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NEW DERIVATIVES OF BENZYLAMIDE WITH ANTICONVULSANT ACTIVITY

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Abstract: Previously obtained picolinic acid benzylamide is a potent anticonvulsant with low neurotoxicity. In search for new effective anticonvulsants twelve new benzylamides (**1-12**) were synthesized and preliminary evaluated in the Anticonvulsant Screening Program (ASP) of Antiepileptic Drug Development Program (ADDP) of NIH. Two of them appeared the most promising: 1-cyclopentenecarboxylic acid benzylamide (1-Cpc-BZA) (**9**) showed MES ED_{50} = 85,36 mg/kg (PI = 2,49), scPTZ ED_{50} = 1,37 mg/kg (PI = 1,37), 6Hz-EST ED_{50} = 50,29 mg/kg and cyclopentanecarboxylic acid benzylamide (Cpc-BZA) (**11**) showed pilocarpine ED_{50} = 154.75 mg/kg and pilocarpine ED_{97} = 270.95 mg/kg.

Keywords: anticonvulsants, synthesis, activity, neurotoxicity

A number of aromatic amides of aromatic and heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity (1 – 3). Some of them have been reported previously to be a potent agents. Generally, benzylamides were found to be more active than other amides. On the other hand, the most effective appeared amides of acids: picolinic, nicotinic, isonicotinic, nipecotic and isonipecotic. The most effective anticonvulsants came out to be picolinic acid benzylamide (Pic-BZA, PI against MES > 28.0) (1) and nicotinic acid benzylamide (Na-BZA, PI against MES = 4.70) (2). Some of derivatives of those compounds substituted in both rings were designed, prepared and pharmacologically evaluated. The best were: picolinic acid 2-fluorobenzylamide (Pic-2-F-BZA, PI against MES = 3.40) (3) and nicotinic acid benzylamide N-oxide (Nic-O-BZA, PI against MES < 5.6) (2).

This work was designed to extend the investigation to synthesis of derivatives of picolinic, nicotinic and isonicotinic acid benzylamides substituted with Cl, F and CF_3 in the rings. In the structure of compound **8** the distance between aromatic ring and amide group was prolonged with one CH_2 group to see the influence of this modification upon activity in comparison with picolinic acid benzylamide. Also was attempted preparation of series of benzylamides of the acids containing 5- or 7-member rings in the structure. The following compounds were obtained: picolinic acid 2-chlorobenzylamide (Pic-2-Cl-BZA, **1**), picolinic acid 3-chlorobenzylamide (Pic-3-Cl-BZA, **2**), picolinic acid 4-chlorobenzylamide (Pic-4-Cl-BZA, **3**), picolinic acid 2-fluoro-3-trifluoromethylbenzylamide (Pic-2F-3-TFM-BZA, **4**), 2-chloronicotinic acid benzylamide (2-Cl-Na-BZA, **5**), 6-chloronicotinic acid benzylamide (6-Cl-Na-BZA, **6**), 2-chloroisonicotinic benzylamide (2-Cl-iNa-BZA, **7**), picolinic acid phenyletylamide, **8**), 1-cyclopentene-1-carboxylic acid benzylamide, 1-Cpc-BZA, **9**), 3-cyclopentene-1-carboxylic acid benzylamide (3-Cpc-BZA, **10**), cyclopentanecarboxylic acid benzylamide (Cpc-BZA, **11**), cyclohexancarboxylic acid benzylamide (Chc-BZA, **12**). One of the prerequisites of the compounds activity is a correct lipophilicity of the molecule assuring a good biological barrier penetration. Therefore log P values of the partition coefficient between *n*-octanol and water of the designed compounds were taken under consideration. These values as a measure of lipophilicity were moderate for all synthesized compounds, that suggests a good penetration across biological barriers and therefore rather good activity.

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EXPERIMENTAL

Chemistry

General

All used acids (Pic, 2-Cl-Na, 6-Cl-Na, 2-Cl-iNa, 1-Cpc, 3-Cpc, Cpc and Chc) as well as isobutyl chloroformate were purchased from Aldrich. N-methylmorpholine and benzylamine were supplied from Merck. DMF and THF were from POCH Gliwice.

^{1}H NMR spectra were recorded on a Bruker DM 400 MHz spectrometer. Chemical shifts were measured as δ units (ppm) relative to tetramethylsilane. TLC was carried out on a 0.25 mm thickness silica gel plates (Merck Kieselgel 60 F-254). The spots were visualized in UV light or with 0.3% ninhydrin in EtOH (97:3). CC was carried out under gravity on silica gel (Merck, grade 230 – 400 mesh). The solvent system used in TLC and CC was $CHCl_3$/MeOH in different volume ratios. HPLC was performed on a chromatograph equipped with pump (Techma-Robot, Warsaw), UV detector LCD 2040 (Laboratorni Přistroje, Praha) and a computer registrator/recorder (CHROMA POLLAB, Warsaw). The peaks were recorded at 210 nm. Elemental analyses were performed on a Perkin-Elmer Microanalyser. Melting points were determined in a Böetius apparatus.

Synthesis of amides

The compounds **1-12** were synthesized using the mixed anhydrides method (4) of peptide synthesis. Suitable acid (Pic, 2-Cl-Na, 6-Cl-Na, 2-Cl-iNa, 1-Cpc, 3-Cpc, Cpc or Chc) (10 mmol) was dissolved in DMF (15 mL) and THF (15 mL) was added. Next, N-methylmorpholine (10 mmol, 1.1 mL) was added and the mixture was stirred under nitrogen and chilled to -15°C. Isobutyl chloroformate (10 mmol, 1.3 mL) was added dropwise to keep the temperature below -15°C. Then, benzylamine (BZA) (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 EtOAc (20 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 by crystallization with MeOH/Et_2O, acetone/hexane and/or by CC ($CHCl_3$/MeOH, 95:5, v/v).

Computer calculations

The HyperChem 4.5 (Hypercube, Inc.) program was used. The semiempirical method PM 3 was applied for a single point calculation. Geometry

Table 1. Physical and analytical data of the synthesized compounds

No.	Compound	Formula	M.w.	Yield %	m.p. °C	R_f	log P
1	Pic-2-Cl-BZA	$C_{13}H_{11}ON_2Cl$	246.68	65	84-85	0.56 (A)	2.84
2	Pic-3-Cl-BZA	$C_{13}H_{11}ON_2Cl$	246.68	72	oil	0.61 (A)	2.84
3	Pic-4-Cl-BZA	$C_{13}H_{11}ON_2Cl$	246.68	65	83-85	0.65 (A)	2.84
4	Pic-2F-3-TFM	$C_{14}H_{11}ON_2F_4$	298.23	58	96-97	0.76 (B)	3.35
5	2-Cl-Na-BZA	$C_{13}H_{11}ON_2Cl$	246.68	82	118-120	0.61 (C)	2.81
6	6-Cl-Na-BZA	$C_{13}H_{11}ON_2Cl$	246.68	83	112-113	0.64 (C)	2.81
7	2-Cl-iNa-BZA	$C_{13}H_{11}ON_2Cl$	246.68	78	99	0.49 (C)	2.81
8	Pic-PEA	$C_{14}H_{14}ON_2$	226.27	88	oil	0.80 (A)	2.58
9	1-Cpc-BZA	$C_{13}H_{15}ON$	201.26	64	114-116	0.68 (B)	2.60
10	3-Cpc-BZA	$C_{13}H_{15}ON$	201.26	78	115-117	0.82 (B)	2.54
11	Cpc-BZA	$C_{13}H_{17}ON$	203.28	73	92-93	0.80 (B)	2.80
2	Chc-BZA	$C_{15}H_{21}ON$	231.35	71	117-118	0.76 (B)	3.60

The elemental analyses were within ± 0.4% of the theoretical value. ^{1}H-NMR data clearly confirmed the proposed structure. Hydrophobicity of the compounds expressed as log P value was calculated by a computer method. HPLC purity of all compounds is 100%. A = $CHCl_3$/MeOH, 98:2, v/v; B = $CHCl_3$/MeOH, 99.5:0.5, v/v; C = $CHCl_3$/MeOH, 95:5, v/v.

BZA = benzylamide; PEA = phenylethylamide; TFM = trifluoromethyl group; Pic = picolinic acid; Na = nicotinic acid; iNa = isonicotinic acid; Cpc = cyclopentanecarboxylic acid; 1-Cpc = 1-cyclopentene-1-carboxylic acid; 3-Cpc = 3-cyclopentene-1-carboxylic acid; Chc = cycloheptanecarboxylic acid.

Table 2. Preliminary pharmacological evaluation (ASP) of the synthesized compounds

Comp. no.	Mice *i.p.* Identification ASP no. 1 Test Class of ASP		Rats *p.o.* Identification Rats *i.p* ASP no. 2 Test Class of ASP			Anticonvulsant Identification ASP no. 8 Test (30 mg/kg) Class of ASP		
	MES	scPTZ	MES	scPTZ	TOX	MES	scPTZ	TOX
1	II	II	–	–	–	–	–	–
2	III	III	–	–	–	–	–	–
3	III	III	–	–	–	–	–	–
4	III	III	–	–	–	–	–	-
5	III	III	–	–	–	–	–	-
6	II	III	I	I	0	I	I	0
7	II	II	I	I	0	0	–	0
8	II	II	0	0	0	–	–	–
9	II	III	0	0	0	I	0	0
10	III	III	–	–	–	–	–	-
11	III	III	–	–	–	–	–	-
12	III	III	–	–	–	–	–	-

MES = maximal electroshock seizure test; PTZ = pentetrazole; 0 = inactive or not neurotoxic at the dose used. Class I – anticonvulsant activity at a dose of 100 mg/kg or less, class II – anticonvulsant activity at a dose greater than 100 mg/kg and class III – no activity at a dose up to and including 300 mg/kg.

optimization was performed by a Polak-Ribiere algorithm. Afterwards, the QSAR Properties module using atomic parameters derived by Ghose et al. (5) was applied to calculate log P values as a measure of hydrophobicity of the optimized structures of the compounds.

Pharmacology

All synthesized compounds (**1-12**) were evaluated in mice in Identification ASP no. 1 Test according to the method described by Krall et al. (6). MES, scPTZ and TOX tests were performed after *i.p.* administration. On the basis of the findings, the compounds were included in one of three classes. Class I – anticonvulsant activity at a dose of 100 mg/kg or less, class II – anticonvulsant activity at a dose greater than 100 mg/kg and class III – no activity at a dose up to and including 300 mg/kg. (Table 2). Four selected compounds (**6, 7, 8, 9**) were evaluated in rats in Identification ASP no. 2 Test after *p.o.* administration at a dose of 30 mg/kg (Table 2) and three compounds (**6, 7, 9**) in rats in Anticonvulsant Identification ASP no. 8 Test after *i.p.* administration at a dose 30 mg/kg (Table 2). Both experiments were performed also according to the method of Krall et al. (6). The MES ED_{50}, scPTZ ED_{50} and TOX TD_{50} of the most promising compound (**9**) were determined in mice in Quantification ASP no. 4 Test, according to the method reported by Swinyard et al. (7), after *i.p.* administration (Table 3). Activity of compound (**9**) was examined in mice in Anticonvulsant Evaluation ASP no. 7 Minimal Clonic Seizure (6 Hz) Test (6Hz-EST) by procedure described by Barton et al. (8) after *i.p.* administration (Table 3). Pharmacological evaluation of potential anticonvulsant activity of compounds **9** and **11** was accomplished using method of Racine (9) of pilocarpine induced seizures. Prevention of Pilocarpine Induced Status, ASP no. 71 Test (rats, *i.p.*, time 0 min.) and Pilocarpine-Induced Status, ASP no. 72 Test (rats, *i.p.*, time 30 min.) were performed (Table 3).

RESULTS

Physico-chemical data of the synthesized compounds are given in Table 1 and the results of preliminary pharmacological tests in Tables 2 and 3.

DISCUSSION

Of the compounds obtained in the present study Pic-2-Cl-BZA (**1**), 6-Cl-Na-BZA (**6**), 2-Cl-iNa-BZA (**7**) and Pic-PEA (**8**) demonstrated a potent

Table 3. Preliminary pharmacological evaluation (ASP) of the synthesized compounds

Compd.	Mice *i.p.* Quantification ASP no. 4	PI	Mice *i.p.* Anticonvulsant Evaluation Test ASP no. 7	Rats *i.p.* pilocarpine-induced status Test ASP no. 71	Rats *i.p.* Prevention of pilocarpine-induced status Test ASP no. 72 Test
9	MES ED_{50} (mg/kg) 85.36 scPTZ ED_{50} (mg/kg) 154.74 TD_{50} (mg/kg) 212.60	ag. MES 2.49 ag. scPTZ 1.37	6Hz-EST ED_{50} (mg/kg) 50.29 – –	time 0 min. (mg/kg.) 300 – –	time 30 min. (mg/kg) 200 – –
11	Pilocarpine ED_{50} (mg/kg) 154.75 Pilocarpine ED_{97} (mg/kg) 270.95		– –	300 –	225 –
Valproic acid	MES ED_{50} (mg/kg) 425.1 TD_{50} (mg/kg) 243.0	ag. MES 0.6	– –	– –	– –
Pheno-barbital	MES ED_{50} (mg/kg) 9.1 TD_{50} (mg/kg) 61.1	ag. MES 6.7	– –	– –	– –

MES = maximal electroshock seizure test (60 Hz); 6Hz-EST = minimal electroshock seizure test; PI = TD_{50} (against MES or scPTZ) / ED_{50}

anticonvulsant activity. However, their neurotoxicity was high (4/8 to 8/8 mice at a dose 100 mg/kg). Instead, the lowest neurotoxicity showed 1-Cpc-BZA (**9**) and Cpc-BZA (**11**) (both 0/8 mice at a dose 100 mg/kg). Among them compound **9** displaied stronger activity (3/3 mice at a dose 100 mg/kg), than compound **11** (1/3 mice at the same dose). MES ED_{50} and scPTZ ED_{50} of both are presented in Table 2. Because some clinically useful anticonvulsants can be poorly active in the standard MES and scPTZ tests but have anticonvulsant activity *in vivo,* activity of compound (**9**) was examined in Minimal Clonic Seizure (6 Hz) Test (6Hz-EST) (Table 3). Pharmacological evaluation of potential anticonvulsant activity of compounds **9** and **11** was accomplished in pilocarpine induced seizures. Prevention of Pilocarpine Induced Status showed both compounds at similar doses. Pilocarpine ED_{50} and ED_{97} of compound **11** were determined and are reported (Table 3). Pharmacological tests of the synthesized potential anticonvulsants seem to indicate that reduction of 6-member ring of the acidic fragment of the compound to 5-member one is leading to an increased activity and decreased neurotoxicity (**9, 11**). On the contrary, enlargement of the ring to a 7-member one decreases anticonvulsant activity (**12**) (Table 3). Based on the reported results one can conclude that reduction of the compound molecular size caused by reduction of ring size might be advantageous assuring a better biological barriers

penetration. Pharmacological tests also showed, that enlargement of the distance between both rings in picolinic acid benzylamide (introduction of additional CH_2 group in picolinic acid phenylethylamide in compound **8**) reduces antiepileptic activity. This observation seems to show a decrease of receptor affinity of such structure.

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