f =0.76 (S_1); HPLC: t_R=6.57 min.; mp: 124–126°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.4 Hz), 1.37 (s, 3H, CH_3), 1.54–1.80 (m, 2H, CH_2), 2.51 (d, 1H imide, *J*=18.5 Hz), 2.74 (d, 1H, imide, *J*=18.2 Hz), 3.03 (t, 2H, piperazine, *J*=4.9 Hz), 3.11 (t, 2H, piperazine, *J*=4.9 Hz), 3.66 (t, 2H piperazine, *J*=5.4 Hz), 3.77 (t, 2H, piperazine, *J*=4.8 Hz), 4.33 (s, 2H, CH_2), 7.00–7.03 (m, 2H, ArH), 7.21–7.40 (m, 2H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_3Cl_1$: 378.15, found: 378.08.

4.2.2.16. 1-{2-[4-(3-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (20). White powdery crystals; yield: 65%; R_f =0.83(S_1); HPLC: t_R=6.59 min.; mp: 107–109°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.4 Hz), 1.36 (s, 3H, CH_3), 1.62–1.84 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.5 Hz), 3.18 (t, 2H, piperazine, *J*=5.1 Hz), 3.25 (t, 2H, piperazine, *J*=5.0 Hz), 3.63 (t, 2H, piperazine, *J*=5.1 Hz), 3.75 (t, 2H, piperazine, *J*=4.6 Hz), 4.32 (s, 2H, CH_2), 6.77–6.88

(m, 3H, ArH), 7.16–7.19 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.70, 23.92, 30.80, 39.36, 40.43, 41.93, 44.43, 44.54, 48.70, 48.81, 114.50, 116.47, 130.20, 135.02, 151.76, 163.44, 175.81, 182.49; MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_3Cl_1$: 378.15: found: 378.08.

4.2.2.17. 1-{2-[4-(4-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (21). White powdery crystals; yield: 57%; R_f =0.62 (S_1); HPLC: t_R=6.51 min.; mp: 141–143°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.4 Hz), 1.36 (s, 3H, CH_3), 1.67–1.84 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.2 Hz), 3.13 (t, 2H, piperazine, *J*=5.4 Hz), 3.21 (t, 2H, piperazine, *J*=5.1 Hz), 3.63 (t, 2H piperazine, *J*=5.3 Hz), 3.75 (t, 2H, piperazine, *J*=5.1 Hz), 4.32 (s, 2H, CH_2), 6.82–6.87 (m, 2H, ArH), 7.21–7.26 (m, 1H, ArH); MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{24}N_3O_3Cl_1$: 378.15, found: 378.08.

4.2.2.18. 1-{2-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (22). White powdery crystals; yield: 58%; R_f =0.76 (S_1); HPLC: t_R=7.15 min.; mp: 148–150°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.5 Hz), 1.37 (s, 3H, CH_3), 1.67–1.85 (m, 2H, CH_2), 2.51 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.2 Hz), 3.02 (t, 2H, piperazine, *J*=5.1 Hz), 3.10 (t, 2H, piperazine, *J*=5.2 Hz), 3.66 (t, 2H piperazine, *J*=5.1 Hz), 3.78 (t, 2H, piperazine, *J*=4.7 Hz), 4.33 (s, 2H, CH_2), 6.92–6.95 (m, 1H, ArH), 7.17–7.21 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.70, 23.92, 30.81, 39.41, 39.41, 40.45, 42.48, 44.55, 44.99, 50.89, 51.29, 118.83, 125.35, 127.60, 134.18, 150.38, 163.46, 175.85, 182.52; MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}N_3O_3Cl_2$: 412.11, found: 412.04.

4.2.2.19. 1-{2-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (23). White powdery crystals; yield: 71%; R_f =0.84 (S_1); HPLC: t_R=7.15 min.; mp: 140–142°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.5 Hz), 1.36 (s, 3H, CH_3), 1.59–1.84 (m, 2H, CH_2), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.2 Hz), 3.16 (t, 2H, piperazine, *J*=4.9 Hz), 3.23 (t, 2H, piperazine, *J*=4.9 Hz), 3.63 (t, 2H piperazine, *J*=5.4 Hz), 3.74 (t, 2H, piperazine, *J*=4.9 Hz), 4.32 (s, 2H, CH_2), 6.74 (dd, 1H, ArH, *J*=2.9 Hz), 6.96 (d, 1H, ArH, *J*=2.8 Hz), 7.30 (d, 1H, ArH, *J*=9.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.71, 23.93, 30.81, 39.35, 40.43, 41.85, 44.35, 44.56, 48.71, 48.83, 115.94, 118.00, 123.33, 130.62, 132.95, 150.09, 163.44, 175.82, 182.50; MS: *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}N_3O_3Cl_2$: 412.11, found: 412.04.

4.2.2.20. 1-{2-[4-(3-Trifluoromethylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (24). White powdery crystals; yield: 65%; R_f =0.73 (S_1); HPLC: t_R=6.88 min.; mp: 128–130°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.5 Hz), 1.36 (s, 3H, CH_3), 1.67–1.85 (m, 2H, CH_2), 2.51 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.2 Hz), 3.23 (t, 2H, piperazine, *J*=5.2 Hz), 3.30 (t, 2H, piperazine, *J*=5.1 Hz), 3.66 (t, 2H piperazine, *J*=5.0 Hz), 3.77 (t, 2H, piperazine, *J*=5.1 Hz), 4.33 (s, 2H, CH_2), 7.05–7.16 (m, 3H, ArH), 7.38 (t, 1H, ArH, *J*=7.90 Hz); MS: *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}N_3O_3F_3$: 412.18, found: 412.11.

4.2.2.21. 1-{2-[4-(2-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (25). White powdery crystals; yield: 60%; R_f =0.53 (S_1); HPLC: t_R=6.65 min.; mp: 137–139°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.5 Hz), 1.37 (s, 3H, CH_3), 1.67–1.85 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.51 (d, 1H, imide, *J*=18.2 Hz), 2.74 (d, 1H, imide, *J*=18.2 Hz), 2.89 (t, 2H, piperazine, *J*=5.0 Hz), 2.97 (t, 2H, piperazine,

J=5.0 Hz), 3.62 (t, 2H piperazine, *J*=5.0 Hz), 3.74 (brs, 2H, piperazine), 4.33 (s, 2H, CH_2), 6.98–7.05 (m, 2H, ArH), 7.18–7.21 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 8.72, 17.78, 23.94, 30.81, 39.47, 40.45, 42.80, 44.55, 45.27, 51.48, 51.66, 119.29, 123.92, 126.73, 131.17, 132.72, 150.67, 163.42, 175.89, 182.56; MS: *m/z* $[M+H]^+$ calcd for $C_{20}H_{27}N_3O_3$: 358.21, found: 358.14.

4.2.2.22. 1-{2-[4-(3-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3-ethyl-3-methyl-pyrrolidine-2,5-dione (26). White powdery crystals; yield: 52%; R_f =0.38 (S_1); HPLC: t_R=5.94 min.; mp: 110–112°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, CH_3, *J*=7.7 Hz), 1.36 (s, 3H, CH_3), 1.62–1.84 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.50 (d, 1H imide, *J*=18.2 Hz), 2.73 (d, 1H, imide, *J*=18.2 Hz), 3.16 (t, 2H, piperazine, *J*=5.2 Hz), 3.23 (t, 2H, piperazine, *J*=5.0 Hz), 3.63 (t, 2H piperazine, *J*=5.2 Hz), 3.75 (t, 2H, piperazine, *J*=5.0 Hz), 4.33 (s, 2H, CH_2), 6.72–6.75 (m, 3H, ArH), 7.17 (t, 1H, ArH, *J*=7.7 Hz); MS: *m/z* $[M+H]^+$ calcd for $C_{20}H_{27}N_3O_3$: 358.21, found: 358.14.

4.3. Pharmacology

Male albino mice (CD-1 strain) weighing 16–26 g were used as experimental animals. The animals were housed in cages at room temperature of 22 ± 2°C, under a light/dark (12/12) cycle and had free access to food and water. Ambient temperature of the room and humidity were kept consistent throughout all tests. For the experiments, the animals were randomly selected. Each group consisted of three-four animals (anticonvulsant screening) and each mouse was used only once. The compounds were suspended in 0.5% methylcellulose/water mixture. 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 hours after administration. Compounds **5**–**8**, **10**, and **13** were pharmacologically pre-evaluated

within the Antiepileptic Drug Development (ADD) Program, realized in NINDS/NIH, Rockville, USA, using procedures described elsewhere.[28] Compounds **9**, **11**, **12**, and **14**–**26** were tested at the Department of Pharmacodynamics, Jagiellonian University Medical College. All the procedures were approved by the Local Ethical Committee on Animal Testing at the Jagiellonian University in Krakow (no 16/2014 and 100/2014).

4.3.1. The maximal electroshock test (MES)

In the MES screen, electroconvulsions were produced by an electrical stimulus of 0.2 s in duration at a frequency of 50 Hz delivered via standard auricular electrodes by an electroshock generator (Hugo Sachs rodent shocker, Germany). The tonic hind-limb extension was taken as the end-point. Mice not displaying hind-limb tonic extension were considered protected from seizure.[29]

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

*Sc*PTZ-induced seizure was performed by subcutaneously injection of pentylenetetrazole (Sigma Aldrich, Poland) at a dose of 85 mg/kg. This produced clonic convulsions lasting for at least five seconds in 97% of animal tested. The absence of clonic convulsions in the observed time period of 30 min was interpreted as the compound's ability to protect against PTZ-induced seizure.[30]

4.3.3. The neurological toxicity (NT)

Neurological toxicity induced by compound was detected in mice using standardized rotarod test. Untreated control mice 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 animal to maintain equilibrium on the rod revolting at 10 rpm for at least 1 minute.[31]

4.3.4. The 6–Hz model

This screen was carried out according to the protocol originally described by Brown et al.[32] and more recently by Barton et al.[33] and Kaminski et al.[34] 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.[34]

4.3.5. Quantification Studies

The ED_{50} is defined as the dose of a drug protecting 50% of animals against the MES, *sc*PTZ and 6-Hz seizures. Groups of six animals received various doses of the compound until at least three points were established in the range of 10-90% seizure protection or minimal motor impairment. Both ED_{50} and TD_{50} values with 95% confidence limits were calculated by probit analysis.[35] The PI (protective index) value was calculated as the ratio of TD_{50} to ED_{50} ($PI = TD_{50}/ED_{50}$).

4.3.6. Antinociceptive activity – the formalin test

Antinociceptive activity in the formalin hind paw test was examined according to the method previously described.[36] In mice intraplantarly injection of diluted formalin produces a

biphasic nocifensive behavioural response (i.e., licking or biting the injected hind paw). The acute nociceptive phase lasts for the first 5 min, whereas the second inflammatory phase occurs between 15 and 30 min after formalin injection. The mice were pretreated with the test compounds or the vehicle 30 min (**9**, **18** and **24**), 1 h (**7**) or 4 h (**10** and **12**) before the experiment at the doses equal to the respective ED_{50} values obtained in MES test. Then, 20 µl of a 5% formalin solution was injected intraplantarly into the right hind paw of the mouse. Immediately after formalin injection, the animals were placed individually into transparent Plexiglas cages and were observed during the next 30 min. Time (in seconds) spent on licking or biting the injected hind paw in selected intervals, 0–5, 15–20, 20–25, and 25–30 min, was measured in each experimental group and was indicator of nociceptive behaviour.

4.3.7. Local anesthetic activity–the tail immersion test

The heat method used for evaluating the systemic analgesic activity can also be used with a slight modification to determine whether a compound possesses local anesthetic activity. The method was performed by subcutaneously (*sc*) injecting the investigated substance in a constant volume of 0.2 ml about 1 cm from the root of the mouse tail and waiting 15 min. The 3 cm distal part of the tail was immersed into temperature controlled water (50 ± 0.5°C). The reaction time (the length of time until the tail is pulled away) was measured by the means of a chronometer. The observation time was limited to 20 s.[37]

4.3.8. *In vitro* pharmacology

Sodium and calcium channels binding assay. The radioligand binding studies were performed commercially in Cerep Laboratories (Poitiers, France) using testing procedures described elsewhere (Na^+ channel,[38] L-type Ca^{2+} channel [39]).

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