Amino Acid Derivatives with Anticonvulsant Activity

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A series of benzylamides of *N*-alkylated, *N*-acylated or free nine cyclic and one linear amino acids as potential anticonvulsants have been synthesized. The structures of the obtained compounds were designed on the basis of the previously determined structure and physicochemical properties/anticvonvulsant activity relationship of the formerly synthesized compounds of this type. The obtained compounds were evaluated in mice after intraperitoneal (ip) administration, by maximal electroshock seizure test (MES test), subcutaneous (sc) pentylenetetrazol test (sc PTZ test) and by the rotarod neurotoxicity test (Tox test). The results were the basis for their classification into one of three classes of the Anticonvulsant Screening Project (ASP) of the Antiepileptic Drug Development Program (ADDP) of the NIH. Three selected compounds were tested quantitatively in rats after oral administration. The MES ED_{50}, sc PTZ ED_{50}, Tox TD_{50} were determined and their protective index (PI) values were calculated. Anticonvulsant activity of the most promising compound (15) was examined in different seizure models. The respective ED_{50} and PI values of this compound were as follows: against bicuculline, 73 and 1.4; against PTZ, 47 and 2.2; against strychnine, 73 and 1.4; against pilocarpine 156, and 0.7; against kainic acid (2-carboxy-4-isopropenyl-3-pyrrolidineacetic acid), 39 and 2.6; against AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), 10 and 10.3 and against NMDA (*N*-methyl-D-Aspartic acid), 114 and 0.9.

Key words anticonvulsants; cyclic amino acid derivative

In previous papers[1—3] we reported the syntheses, pharmacological evaluation and classification of series of aromatic amides of *N*-alkylated and *N*-acylated amino acids, according to the Anticonvulsant Screening Project (ASP). All of them, with one exception, were derivatives of linear amino acids. This exception was (*R*)-acetylproline benzylamide ((*R*)-Ac-Pro-BZA), which has been demonstrated as effective in mice against the maximal electroshock seizure (MES) ED_{50}= 67.4 mg/kg body weight (b.w.), against the subcutaneous pentylenetetrazol (sc PTZ) ED_{50}=183.8 mg/kg b.w., the rotarod neurotoxicity (Tox) TD_{50}=303.8 mg/kg b.w. and the protective index (PI), (MES)=4.5.[2] This strong anticonvulsant activity of proline benzylamide, as well as the cyclic structure of many antiepileptic drugs, caused us to focus attention on cyclic amino acid derivatives. Therefore, we have now designed structures of derivatives of nine of this type amino acids. The calculated log P value of the partition coefficient between *n*-octanol and water (log P) of five compounds (**2**, **5**, **7**, **11**, **14**) was moderate and we thus presumed their high activity. According to our observation, aromatic amides of *N*-alkylated or *N*-acylated amino acids of a mean hydrophobicity show strong anticonvulsant activity.[4] Too low or too high a log P value of three other compounds (**4**, **10**, **15**) could suggest the lack of activity. However, they were synthesized to verify our hypothesis, that it is possible to predict activity of this class of compounds on the basis of their hydrophobicity. This hypothesis could be right if the anticonvulsant effect was exerted by unmetabolized compounds. Hydrophobicity has no meaning if metabolites are involved in the effect. However, strong but short lasting activity seems to suggest that the unmetabolized compound is the acting one. Compound **9** was synthesized to confirm our observation that anticonvulsant activity demands the presence of amide nitrogen. Taurine (Tau) derivative (**16**) was synthesized because Tau is present in the brain in a significant concentration, though its function is not yet clear. It has been reported that Tau and growth factors protect the brain against excitotoxicity.[5] It is supposed that Tau acts on excitatory amino acid (EAA) receptors and therefore it would be worth to obtain and the pharmacological properties of taurine benzylamide, a potential EAA receptor antagonist with anticonvulsant activity.

Chemistry Syntheses were carried out as follows:

2: (*R*)-Pro-OH→(*R*)-MePro-OH→(*R*)-MePro-BZA (**2**); **4**: (2*S*,4*R*)-Pro(4-OH)-OH→(2*S*,4*R*)-Boc-Pro(4-OH)-OH→(2*S*,4*R*)-Boc-Pro(4-OH)-BZA→HCl. (2*S*,4*R*)-Pro(4-OH)-BZA→(2*S*,4*R*)-Ac-Pro(4-OH)-BZA (**4**); **5**: HCl. (2*S*,4*R*)-Pro(4-OH)-BZA→(2*S*,4*R*)-MePro(4-OH)-BZA (**5**); **7**: (*S*)-Glu $(OH)_2$→(*RS*)-<Glu-OH→(*RS*)-Ac-<Glu-OH→(*RS*)-Ac-<Glu-BZA (**7**); **9**: (*S*)-Glu-$(OH)_2$→(*RS*)-<Glu-OH→(*RS*)-<Glu-OBzl→(*RS*)-Ac-<Glu-OBzl (**9**); **10**: Paz-$(OH)_2$→Paz-$(BZA)_2$ (**10**); **11**: Pyr-OH→Pyr-BZA (**11**); **14**: (*RS*)-Pip-OH→(*RS*)-Boc-Pip-OH→(*RS*)-Boc-Pip-BZA→HCl. (*RS*)-Pip-BZA→(*RS*)-Ac-Pip-BZA (**14**); **15**: Pic-OH→Pic-BZA (**15**); **16**: Tau-OH→Cbz-Tau-ONa→Cbz-Tau-OH→Cbz-Tau-BZA (**16**). (Boc=*N*-*tert*-butoxycarbonyl group, Cbz=*N*-benzyloxycarbonyl group, BZA=benzylamide group, Paz=pyrazol-3,5-dicarboxylic acid, Pyr=pyrazine-2-carboxylic acid, Pip=pipecolinic acid, Pic=picolinic acid, <Glu=pyroglutamic acid, Bzl=benzyl group.)

General methods of synthesis, as well as untypical proceedings are given in the Experimental part. All the products were purified by crystallization or column chromatography (CC) and characterized by TLC, HPLC, ^{1}H-NMR, elemental analysis, and if necessary, by optical rotation determination. Physical and analytical data of the synthesized compounds are given in Tables 1 and 2.

Pharmacology Compounds: **2**, **4**, **5**, **7**, **9**—**11**, **14**—**16** were evaluated in mice after ip administration in the MES test, sc PTZ test and Tox test. The results are a basis for ASP

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Table 1. Physical and Analytical Data and Preliminary Pharmacological Evaluation (ASP, Phase I Identification, Mice, ip) of the Synthesized Compounds

	Compound	Formula	m.w.	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ (*c*, MeOH)	Class[a] of ASP	Log P[b]
1	(*R*)-MePro-OH	$C_6H_{11}NO_2$	(129.16)	96	140—143	+97.8 (1.3)	—	—
2	(*R*)-MePro-BZA	$C_{13}H_{18}NO_2$	(218.29)	70	62—63	+99.3 (3.0)	I	1.71
3	(2*S*,4*R*)-Boc-Pro-(4-OH)-BZA	$C_{17}H_{24}N_2O_4$	(320.38)	68	111—114	−44.0 (1.0)	—	—
4	(2*S*,4*R*)-Ac-Pro-(4-OH)-BZA	$C_{14}H_{18}N_2O_3$	(262.30)	56	147—148	−32.7 (1.5)	III	0.64
5	(2*S*,4*R*)-MePro-(4-OH)-BZA	$C_{13}H_{18}N_2O_2$	(234.29)	66	219	−5.3 (1.0)	II	0.89
6	(*RS*)-Ac-<Glu-OH	$C_7H_9NO_4$	(171.15)	72	Semisolid	—	—	—
7	(*RS*)-Ac-<Glu-BZA	$C_{14}H_{16}N_2O_3$	(260.29)	36	122	—	I	1.0
8	(*RS*)-<Glu-OBzl	$C_{12}H_{13}NO_3$	(219.23)	86	Semisolid	—	—	—
9	(*RS*)-Ac-<Glu-OBzl	$C_{14}H_{15}NO_4$	(261.27)	94	Oil	—	III	1.37
10	Paz-$(BZA)_2$	$C_{19}H_{18}N_4O_2$	(334.37)	28	284—287	—	III	2.89
11	Pyr-BZA	$C_{12}H_{11}N_3O$	(213.24)	80	114—115	—	I	1.01
12	(*RS*)-Boc-Pip-OH	$C_{11}H_{19}NO_4$	(229.27)	94	115—119	—	—	—
13	(*RS*)-Boc-Pip-BZA	$C_{18}H_{26}N_2O_3$	(318.41)	75	123—126	—	—	—
14	(*RS*)-Ac-Pip-BZA	$C_{15}H_{20}N_2O_2$	(260.33)	63	106—109	—	II	1.86
15	Pic-BZA	$C_{13}H_{12}N_2O$	(212.25)	85	81—84	—	I	2.32
16	Cbz-Tau-BZA	$C_{17}H_{20}N_2O_4S$	(348.26)	78	105—107	—	I	2.92

HPLC purity of compounds **2**, **4**, **5**, **7**, **8**, **10**, **11**, **14**—**16** is not less than 99%. The elemental analyses were within ±0.4% of the theoretical value. *a*) Class I=anticonvulsant activity at dose of 100 mg/kg or less, class II=anticonvulsant activity at a dose greater than 100 mg/kg, class III=no anticonvulsant activity at a dose up to an including 300 mg/kg. *b*) Hydrophobicity of the compounds expressed as log P value calculated by a computer method (HyperChem (Hypercube, Inc.), ChemPlus, QSAR Properties Program).

Table 2. ^{1}H-NMR Spectra of the Synthesized Compounds

Compound	Chemical shifts δ (ppm):
2	$CDCl_3$: 1.70—1.95 (4H, m), 2.35 (3H, s), 2.90—3.12 (3H, m), 4.45 (2H, d, *J*=7.0 Hz), 7.31 (5H, s), 7.67 (1H, br s)
4	D_2O: 2.12 (3H, s), 2.27—2.42 (2H, m), 3.61, 3.67 (1H total, dt, *J*=1.5, 1.5 Hz), 3.78, 3.84 (2H total, dd, *J*=3.5, 3.5 Hz), 4.40 (2H, s), 7.27—7.47 (5H, m)
5	$CDCl_3$: 2.16—2.53 (2H, m), 2.88 (3H, s), 3.18, 3.90 (2H total, dd, *J*=14, 14 Hz), 4.38 (2H, br s), 4.48 (1H, br s), 7.24—7.30 (5H, m)
7	$CDCl_3$: 2.08—3.06 (7H, m), 4.44 (2H, d, *J*=6.0 Hz), 4.61—4.69 (1H, m), 6.46 (1H, br s), 7.22—7.40 (5H, m)
9	$CDCl_3$: 1.98—2.50 (2H, m), 2.54 (3H, s), 2.62—2.78 (2H, m), 5.21 (2H, s), 7.36 (5H, s)
10	DMSO-d_6: 3.32 (1H, s), 4.45 (4H, d, *J*=6.0 Hz), 7.18—7.35 (10H, m), 8.97 (2H, s), 13.99 (1H, s)
11	$CDCl_3$: 4.68 (2H, d, *J*=6.0 Hz), 7.26—7.39 (5H, m), 8.12 (1H, br s), 8.50 (1H total, dd, *J*=2.0, 2 Hz), 8.74 (1H, d, *J*=2.5 Hz), 9.45 (1H, d, *J*=1.5 Hz)
14	$CDCl_3$: 1.32—2.64 (6H, m), 2.12 (3H, s), 3.15 (1H, t, *J*=20.0 Hz), 3.62—3.78 (2H, m), 5.23 (2H, d, *J*=7.0 Hz), 6.54 (1H, br s), 7.17—7.38 (5H, m)
15	$CDCl_3$: 4.68 (2H, d, *J*=5.8 Hz), 7.20—7.46 (6H, m), 7.90 (1H, t, *J*=7.0 Hz), 8.24 (1H, d, *J*=7.5 Hz), 8.40 (1H, br s), 8.52 (1H, t, *J*=7.0 Hz)
16	$CDCl_3$: 3.07 (2H, t, *J*=7 Hz), 3.57 (2H, q, *J*=7.0 Hz), 4.26 (2H, d, *J*=6.0 Hz), 5.08 (2H, s), 5.43 (1H, t, *J*=7.0 Hz), 7.30 (10H, s), 7.70 (1H, br s)

classification into one of three classes, as shown in Table 1. Compounds **7**, **11** and **15** were tested quantitatively in rats after oral administration. MES ED_{50}, and Tox TD_{50} were determined, PI calculated and the values are given in Table 3 in comparison with those for phenytoin, phenobarbital and valproic acid. The anticonvulsant activity of compound **15** was examined in different seizure models, and ED_{50} and PI values are given in Table 4.

Table 3. Pharmacological Evaluation of the Selected Compounds Administered Orally in Rats (ASP, Phase VIb Quantification)

Compound	ED_{50} against MES (mg/kg)	TD_{50} (mg/kg)	PI[a]
7	35.8	>500	>14
11	10.4	>250	>24
15	17.8	>250	>28
Phenytoin[6]	29.8	>3000	>100
Phenobarbital[6]	9.1	61.1	6.7
Valproic acid[6]	425.1	243	0.6

a) PI=TD_{50}/ED_{50} against MES.

Table 4. Anticonvulsant Action of Compound **15** in Different Seizure Models (Mice, ip)

Convulsant	ED_{50} (mg/kg)	PI[a]
Bicuculline	73	1.4
PTZ	47	2.2
Strychnine	73	1.4
Pilocarpine	156	0.7
Kainic acid	39	2.6
AMPA	10	10.3
NMDA	114	0.9

a) PI=TD_{50}/ED_{50} against convulsant.

Experimental

Elemental analyses were performed on a Perkin-Elmer Microanalyzer. Melting points were determined in a Böetius apparatus. ^{1}H-NMR spectra were recorded on a Varian, Unity 200 or 500 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 with 0.3% ninhydrin in EtOH/AcOH (97 : 3) and 7% phosphomolybdic acid in EtOH. HPLC was performed on a Techma-Robot Type 302 apparatus equipped with a UV detector LCD 2040 (Laboratorni Pristroje, Praha) and a computer registrator/recorder CHROMA (POLLAB Warsaw). The peaks were recorded at 210 nm, CC was carried out under gravity on silica gel (Merck, grade 230 to 400 mesh). The solvent systems used in TLC and CC were: $CHCl_3$/MeOH (50 : 50) (A),

(80 : 20) (B), (90 : 10) (C), (95 : 5) (D), (98 : 2) (E), *n*-butanol/AcOH/H_2O (4 : 1 : 5, organic phase) (BAW) (F), *n*-butanol/pyridine/H_2O (65 : 35 : 65, organic phase) (BPW) (G), phenol saturated with H_2O (H).

Preparation of Boc-Amino Acids and Removal of Boc Group The Boc group was introduced by the method of Schwyzer *et al*.[7] using Boc-azide and was removed with HCl/dioxane in the typical manner.

Acetylation (2*S*,4*R*)-Ac-Pro(4-OH)-BZA (**4**) and (*RS*)-Ac-Pip-BZA (**14**) were obtained by acetylation of HCl×(2*S*,4*R*)-Pro(4-OH)-BZA or HCl ×(*RS*)-Pip-BZA with acetic anhydride in methylene chloride at room temperature for 4 h. Then the solution was concentrated *in vacuo*, the residue was crystallized with EtOAc/hexane and dried *in vacuo*. (*RS*)-Ac-<Glu-OH (**6**) and (*RS*)-Ac-<Glu-OBzl (**9**) were obtained by the acetylation of (*RS*)-<Glu-OH and (*RS*)-<Glu-OBzl with boiling acetic anhydride for 15 min. Then the solution was concentrated *in vacuo* and several times concentrated with MeOH until the acetic acid smell disappeared and then it was dried *in vacuo* over solid NaOH.

Cbz-Tau-OH This compound was obtained by the modified Bergmann *et al.* method.[8] Tau-OH was dissolved in 4 M NaOH. The solution was cooled to 0°C and stirred with an excess of Cbz-Cl chloride for 2 h and at room temperature overnight. The mixture was then extracted 3 times with ethyl ether. The alkaline solution was acidified to pH 6.0 and concentrated. The residue was extracted with dimethylformamide (DMF), the solution was filtered and concentrated *in vacuo* to a small volume. A four times volume of ethyl ether was added into this solution and the mixture was left for 12 h in refrigerator. The obtained crystals were filtered, dissolved in dry DMF, treated with ethyl ether and again left to crystallize. Crystals of Cbz-Tau-ONa were dried *in vacuo*, dissolved in H_2O and filtered on a column with Amberlite IRC 50 in the H^+ form. The filtrate was concentrated *in vacuo*, then concentrated several times with MeOH and benzene and dried *in vacuo.* The obtained raw product was used without further purification.

Cbz-Tau-Cl Raw Cbz-Tau-OH was dissolved in dry benzene and an excess of thionyl chloride was added. The mixture was stirred overnight at room temperature and then boiled for 1 h. This solution was concentrated *in vacuo* and the oily residue was dissolved in dry benzene. The solution was filtered, concentrated *in vacuo*, treated with hexane and left for several hours in a refrigerator. The obtained crystalline raw material was dried *in vacuo*.

Cbz-Tau-BZA Raw Cbz-Tau-Cl (0.97 g, 3.4 mmol) was dissolved in ethyl ether (20 ml) and benzylamine (0.72 g, 6.8 mmol) in ethyl ether was added. This solution was stirred at room temperature for 6 h, then filtered and concentrated *in vacuo*. The oily residue was dissolved in ethyl ether (20 ml) and washed with 20 ml portions of 2 M HCl, saturated $NaHCO_3$ solution and saturated NaCl solution. The ethereal solution was dried with anhydrous $MgSO_4$ and concentrated *in vacuo*. The obtained product was purified by CC. Physicochemical data are given in Tables 1 and 2 .

***N*-Methylation** (*R*)-Pro-OH and HCl×(2*S*,4*R*)-Pro-(4-OH)-BZA were *N*-methylated using formaldehyde solution and catalytical hydrogenation according to the method of Moore.[9]

(RS)-<Glu-OBzl Benzyl ester group was introduced by the procedure of Wang *et al*.[10] Physicochemical data are given in Tables 1 and 2.

Preparation of Benzylamides A BZA group was introduced by the following procedure: An amino acid or its derivative with a free carboxyl function (10 mmol) was dissolved in dry tetrahydrofuran (THF) or a mixture of THF/DMF (40 ml). Then *N*-methylmorpholine (NMM) (10 mmol) was added and the mixture was stirred under nitrogen and chilled to −15 °C. Isobutyl chloroformate (10 mmol) in THF (4 ml) was added dropwise to keep the temperature below −15 °C. BZA (10 mmol) in THF (4 ml) 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 residue was crystallized with EtOAc/hexane.

Pharmacology. Preliminary Evaluation of Anticonvulsant Activity and Neurotoxicity in Phase I Identification (ASP) The synthesized compounds **2**, **4**, **5**, **7**, **9**—**11**, **14**—**16** were tested in mice after ip administration. Tests of MES, sc PTZ and Tox were performed according to the method given by Krall *et al.*[11] as described in the previous paper.[1] The results qualifying the compounds into class I, II or III of ASP are given in Table 1.

Quantitative Assessment of Anticonvulsant Activity in Phase VIb Quantification (ASP) Three compounds (**7**, **11**, **15**) were examined in rats after oral administration using the method of Krall *et al*.[11] described in the previous paper.[1] The results are given in Table 3.

Determination of Anticonvulsant Activity of Compound 15 This compound was administered ip in adult male Swiss mice. Pilocarpine and strychnine were injected ip. Bicuculline and PTZ were administred sc. Kainic acid, NMDA and AMPA were applied intracerebroventricularly (icv). All convulsants were administered at the dose inducing seizures in 97% of tested animals. A detailed description of the methods was published previously.[12—14] The results are given in Table 4.

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