SYNTHESIS AND STUDY OF SUBSTITUTED AMIDES OF SOME 5-MEMBERED ISOCYCLIC AND HETEROCYCLIC ACIDS AS POTENTIAL ANTICONVULSANTS

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Abstract: A series of substituted amides of isocyclic and heterocyclic acids have been synthesized. All obtained compounds were evaluated qualitatively for their anticonvulsant activity in the maximal electroshock seizure test (MES test) and psychomotor seizure test (6 Hz test) in mice after intraperitoneal administration. The neurological toxicity was determined in the rotorod neurotoxicity test (Tox test). Two of these compounds appeared most promising: 3-oxo-1-cyklopentanecarboxylic acid 2-fluorobenzylamide (**5**), 1-cyclopentenecarboxylic acid 2-(trifluoromethyl)benzylamide (**8**) and were tested quantitatively in MES test in mice after oral administration (o.p.) and in 6 Hz test in mice after intraperitoneal administration (i.p.). Compound (**5**) showed MES ED_{50} = 82.65 mg/kg and compound (**8**) showed 6 Hz ED_{50} = 84.13 mg/kg.

Keywords: anticonvulsant activity , substituted benzylamides of isocyclic acids, substituted benzylamides of heterocyclic acids, the minimal clonic seizure test, maximal electroshock seizure test

Epilepsy is the main chronic neurological disease that affects more than 1% of the world's population. It consists of various sudden, unprovoked and chronic seizures, which are a result of heightened excitability of connected groups of neurons that discharge electrically in a spontaneous and synchronic way. The purpose of the therapy of epilepsy is to obtain full control of the occurrence of seizures and their complete management or partial reduction of the occurrence of seizures together with alleviating the accompanying psychological symptoms and preventing post-seizure effects of brain damage (1-3). The therapy of epilepsy in the majority of cases is based on pharmacological treatment. The currently used antiepileptic drugs (AEDs) are still ineffective for about 25%-40% of patients suffering from epilepsy (4). Besides the inefficiency of the AEDs against the drug-resistant epilepsy, the therapy is limited by their adverse side effects (5). Therefore, there is a need to design and synthesize new anticonvulsant substances which would be safer and more effective in the therapy of epilepsy, especially in case of the drug-resistant type (6, 7).

In previous papers we reported that several compounds with the structure of aromatic amides derivative of heterocyclic and isocyclic acids showed anticonvulsant activity in established animal models of seizures, especially in an electrically induced seizure, and relatively low neurotoxicity (8-10, 11, 13). Picolinic acid benzylamide turned out to be the most effective anticonvulsant, for which the value ED_{50} was 17,8 mg/kg and therapeutic index PI > 28.0 in maximal electroshock seizure test after oral administration in rats, which was comparable with the standard antiepileptic drug phenytoin (ED_{50} = 29.8 mg/kg). However, its half time of action is about 15 min. (8). These findings were the reason why the basic structure of picolinic acid benzylamide has been modified to obtain equally effective anticonvulsants, but with prolonged time of action. Considering the structure-activity relationships (SAR) among the picolinic acid benzylamide analogues it was determined that the position of amide group in relation to the heterocyclic or isocyclic ring of the acidic part and the distance between the ring of the acidic part and the amide group is essential for anticonvulsant activity (9, 13).

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The same effect was observed in case of the increase in the distance between the amide group and phenyl moiety (12). Our previous research also demonstrated that the substitution in the benzyl moiety, especially with an electron-withdrawing fluorine atom at the 2-site, is able to increase the anticonvulsant activity, contrary to electron-donating groups (10, 13). However, when a chlorine atom was incorporated at the same position, the compounds were inactive (11, 13). The significant influence of the substitution with electron-withdrawing groups in benzyl moiety on the anticonvulsant activity among lacosamide derivatives has been reported by Kohn et al. (14, 16-20). According to this research, the introduction of an electron-withdrawing substituent larger than fluorine atom such as trifluoromethyl or trifluoromethoxy group also may cause an increase in the anticonvulsant activity (14, 16). Additionally, the structure of the heterocyclic ring of the acid portion was modified by the reduction of a 6-member ring to a 5-member one. It was found that a 5-membered ring of acids is especially useful. Substituents of the ring, presence of heteroatoms or double bonds have a decisive influence upon the activity (11). In this series of compounds, 3-oxo-1-cyklopentanecarboxylic acid benzylamide (1), 1-cyclopentenecarboxylic acid benzylamide (2) and 1-cyclopentenecarboxylic acid 2-fluorobenzylamide (3) as well

as 2-furoic acid benzylamide (4) seemed most valuable (Fig. 1) (11-13). However, the MES seizure protection values in mice for these compounds were lower than that of the parent compound, picolinic acid benzylamide.

The purpose of the present investigations was to look for new effective anticonvulsants in group of benzylamides of 5-member isocyclic and heterocyclic acids. Taking into consideration the increase in activity of previously obtained compounds with the fluorine substituents, we have designed and synthesized a series of analogues of compounds 1-3 (Fig. 1), differently substituted in benzyl moiety. Additionally, in the structure of the analogues of compound 4, apart from the substitution in benzyl portion, the position of the amide group in relation to the oxygen atom of the 5-member ring was changed.

One of the prerequisites of these compounds' activity is also an appropriate lipophilicity of the molecule assuring a penetration of biological barriers and a good solubility in body fluids. Furthermore the protein binding and the receptor affinity of the compound is also associated with the lipophilicity of a molecule. As it was found previously, the optimal log P value of active benzylamide anticonvulsants ought to be > 0 and probably near 3. However, it has been observed, that compounds with higher log P


(1)
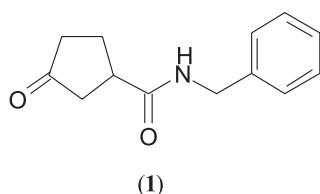
MES ED_{50} = 80.32 mg/kg [*]

TD_{50} = 253.89

PI = 3.2 (TD_{50}/ED_{50})
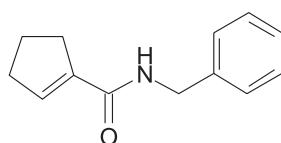
(2)

MES ED_{50} = 85.36 mg/kg [*]

TD_{50} = 212.60, PI = 2.5 (TD_{50}/ED_{50})

6 Hz ED_{50} = 50.29 mg/kg [*]

(3)
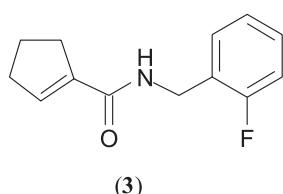
MES ED_{50} = 54.05 mg/kg [*]

TD_{50} = 312.04

PI = 5.8 (TD_{50}/ED_{50})

(4)
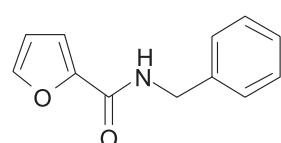
MES ED_{50} = 36.5 mg/kg [*]

TD_{50} = 269.75

PI = 7.4 (TD_{50}/ED_{50})

Figure 1. Chemical structure of parent compounds **1-4** (*mice, i.p)
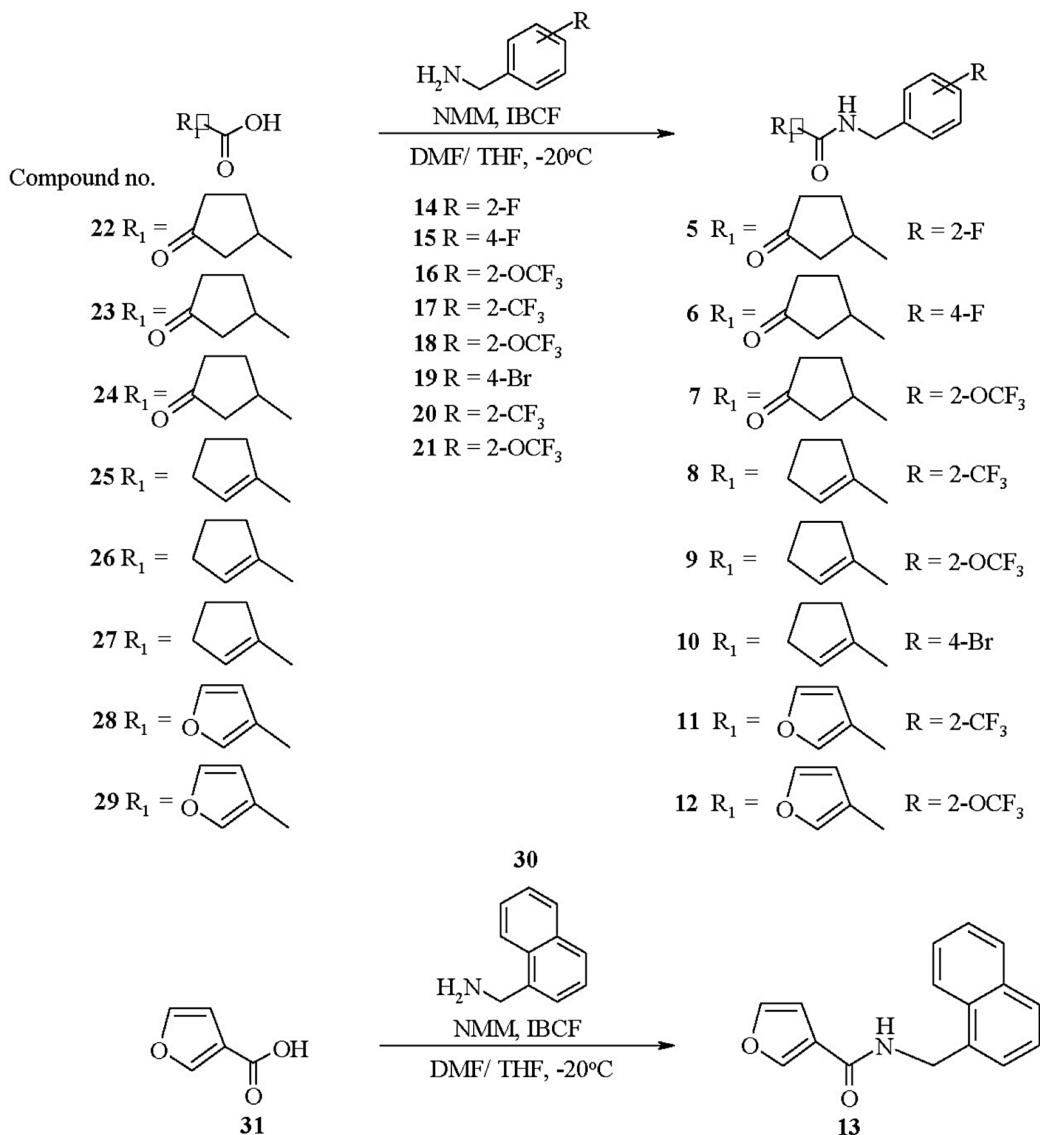
Scheme 1. General procedure for the synthesis of compounds

value can also show anticonvulsant activity. Therefore log P values of the partition coefficient between n-octanol and water of these compounds were calculated.

EXPERIMENTAL

Chemistry
Methods and materials

Melting points were determined in open capillary tubes using a DigiMelt MPA 161 melting point apparatus (SRS, USA) and are uncorrected. Reactions were monitored by analytical thin-layer chromatography (TLC) carried out on a 0.25 mm thickness silica gel plates (Merck Kieselgel 60 F-254). The spots were visualized in UV light (254 nm) or with 0.3% ninhydrin in ethanol:CH_3COOH (97 : 3 v/v). The column chromatography (CC) was performed under gravity on silica gel column (1.8 x 45 cm^3, Merck, grade 230 to 400 mesh). The solvent systems used in TLC and CC were chloroform: methanol in different ratios. HPLC was performed on a Shimadzu chromatograph (Shimadzu Corp., Japan) equipped with LC-10AT pump, SPD-10A UV spectrophotometer and a computer registrator/ recorder (ChromaX 2010 POL-LAB, Poland) by

Table 1. Physical and analytical data of the synthesized compounds **5-13**.

structure A   structure B   structure C

Compound no.	Structure	Aryl site	Formula R	Yield Mol. Weight	M.p (%)	(°C)	R_f [b]	Log P [c]
5.	A	2	F	$C_{13}H_{14}NO_2F$ 235.26	65.5	74-75	0.67	1.65
6.	A	4	F	$C_{13}H_{14}NO_2F$ 235.26	30.5	116-117	0.60	1.65
7.	A	2	OCF_3	$C_{14}H_{14}NO_3F_3$ 301.28	32.0	70-71	0.71	3.30
8.	B	2	CF_3	$C_{14}H_{14}NOF_3$ 269.28	37.0	91-92	0.85	3.25
9.	B	2	OCF_3	$C_{14}H_{14}NO_2F_3$ 285.28	30.7	83-84	0.85	3.95
10.	B	4	Br	$C_{13}H_{14}NOBr$ 280.16	44.3	141-142	0.79	3.16
11.	C	2	CF_3	$C_{13}H_{10}NO_2F_3$ 269.24	23.0	109-111	0.89	1.20
12.	C	2	OCF_3	$C_{13}H_{10}NO_3F_3$ 285.24	40.3	83-85	0.81	2.00
13.	C	[a]1-naphthylmethyl		$C_{16}H_{13}NO_2$ 251.29	50.9	102-104	0.74	2.22

[a]The benzyl fragment was replaced with 1-naphthylmethyl group. [b]Solvent system for TLC was chloroform: methanol (95: 5). [c]The lipophilicity of the compounds expressed as log P value calculated by a computer method

Table 2. ^{1}H NMR spectra in $CDCl_3$ of the synthesized compounds.

Compound No.	Chemical shift in δ (*ppm*) in $CDCl_3$
5.	2.08-2.62 (m, 6 H, 3x CH_2), 2.89 (p, 1H, CH), 4.51 (d, J = 6 Hz, 2H, CH_2), 5.98 (br s, 1H, NH), 7.01-7.16 (m, 2H, ArH), 7.32-7.38 (m, 2H, ArH);
6.	2.10-2.65 (m, 6H, 3x CH_2), 2.89 (p, 1H, CH), 4.44 (d, J = 5.7 Hz, 2H, CH_2), 5.82 (br s, 1H, NH), 6.98-7.08 (m, 2H, ArH), 7.22-7.30 (m, 2H, ArH);
7.	2.08-2.62 (m, 6H, 3x CH_2), 2.89 (p, 1H, CH), 4.54 (d, J = 6 Hz, 2H, CH_2), 5.91 (br s, 1H, NH), 7.23-7.38 (m, 3H, ArH), 7.41 (d, J = 7.5, 1H, ArH);
8.	1.98 (p, 2H, CH_2), 2.44-2.60 (m, 4H, 2x CH_2), 4.69 (d, J = 6 Hz, 2H, CH_2), 5.98 (br s, 1H, NH), 6.56 (br s, 1H, CH), 7.38 (t, J = 7.8 Hz, 1H, ArH), 7.52 (t, J = 7.8 Hz, 1H, ArH), 7.59-7.66 (m, 2H, ArH);
9.	1.99 (p, 2H, CH_2), 2.44-2.60 (m, 4H, 2x CH_2), 4.58 (d, J = 6 Hz, 2H, CH_2), 5.97 (br s, 1H, NH), 6.56 (t, J = 2.4, 1H, CH), 7.22-7.36 (m, 3H, ArH), 7.46 (d, J = 7.5 Hz, 1H, ArH);
10.	1.99 (p, 2H, CH_2), 2.45-2.60 (m, 4H, 2x CH_2), 4.46 (d, J = 6 Hz, 2H, CH_2), 5.94 (br s, 1H, NH), 6.57 (t, J = 2,4 Hz, 1H, CH), 7.18 (d, J = 8.1 Hz, 2H, ArH), 7.45 (d, J = 8.4 Hz, 2H, ArH);
11.	4.75 (d, J = 6 Hz, 2H, CH2), 6.19 (br s, 1H, NH), 6.65 (s, 1H, CH), 7.39 (t, J = 6 Hz, 2H, 2x CH), 7.53 (t, J = 9 Hz, 1H, CH), 7.60-7.70 (m, 2H, 2x CH), 7.93 (s, 1H, CH);
12.	4.65 (d, J = 3 Hz, 2H, CH_2), 6.22 (br s, 1H, NH), 6.65 (s, 1H, CH), 7.20-7.38 (m, 3H, 3x CH), 7.40-7.50 (m, 3H, 3x CH);
13.	5.02 (d, J = 5.1 Hz, 2H, CH_2), 6.02 (br s, 1H, NH), 6,56 (s, 1H, H, furan), 7.37-7.59 (m, 5H, H, furan, 4x H, naphthalene), 7.81-7.93 (m, 3H, H, furan, 2x H, naphthalene), 8.06 (d, J = 4.5 Hz, 1H, H, naphthalene);

using Waters Symmetry C 18 column (5 μm, 4.6 mm x 150 mm) and methanol: water (60 : 40 or 70 : 30 v/v) as eluents at a flow rate of 1 mL/min. The peaks were recorded at 262 nm for all compounds. ^{1}H NMR spectra were recorded at 300 Hz using tetramethylsilane (TMS) as a internal standard as

well as $CDCl_3$ as solvent and were obtained in a Varian VNMRS 300 MHz Oxford spectrometer (Varian Inc., USA). The chemical shifts (δ) were reported in parts per million (ppm) from tetramethylsilane. Signals multiplicities were represented by following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), m (multiplet). Coupling constants (*J*) were given in Hertz (Hz). Elemental analyses for C, H, N and Br were performed on an Elementar– Vario El III Element Analyzer (Elementar Analysensysteme GmbH, Germany). The mass spectra were taken on Qualtra LC.LR spectrometer and 4000 QTRAP spectrometer. All chemicals were purchased from Sigma Aldrich (St. Louis, USA) and were used without further purification. The solvents were obtained from POCH (Gliwice, Poland). All analytical data (^{1}HNMR, ESI-MS, HPLC, elemental analyses) clearly confirmed the structure and purity of the obtained compounds. The elemental analyses were within ± 0.4 % of the theoretical value. The purity of the products was ≥ 95%.

Table 3. Mass spectra of the synthesized compounds.

Compound No.	ESI-MS (m/z, M+Na$^+$)
5.	258.12
6.	258.11
7.	324.31
8.	292.15
9.	308.32
10.	302.07 for (79) Br, 304.07 for (80) Br
11.	292.00
12.	308.10
13.	274.16

Table 4. Anticonvulsant identification. Qualitative tests of synthesized compounds **5-13** in mice.

Compound no.	MES^b (0.5 h)		6 Hz^c (32 mA, 0.5 h)		Tox^d (0.5 h)	
	Dose (mg/kg)	N/F^a	Dose (mg/kg)	N/F^a	Dose mg/kg	N/F^a
5.	100	1/4	100	0/4	100	0/8
	300	4/4 (3/4^e)	300	4/4	300	4/8* (0/8)
6.	100	0/4	100	0/4	100	0/8
	300	3/4 (2/4^e)	300	4/4	300	3/8 (0/8^e)
7.	100	2/4	100^f	0/4	100	0/8
	300	4/4 (2/4^e)	300^f	2/4	300	3/8**
8.	100	2/4	100	4/4	100	0/8
	300	4/4 (4/4^e)	300	4/4 (3/4^e)	300	7/8* (4/8^e)
9.	100	2/4	100^f	0/4	100	0/8
	300	3/4 (3/4^e)	300^f	0/4	300	0/8 (0/8^e)
10.	100	0/4	100	0/4	100	0/8
	300	1/4	300	2/4 (2/4^e)	300	0/8 (0/8^e)
11.	100	0/4	100^f	0/4	100	0/8
	300	4/4	300^f	4/4	300	3/8
12.	100	1/4	100^f	3/4	100	0/8
	300	4/4 (4/4^e)	300^f	4/4 (4/4^e)	300	7/8* (0/8^e)
13.	100	2/4	100^f	1/4	100	0/8
	300	4/4 (4/4^e)	300^f	4/4 (4/4^e)	300	0/8 (1/8)

[a]Doses of 30, 100 and 300 mg/kg were administered intraperitoneally to mice. The animals were examined at 0.5 and 2 h. The data were presented as N/F, where N is the number of animals protected or toxic and F is the number of animals tested. [b]Maximal electroshock test. [c]Psychomotor seizure test with a 32 mA current. [d]Tox- neurological toxicity rotorod test. [e]The animals were examined at 2 h. [f]Psychomotor seizure test with a 44 mA current. [*]The animals were unable to grasp rotorod, [**]The animals were sedated

General procedure for synthesis of amides

The compounds **5-13** were synthesized using the mixed anhydrides method (21) of peptide synthesis. The suitable acid (10 mmol) was dissolved in dry tetrahydrofuran (THF) or a mixture of dimethylformamide (DMF)/tetrahydrofuran (30 mL). Next, *N*-methylmorpholine (NMM) (10 mmol, 1.1 mL) was added and the mixture was stirred under nitrogen and chilled to -20°C. Isobutyl chloroformate (IBCF) (10 mmol, 1.3 mL) was added dropwise to keep the temperature below -15 °C. Then the suitable amine: 2- or 4-fluorobenzylamine; 2- trifluoromethoxybenzylamine; 2-trifluoromethylbenzylamine, 4-bromobenzylamine or 1-naphthylmethylamine (10 mmol) in THF was added in small portions and the reaction mixture was stirred at -15°C for 30 min. and at room temperature for 1 h. The solution was concentrated *in vacuo* and the residue was dissolved in $CHCl_3$ (40 mL). This solution was washed with 20 mL portions of 1 M HCl, saturated $NaHCO_3$ solution and saturated NaCl solution, then dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The obtained compounds were purified as follows: **5, 6, 8, 11, 12** and **13** by crystallization from ethyl acetate/hexane, **10** by crystallization from ethyl acetate and **7, 9** by column chromatography in chloroform as eluent and then by crystallization from ethyl acetate/hexane. All stages of the synthesis were controlled by the thin-layer chromatography. The general procedure for the synthesis of the obtained compounds is shown in Scheme 1.

Pharmacology

The initial pharmacological evaluation of compounds **5-13** was performed in three tests: maximal electroshock seizure (MES), psychomotor seizure (6 Hz) and neurological toxicity (Tox), according to the standard procedures proposed by the National Institute of Neurological Disorders and Strokes, within the Antiepileptic Drug Development (ADD) Program. The MES and 6 Hz tests have clearly different pharmacological profiles. The MES test is used to assess a compound's efficacy against generalized tonic-clonic seizure while the 6 Hz test indicates compounds with activity against complex partial seizures and is considered a model of resistant epilepsy (22, 23). The maximal electroshock (MES) test was performed using the procedures described subsequently by Krall et al. (24), Swinyard et al. (25), White et al. (26) and White et al. (27). In the psychomotor seizure (6 Hz) test, the procedure originally described by Brown et al. (28) and subsequently by Barton et al. (29) and Kaminski et al. (30) was used. The neurological toxicity was assessed in rotorod test according to the procedure described by Dunham et al. (31). All compounds were tested qualitatively after intraperitoneal injection (*i.p.*) in mice at three dosage levels: 30 mg/kg,

Table 5. Quantification studies of compounds **5, 8** in the MES test in mice [a].

Compound no.	MES ED_{50} (mg/kg, 0.25 h[b])	Tox[c] TD_{50} (mg/kg, 0.25 h[b])	PI[d]
5	82.65 (64.05-104.45)[e]	ND	ND
8	186.65 (120.18-266.4)	ND	ND
Phenytoin [f]	5.64 (4.74-6.45)	41.0	7.3
Valproic acid [f]	263 (237-282)	-	1.5

[a]The compounds were administered orally to mice. [b]Time to peak effect. [c]Tox- neurological toxicity rotorod test. [d]Protective index value, PI (TD_{50}/ED_{50}). [e]Numbers in parentheses are 95% confidence intervals. [f]Reference (29)

Table 6. Quantification studies of compounds **5, 8** in psychomotor seizure test (6 Hz, current 44 mA, mice[a].

Compound no.	6 Hz ED_{50} (mg/kg, 0.25h[b])	Tox[c] TD_{50} (mg/kg, 0.25 h[b])	PI[d]
5	208.24 (136.08-297.9)[e]	435.25 (392.47-520.59)	2.1
8	84.13 (79.28-87.7)	211.02 (172.79-261.63)	2.5
Levetiracetam [f]	1089 (787-2650)	>500	-
Valproic acid [f]	310 (258-335)	398 (356-445)	1.3

[a]The compounds were administered intraperitoneally to mice. [b]Time to peak effect. [c]Tox- neurological toxicity rotorod test. [d]Protective index value, PI (TD_{50}/ED_{50}). [e]Numbers in parentheses are 95% confidence intervals. [f]Reference (29)

100 mg/kg and 300 mg/kg. The tested compounds were solubilized in 0.5% methylcellulose/water suspension. Anticonvulsant activity and neurotoxicity were noted at 0.5 h and 2 h after administration. The results from these tests are expressed as the number of animals protected in the MES test, 6 Hz test or exhibiting neurotoxicity in the rotorod test divided by the number of animals tested. The obtained pharmacological data from the above tests are summarized in Table 4. Two selected compounds **5** and **8** were assessed quantitatively in both the MES (mice, *p.o*) and the psychomotor seizure (6 Hz, mice, i.p) assays. These compounds were solubilized as in the initial evaluation. In the quantitative determination of ED_{50} and TD_{50} values by the respective anticonvulsant procedure, eight animals received various doses of the compound *via* the *i.p.* or *p.o.* route and were tested in the respective anticonvulsant test at previously estimated time of peak effect. This procedure was repeated until at least three points were established between the dose level which protected 0% of the animals and the dose which protected 100% of the animals or between the dose level which induced no signs of toxicity in any of the animals and the dose which was toxic to all of the animals. From the data obtained, the respective ED_{50} and TD_{50} values, 95% confidence intervals, the slope of the regression line and the standard error of the slope were calculated. The results from these tests are presented in Tables 5 and 6.

RESULTS

Physical and analytical data of the synthesized compounds are given in Tables 1, 2 and 3. The results of pharmacological tests are presented in Tables 4-6.

DISCUSSION AND CONCLUSION

The result of our previous investigations indicated that the modification of the previously obtained most active structures by the reduction of a 6-member ring to a 5-member one in their acid portion yielded derivatives with anticonvulsant activity (11). However, a slight decrease in the anticonvulsant activity was observed. It seems to be caused by the lack of a nitrogen atom in the ring, because among benzylamides of monocyclic 6-membered heterocyclic acids containing nitrogen atom there are anticonvulsants of high effectiveness (8-10). However, there is no explanation at the moment of the difference between the activity of 6-member and 5-member structures. Additionally, it has been

reported that electron-withdrawing groups on the benzylamide site are able to increase the anticonvulsant activity. Our earlier study also indicated the benefit of incorporating fluorine substituents to N-benzylamide moiety (10, 13). Therefore, it was decided to prepare halogen-substituted amides of 5-membered isocyclic (**5, 6, 7, 8, 9, 10**) and heterocyclic acids (**11, 12**). The fluoro (**5, 6**) , the trifluoromethylo (**8, 11**), the trifluoromethoxy (**7, 9**) and bromo (**10**)) substituents were selected for this study. All the synthesized compounds were screened in qualitative tests : MES-test, 6 Hz-test (32 mA or 44 mA) and Tox test. Compounds **5, 7, 8, 9** and **13** displayed protection against MES- induced seizures (2/4 mice at a dose of 100 mg/kg) as well as compounds **8** and **12** were more potent in 6 Hz test (4/4 mice at the same dose), but these activities were noticed only after 0.5h administration (Table 4). Higher protection (4/4 or 3/4 mice) in the MES test was observed for all compounds at a maximum dose (300 mg/kg) in both time intervals 0.5 h and 2 h. Except the compound **10** which was ineffective. Similar results were obtained in the psychomotor (6 Hz) seizure test (Table 4). The compounds **5** and **8** were recognized as most promising and put to further tests. For these compounds there were estimated the median effective (ED_{50}) and toxic (TD_{50}) doses in mice after oral (*p.o.*) administration in MES test as well as in mice after *i.p.* administration in 6 Hz test. The results of the quantitative MES test for compounds **5** and **8** were compared with the data for phenytoin and valproic acid as reference antiepileptic drugs (Table 5). Both compounds displayed a markedly higher median effective doses than that of phenytoin. In contrast, the anticonvulsant activity of compounds **5** and **8** were noticeably better than that of the anticonvulsant drug, valproic acid. In the quantitative 6 Hz test, for comparison the data for known anticonvulsant drugs: levetiracetam and valproic acids were given. The results of the quantification studies in the psychomotor seizure test (44 mA) revealed higher activity of both compounds and lower toxicity than that of the reference drugs (Table 6). However, it has been reported that with the increase in the current intensity to 44 mA in 6 Hz test, there was observed a progressive decrease in potency for some anticonvulsants, including valproic acid and levetiracetam (29). The comparison of the anticonvulsant activities in MES test of fluoro-substituted derivate **5** with unsubstituted parent compound **1** showed a slight decrease in activity of compound **5**. However, in our previous investigations it has been noted that substitutions with this group made at this specific site are able to increase

the anticonvulsant activity noticeably (MES ED_{50} = 85.36 mg/kg, PI = 2.5 for **2** and MES ED_{50} = 54.05 mg/kg, PI = 5.8 for **3** (Fig 1)). On the other hand the incorporation of the larger halogen group bromine (**10**) resulted in the complete loss of anticonvulsant activity in the MES test. A similar trend was observed earlier for the substitution with chlorine group (**11**, **13**). Accordingly, the progressive decrease in activity was noticed for compounds containing the trifluoromethylo (**11**) or the trifluoromethoxy (**7**, **9**) substituents, but the compound **8** showed rather slightly lower activity than that of the parent compound **2**.

In conclusion, the increase in the size of the electron-withdrawing substituents turned out to be unfavourable for the anticonvulsant activity of these compounds. The obtained results seem to confirm that the incorporation of the small halogen fluoro group into the benzyl ring at the 2-site of previously obtained pharmacologically active compounds is beneficial for the activity and yields analogs with higher or comparable anticonvulsant activity to the parent compounds. It confirms our assumptions that, besides the electron effects and hydrophobic interactions, the size of the substituent is significant for the anticonvulsant activity of these compounds.

Acknowledgments

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