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SYNTHESIS AND RESEARCH OF BENZYLAMIDES OF SOME ISOCYCLIC AND HETEROCYCLIC ACIDS AS POTENTIAL ANTICONVULSANTS

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Abstract: A series of benzylamides of isocyclic and heterocyclic acids was synthesized and tested in Anticonvulsant Screening Project (ASP) of Antiepileptic Drug Development Program (ADDP) of NIH. Near all synthesized derivatives of heterocyclic acids showed activity. All obtained derivatives of mono- and bicyclic isocyclic acids were inactive. The power of action of heterocyclic acids derivatives seems does not depend upon kind of heteroatom (N, O or S). One of the compounds (2-furoic acid benzylamide (**4**)) appeared most promising. It showed in minimal clonic seizure (6Hz) test (ASP) in rats after *i. p.* administration: MES ED_{50} = 36.5 mg/kg, TOX TD_{50} = 269.75 mg/kg, and PI = 7.39.

Keywords: Anticonvulsant, isocyclic, heterocyclic benzylamides, hydrophobicity

In 1985, Kohn et al. discovered a novel class of anticonvulsants which were derivatives of amino acids called functionalized amino acids (1). They modified structures of these derivatives to study the role of different substituents. They found significant role of substituted or unsubstituted benzyl moiety of these compounds (2, 3). They also observed that electron-withdrawing groups on benzylamide site are able to increase anticonvulsant activity, on the contrary to electron-donating groups (4). Over the past years, a number of amides of heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity (5-7). Benzylamides have been found more active than other amides. Some of them have been reported previously as potent agents (5, 6). The purpose of these investigations was to look for new effective anticonvulsants in group of benzylamides of isocyclic and heterocyclic acids and to find in practical way what structure elements are necessary for anticonvulsant activity. No univocal data have been reported regarding structure of the binding sites of NMDA receptors. It is main difficulty to design the formula of compound with high receptor affinity. Therefore, it was decided to consider three kinds of properties of potential anticonvulsants. It is commonly known, that smaller size molecules better penetrate biological barriers and therefore could be more effective. So, anticonvulsant molecule probably ought not to be of too big size. The other important property related to biomolecular interaction is hydrophobicity of the compound. Its pertinent value assures a better transmembrane transport, protein binding and receptor affinity of the compound. Therefore, log P values of the partition coefficient between *n*-octanol and water of synthesized compounds have been taken under consideration. It seems that optimal log P value of designed benzylamides ought to be > 0 and probably near to 3. This value is probably the best compromise between water and lipids affinity of the designed compound. However, it is observed, that compounds with higher log P value can also show anticonvulsant activity. The third attribute of the molecule could be the presence of heteroatom in the acid structure. It was designed to see what heteroatom is privileged, if at all. This study was undertaken in order to consider the influence of these properties upon anticonvulsant activity of the designed compounds. Benzylamides of four five-membered heterocyclic acids, four bicyclic isocyclic acids, three bicyclic heterocyclic acids and one monocyclic isocyclic acid were synthesized.

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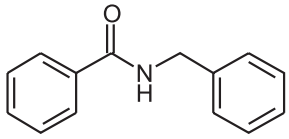
Table 1. Physical and analytical data of the synthesized compounds.

Compound	Formula	M.w.	M.p. ^{o}C	Crystallization solvent system	log P
1.	$C_{14}H_{13}ON$	211.25	103-104	EtOAc/hexane	3.24
2.	$C_{13}H_{13}ONS$	231.31	89-91	EtOAC/hexane	1.70
3.	$C_{12}H_{11}ONS$	217.29	103-105*	EtOAc/hexane	1.55
4.	$C_{12}H_{11}O_2N$	201.24	108-109**	EtOAc/hexane	1.21
5.	$C_{13}H_{13}O_2N$	215.27	88-89	EtOAc/hexane	0.77
6.	$C_{18}H_{15}ON$	261.31	138-140	EtOAc/hexane	4.24
7.	$C_{18}H_{14}ON$	279.30	162-163	EtOAc	4.38
8.	$C_{19}H_{17}ON$	275.34	169-170	MeOH	4.17
9.	$C_{19}H_{17}ON$	275.34	156-157	MeOH	4.17
10.	$C_{17}H_{13}O_3N$	279.2	154-155	EtOAc	2.68
11.	$C_{17}H_{14}ON_2$	262.31	89-90	EtOAc/hexane	3.33
12.	$C_{17}H_{14}ON_2$	262.31	71-73***	EtOAc/hexane	6.74

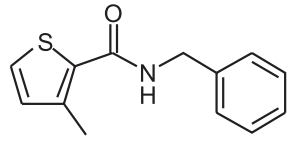
HPLC purity of the all compounds was 100%. The elemental analyses were within ± 0.4% of the theoretical value. ^{1}H NMR data clearly confirm the proposed structure. Hydrophobicity of the compounds is expressed as log P value calculated by a computer method. * M.p. 119.5-120.5^{o}C (crystallization from benzene). Chem. Abstr., 65, 20092 (1966). ** M.p. 112-113^{o}C (crystal. solv. system unknown). Hong Soon Hyeok, Chen Cheng, Zhang Yao, J. Org. Chem., 76, 10005 (2011). *** M. p. 85^{o}C (crystallization from diisopropyl ether). Benincori T., Brenna E., Sannicolo F., J. Chem. Soc., Perkin Trans., 1, 6, 675 (1993). M. p. of **1, 6, 8** and **10** in accordance with literature data, those of **2, 5, 7, 9** and **11** not found.

Table 2. ^{1}H NMR spectra of the synthesized compounds in $CDCl_3$,. Chemical shifts in δ (ppm).

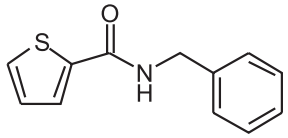
1:	4.61 (d, *J* = 6.0 Hz, 2H, CH_2), 6.80 (s br, 1H, NH), 7.23-7.41 (m, 10H, 2×C_6H_5).
2:	2.53 (s, 3H, CH_3), 4.61 (d, *J* = 6.0 Hz, 2H, CH_2), 6.08 (s br, 1H, NH), 6.89 (d, *J* = 8.6 Hz, 1H, CH), 7.30 (d, *J* = 6.8 Hz, 1H, CH), 7.34-7.36 (m, 5H, C_6H_5).
3:	4.62 (d, *J* = 8.1 Hz, 2H, CH_2), 6.27 (s br, 1H, NH), 7.29-7.34 (m, 1H, CH), 7.33-7.38 (m, 5H, C_6H_5), 7.46; 7.48 (dd, *J* = 1.6 Hz, 1.6 Hz, 1H, CH), 7.49; 7.51 (dd, *J* = 1.6 Hz, 1.6 Hz, 1H, CH).
4:	4.62 (d, *J* = 8.2 Hz, 2H, CH_2), 6.50; 6.51 (dd, *J* = 2.4 Hz, 2.4 Hz, 1H, CH), 6.62 (s br, 1H, NH), 7.14; 7.16 (dd, *J* = 1.1 Hz, 1.1 Hz, 1H, CH), 7.29-7.36 (m, 5H, C_6H_5), 7.41, 7.42 (dd, 1.3 Hz, 1.3 Hz, 1H, CH).
5:	2.31 (s, 3H, CH_3), 4.61 (d, *J* = 7.3 Hz, 2H, CH_2), 6.08, 6.10 (dd, *J* = 0.6 Hz, 0.6 Hz, 1H CH), 6.56 (s, 1H, NH), 7.04 (d, *J* = 4.6 Hz, 1H, CH), 7.27-7.36 (m, 6H, C_6H_5, CH).
6:	4.70 (d, *J* = 5.5 Hz, 2H, CH_2), 6.60 (s br, 1H, NH), 7.30-7.40 (m, 5H, $C_6H_{5)}$, 7.48-7.65 (m, 2H, 2×CH), 7.82-7.92 (m, 4H, 4×CH), 8.30 (s, 1H, CH).
7:	4.70 (d, *J* = 5.7 Hz, 2H, CH_2), 6.55 (s br, 1H, NH), 7.24-7.44 (m, 6H, C_6H_5, CH), 7.48 (d, *J* = 7.2 Hz, 1H, CH), 7.78-7.94 (m, 3H, 3×CH), 8.31 (s, 1H, CH).
8:	4.09 (s, 2H, CH_2 Ac), 4.35 (d, *J* = 8.3 Hz, 2H, CH_2), 5.60 (s br, 1H, NH), 6.99-7.20 (m, 5H, C_6H_5), 7.39-7.46 (m, 2H, 2×CH), 7.54 (t, *J* = 4.7 Hz, 2H, 2×CH), 7.82 (d, *J* = 9.0 Hz, 1H, CH), 7.88 (d, *J* = 12.8 Hz, 1H, CH), 8.00 (d, *J* = 9.6 Hz, 1H, CH).
9:	3.79 (s, 2H, CH_2 Ac), 4.41 (d, *J* = 8.0 Hz, 2H, CH_2), 5.73 (s br, 1H, NH), 7.14-7.30 (m, 6H, C_6H_5, CH), 7.40 (d, *J* = 11.4 Hz, 1H, CH), 7.48 (t, *J* = 5.8 Hz, 2H, 2×CH), 7.79-7.85 (m, 3H, 3×CH).
10:	4.66 (d, *J* = 7.8 Hz, 2H, CH_2), 7.34-7.42 (m, 6H, C_6H_5, CH), 7.65 (d, *J* = 13.1 Hz, 1H), 7.67 (t, *J* = 10.5 Hz, CH, 1H), 7.69 (t, *J* = 12.1Hz, 1H, CH), 7.71 (d, *J* = 12.1 Hz, 1H, CH), 9.17 (s br, 1H, NH).
11:	4.74 (d, *J* = 8.0 Hz, 2H, CH_2), 7.25-7.40 (m, 5H, C_6H_5), 7.69-7.82 (m, 2H, 2×CH), 8.03 (t, *J* = 1.1, 2H, 2×CH), 8.66 (s br, 1H, NH), 8.69 (s, 1H, CH), 9.15 (s, 1H, CH).
12:	4.74 (d, *J* = 7.0 Hz, 2H, CH_2), 7.26-7.46 (m, 5H, C_6H_5), 8.75 (s br, 1H, NH), 7.70-7.81 (m, 2H, 2×CH), 7.87 (t, *J* = 0.9 Hz, 2H, 2×CH), 8.44 (d, *J* = 0.8 Hz, 1H, CH), 9.60 (d, *J* = 1.1 Hz, 1H, CH).



1. benzoic acid benzylamide



2. 3-methyl-2-tiophenecarboxylic acid benzylami



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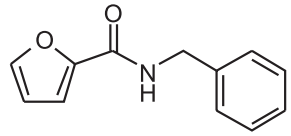

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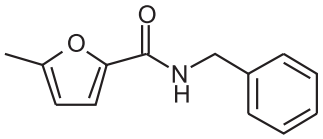
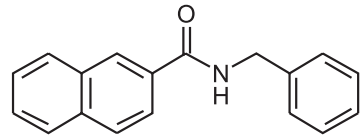
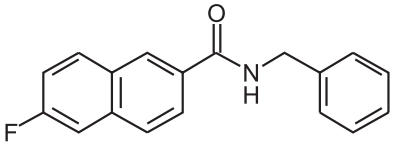


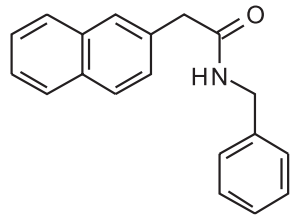
5. 5-methyl-2-furoic acid benzylamide



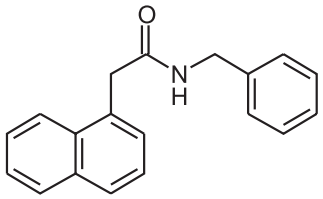
6. 2-naphthoic acid benzylamide



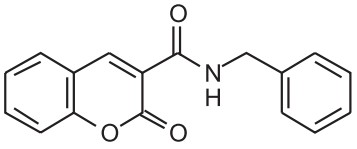
7. 6-fluoro-2-naphthoic acid benzylamide



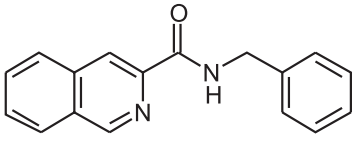
8. 2-naphthaleneacetic acid benzylamide



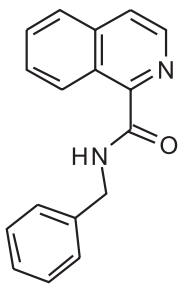
9. 1-naphthaleneacetic acid benzylamide



10. coumarin-3-carboxylic acid benzylamide



11. 3-isoquinolinecarboxylic acid benzylamide



12. 1-isoquinolinecarboxylic acid benzylamide

Figure 1. Structures of the synthesized compounds

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

Comp. No.	Anticonvulsant identification test systems			Pilocarpine induced status test (protected or not protected)	Class (ASP)
	mice *i. p.* (MES) mg/kg	mice *i. p.* (scPTZ) mg/kg	mice *i. p.* (TOX) mg/kg		
1.	NP	NP	NT	ND	III
2.	100	NP	300	protected	I
3.	100	300	300	protected	I
4.	100	300	300	ND	I
5.	100	NP	300	protected	I
6.	NP	NP	NT	not protected	III
7.	NP	NP	NT	not protected	III
8.	NP	NP	NT	not protected	III
9.	NP.	NP	NT	not protected	III
10.	NP*	ND	ND	not protected	III
11.	100*	ND	100*	not protected	I
12.	100*	ND	100*	not protected	I

MES = maximal electroshock seizure test; PTZ = pentetrazole; ND = not determined; NP = no protection up to 300 mg/kg; NT = no toxicity up to 300 mg/kg; * anticonvulsant evaluation in 6Hz (ASP) (11). 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 an including 300 mg/kg.

Table 4. Additional pharmacological evaluation (ASP) of synthesized compounds.

Qualitative (6Hz) test in mice (*i.p.*):
Compound **10** did not show protection. Compound **11** showed protection 3/4 animals (0.25 h), 4/4 (0.5 h) and 1/4 (1.0 h). Compound **12** showed protection 1/4 animals (0.25 h).
Quantitative (6Hz) test in rats (*i.p.*):
Compound **4** showed biological response:

Test	Time (h)	Dose (mg/kg)	Animals prot./used
MES	0.25	15.0	1/8
MES	0.25	30.0	2/8
MES	0.25	45.0	5/8
MES	0.25	60.0	7/8
TOX	0.50	200.0	1/8
TOX	0.50	250.0	4/8
TOX	0.50	300.0	5/8
TOX	0.50	400.0	7/8

Compound **4** showed MES ED_{50} = 36.5 mg/kg, TOX TD_{50} = 269.76, PI = TD_{50}/ED_{50} = 7.39.
Neuroprotection evaluation test: Compounds **4** and **6**-**9** did not show neuroprotrction in *in vitro* hippocampal slice culture neuroprotection test (ASP). Excitotoxins employed: kainic acid (20 μM) and NMDA (10 μM) insult duration also 4 h.

EXPERIMENTAL

Chemistry

General

All used acids: benzoic acid, 3-methyl-2-thiophenecarboxylic acid, 2-thiophenecarboxylic acid, 2-furoic acid, 5-methyl-2-furoic acid, 2-naphthoic acid, 6-fluoro-2-naphthoic acid, 1-naphthaleneacetic acid, 2-naphthaleneacetic acid, coumarin-3-carboxylic acid, 3-isoquinolinecarboxylic acid and isoquinolinecarboxylic acid as well as isobutyl chloroformate were purchased from Aldrich. N-methyl-

morpholine 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). The solvent system used in TLC was $CHCl_3$/MeOH in different ratios. HPLC was performed on a Shimadzu chromatograph equipped with LC-10AT pump, SPD-10A UV spectrophotometer and a computer registrator/recorder (CHROMA POLLAB, Warszawa). 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 of peptide synthesis (8). Suitable acid (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^{o}C. Isobutyl chloroformate (10 mmol, 1.3 mL) was added dropwise to keep the temperature below -15^o. Then, benzylamine (10 mmol) in THF was added in small portions and the reaction mixture was stirred at -15^{o}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. All stages of synthesis were controlled by TLC. The purity of the final compound was determined by HPLC and identity by ^{1}H NMR. The compounds obtained and tested are showed in Figure 1.

Computer calculations

The HyperChem 4.5 (Hypercube, Inc.) program was used. The semiempirical method PM 3 was applied for a single point calculation. Geometry optimization was performed by a Polak-Ribiere algorithm. Afterward, the QSAR Properties module using atomic parameters derived by Ghose et al. (9) was applied to calculate log P values as a measure of hydrophobicity of the optimized structures of the compounds.

Pharmacology

Compounds **1**-**9** were evaluated qualitatively in anticonvulsant identification test systems in mice (MES, scPTZ and TOX tests) after *i.p.* administration. The tests were performed according to the method described by Krall et al. (10). Evaluation of compounds **2**-**12** was accomplished in pilocarpine induced status prevention test (ASP) using the method of Racin (12). In this test, the compound was assessed for pharmacological evaluation of potential activity against nerve agents using the pilocarpine model of epilepsy as introductory screen. Pilocarpine-induced resistant status in rats at time zero was determined. The compound was administered to rat *via* the *i.p.* route. Then, a challenge dose of pilocarpine was administered observing for treatment-effects of the compound. The outcome measures are determination of protected or no protected animals. On the basis of results of both tests the compounds were included in one of three (ASP) classes (Table 3). Compounds **10**-**12** were evaluated qualitatively in minimal clonic seizure (6Hz) test (ASP) in mice after *i. p.* administration according to Barton et al. (11). The results are expressed as the number of animals protected out of the number of animals tested (Table 3). Compound **4** was examined quantitatively in minimal clonic seizures (6Hz) in rats after *i. p.* administration according to Barton et al. (11). This test was performed because some clinically useful compounds could be ineffective in the standard MES and scPTZ tests, but still have anticonvulsant activity *in vivo*. The minimal clonic seizure (6Hz) test (ASP) is used to assess a compound's efficacy against electrically induced seizures but uses a lower 6Hz frequency and longer duration - 3 s. MES ED_{50} and TOX TD_{50} values were determined (Table 4). Compounds **4** and **6**-**9** were examined in neuroprotection evaluation test. Qualitative *in vitro* hippocampal slice culture neuroprotection test (ASP) was performed by method given by Noraberg (13) using excitotoxins – kainic acid and NMDA. The results of the above tests are presented in Table 4.

RESULTS

Physico-chemical data of the synthesized compounds are given in Tables 1 and 2, the results of preliminary pharmacological tests in Tables 3 and 4.

DISCUSSION

A series of benzylamides of mono- and bicyclic, 6-membered isocyclic acids was synthe-

sized (**1, 6, 7, 8, 9**). Preliminary tests show that all these compounds without respect to number of rings in the molecule are inactive and also not toxic. Previously obtained two benzylamides of 5-membered isocyclic acids were inactive in preliminary tests ASP, but the third one, 1-cyclopentene-1-carboxylic acid benzylamide, showed rather high activity: MES ED_{50} = 85.36 mg/kg, scPTZ ED_{50} = 154.74 mg/kg and TD_{50} = 212.60 mg/kg (14). This result was rather a surprise on the ground of a lack of heteroatom in the molecule. The comparison of the tests results show that benzylamides of isocyclic acids are not always inactive. On the basis of the obtained results a hypothesis is put forward, that the reduction of bicyclic 6-membered isocyclic acid into monocyclic moiety and 6-membered into 5-membered ring of acid could cause appearance of anticonvulsant effectiveness even in benzylamide of isocyclic acid.

Also series of benzylamides of heterocyclic, monocyclic 5-membered (**2, 3, 4, 5**) and bicyclic, 6-membered (**10, 11, 12**) acids was synthesized. The accomplished anticonvulsant identification test and pilocarpine induced status test showed, that all synthesized benzylamides of monocyclic 5-membered heterocyclic acids belong to I class ASP and are rather strong anticonvulsants with very low neurotoxicity (Table 3). Two of these compounds contain sulfur heteroatom (**2, 3**) and two (**4, 5**) oxygen heteroatom in 5-membered acid ring. Preliminary tests of activity showed similar results for all these compounds. Compound **4** was recognized as most promising and put to further tests – quantitative (6Hz) and neuroprotection evaluation. Between benzylamides of monocyclic 6-membered heterocyclic acids containing nitrogen atom are anticonvulsants of high effectiveness. These results were already published (6, 7). Considering the results it could be concluded, that the presence of O, S or N heteroatom is usually but not always necessary for activity. Considering the relationship between hydrophobicity and anticonvulsant activity of the tested compounds we have found that five of six compounds included into I class showed log P value in limits 0.8 – 3.3 and only one showed a higher value – 6.7. It confirms our suppositions concerning the role of lipophilicity of the compounds.

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