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Synthesis and anticonvulsant activity of some amino acid derivatives

Part 3: Derivatives of Ala, Arg, Tzl, Gly and χAbu[3]

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Ten amino acid derivatives as antagonists of the excitatory amino acid (EAA) receptor and anticonvulsant activity have been designed. Five of these compounds supposed to show rather strong and five of them rather weak action as it was expected on the base of their hydrophobicity. All the compounds were synthesized and then evaluated in mice in the maximal electroshock seizure (MES) test, the subcutaneous Metrasol seizure threshold (scMet) test and the rotorod neurotoxicity (Tox) test. Four of the obtained componds have shown high activity (three received class I and one class II) and six were classified in class III according to the classification of the Anticonvulsant Screening Project (ASP) of the Antiepileptic Drug Development Program (ADDP) of NINDS. One of the compounds classified in class I (**10**) was tested quantitatively following i.p. administration in mice. It has a MES $ED_{50} = 29.05$ b.w. and protective index (PI) of 3.77.

1. Introduction

In previous papers [1, 2] we reported the synthesis, pharmacological evaluation and ASP classification of N-acylated or N-alkylated amino acid amides as EAA receptor antagonists with anticonvulsant activity (Table 1). Considering the physicochemical properties – activity relationship of these compounds we found that the highly active anticonvulsants show a mean hydrophobicity. Analyzing the synthesized and pharmacologically tested compounds we calculated that a medium partition coefficient logarithm value (log P) of the most active antagonists of the EAA receptor is 1.50.

However, assuming, 25% deviation from the calculated value, a log P in the range of 1.13–1.87 is in our opinion possible for potent anticonvulsants [3]. Considering these results we designed and synthesized five antagonists of supposed high activity (**10**, log P = 1.85; **11**, log P = 1.49; **12**, log P = 1.63; **15**, log P = 1.01 and **16**, log P = 1.26) and five of of supposed low activity (**7**, log P = 3.52; **8**, log P = 3.84; **9**, log P = 0.45; **13**, log P = 6.39 and **14**, log P = 5.50).

2. Investigations and results

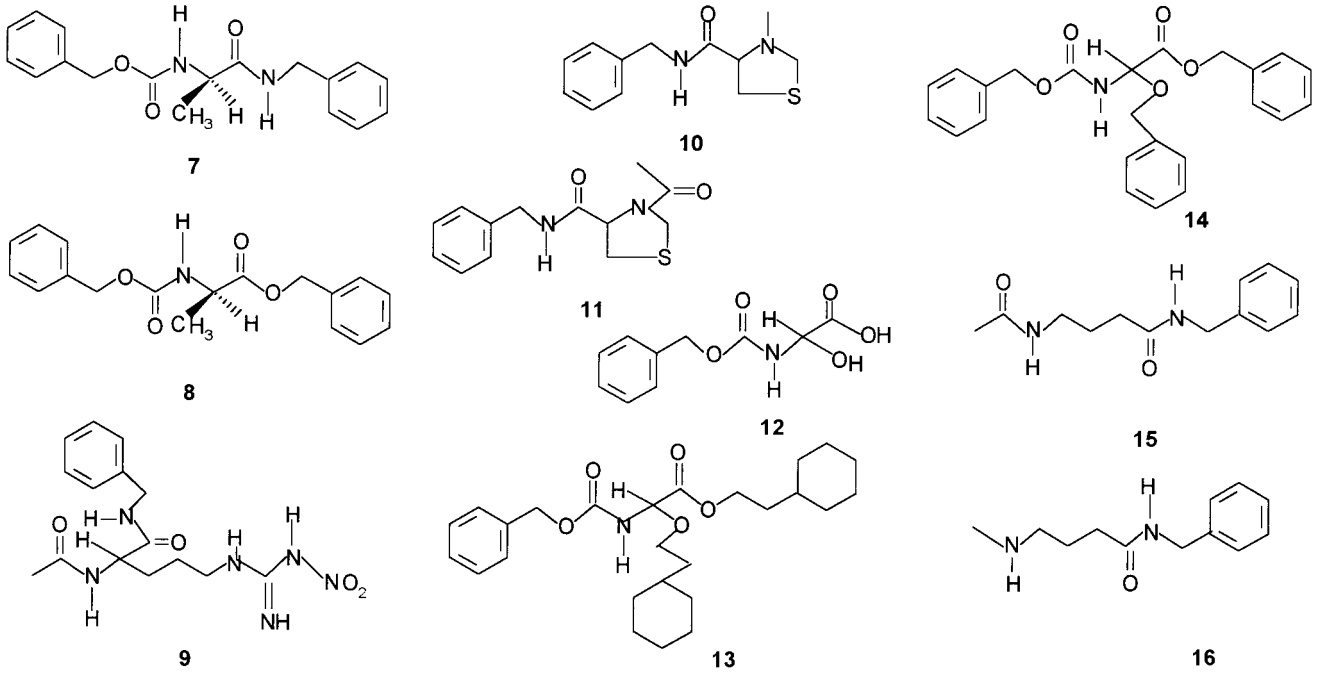
2.1. Chemistry

Syntheses were carried out as follows:
7: (*R*)-Ala-OH → (*R*)-Cbz-Ala-OH → **7**. **8**: (*R*)-Ala-OH → (*R*)-Cbz-Ala-OH → **8**. **9**: (*RS*)-Arg(NO_2)-OH → (*RS*)-Boc-Arg(NO_2)-OH → (*RS*)-Boc-Arg(NO_2)-BZA (**1**) → **9**. **10**: (*RS*)-4-Tzl-OH → (*RS*)-Me-4-Tzl-OH (**2**) → **10**. **11**: (*RS*)-4-Tzl-OH → (*RS*)-Boc-4-Tzl-OH → (*RS*)-Boc-4-Tzl-BZA (**3**) → (*RS*)-4-Tzl-BZA. **12**: Glyoxal + benzyl carbamate → **12**. **13**: (*RS*)-Cbz-Gly(OH)-OH (**12**) → **13**. **14**: (*RS*)-Cbz-Gly(OH)-OH (**12**) → **14**. **15**: χAbu-OH → Boc-χAbu-OH → Boc-χAbu-BZA (**5**) → χAbu-BZA → **15**. **16**: χAbu-OH → Boc-χAbu-OH → Boc-MeχAbu-OH (**4**) → Boc-MeχAbu-BZA (**6**) → **16**. All the products were purified by crystallization or CC. The pure compounds were characterized by TLC, HPLC, ^{1}H NMR, elemental analysis and eventually optical rotation determination. General methods are given in the Experimental part. Physical and analytical data of the synthesized compounds are given in Tables 2 and 3.

Table 1: Compounds previously obtained and described, their classification according to ASP and their log P values

Compd.	class of ASP	Log P	Compd.	Class of ASP	Log P
(*RS*)-Boc-MeAla-BZA [1]	II	2.94	(*R*)-EtAla-BZA [1]	I	1.71
(*RS*)-Ac-Ala-BA [1]	III	0.55	Ac-Sar-BZA [2]	I	0.94
(*R*)-Ac-Ala-BA [1]	III	0.55	(*RS*)-Ac-Leu-BZA [2]	I	2.31
(*RS*)-Ac-Ala-IBA [1]	III	0.56	(*R*)-Ac-Leu-BZA [2]	I	2.31
(*R*)-Ac-Ala-IBA [1]	III	0.56	(*R*)-Ac-MeLeu-BZA [2]	II	2.67
(*RS*)-Ac-Ala-IAA [1]	III	0.88	(*R*)-Ac-Pro-BZA [2]	I	1.46
(*R*)-Ac-Ala-IAA [1]	I	0.88	(*RS*)-Ac-Phe(4 Cl)-BZA [2]	III	3.72
Ac-βAla-BZA [1]	II	0.76	(*S*)-Ac-Trp-BZA [2]	III	2.17
(*RS*)-MeAla-BZA [1]	I	1.37	Ac-Ala(αMe)-BZA [2]	I	1.32
(*R*)-MeAla-BZA [1]	I	1.37	(*R*)-MeAla-FA [2]	II	−0.66
MeβAla-BZA [1]	I	1.01	(*R*)-Ac-MeAla-FA [2]	III	−0.55
(*RS*)-Ac-MeAla-BZA [1]	I	1.48	(*R*)-MeAla-PEA [2]	I	1.62
(*R*)-Ac-MeAla-BZA [1]	I	1.48	(*R*)-MeAla-PMA [2]	III	0.05
Ac-MeβAla-BZA [1]	II	1.12	(*R*)-MeAla-MBZA [2]	I	1.11
(*RS*)-Me_2Ala-BZA [1]	I	1.73	(*R*)-MeAla-FBZA [2]	I	1.51

FA = 2-furfurylamide, PEA = phenylethylamide, PMA = 3-pyridylmethylamide, MBZA = 4-methoxybenzylamide, FBZA = 4-fluorobenzylamide, BA = butylamide, IBA = isobutylamide, IAA = isoamylamide.



2.2. Pharmacology

Compounds **7**–**16** were evaluated in mice after i.p. administration in the MES test, sc Met test and Tox test. The results are a base to ASP classification into one of three classes which is given in Table 2. Compound **10** classified in class I was tested quantitatively in mice after i.p. administration. The MES ED_{50}, Tox TD_{50} and PI were determined and are given in Table 4.

3. Discussion

Looking for amino acidic antagonists of the EAA receptor with anticonvulsant activity we have previously synthesized several amino acid derivatives [1, 2] (Table 1). We also established that they ought to be the compounds of mean hydrophobicity [3]. The first purpose of the present investigation was to obtain new strong anticonvulsants. The second but not less important one was to check experimentally whether the compounds of a mean hydrophobicity (logarithm of the value of partition coefficient n-octanol/water near 1.5) actually show strong activity. We designed structures of five amino acid derivatives suggesting a good activity and of log P values within a range of 1.1–1.9, expecting a strong activity of these compounds. We also designed another five amino acid derivatives with structure also suggesting a good activity, but of log P values higher or lower than those in the first group. In our oppinion, these compounds ought to be of low activity or inactive. The pharmacological examination rather confirmed our assumption relative to both groups of the compounds. We obtained four, not five potent antagonists. The activity of three of them was as expected (log P values 1.85, 1.49 and 1.63 of **10**, **11** and **12**). Surprisingly, com-

Table 2: Physical and analytical data and preliminary pharmacological evaluation (ASP, Phase I Identification, mice, i.p.) of the synthesized compounds

Compd.	Formula (m.w.)	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ (c, MeOH)	TLC, R_f (solv. syst.)	Class[a] of ASP	Log P
1 (*RS*)-Boc-Arg(NO)$_2$-BZA	$C_{18}H_{28}O_5N_6$ (408.5)	91	71–74	–	0.89 (I)	–	–
2 (*RS*)-Me-4-Tzl-OH	$C_5H_9O_2NS$ (147.2)	73	semisolid	–	0.47 (A)	–	–
3 (*RS*)-Boc-4-Tzl-BZA	$C_{16}H_{22}O_3N_2S$ (322.4)	89	86–88	–	0.43 (G)	–	–
4 Boc-MeχAbu-OH	$C_{10}H_{19}O_4N_2$ (217.3)	54	oil	–	0.89 (I)	–	–
5 Boc-χAbu-BZA	$C_{16}H_{24}O_3N_2$ (292.4)	77	114–117	–	0.48 (E)	–	–
6 Boc-MeχAbu-BZA	$C_{17}H_{26}O_3N_2$ (307.4)	70	semisolid	–	0.83 (F)	–	–
7 (*R*)-Cbz-Ala-BZA	$C_{18}H_{20}O_3N_2$ (312.4)	88	138–140	+11.7 (1.3)	0.66 (B)	I	3.52
8 (*R*)-Cbz-Ala-OBz	$C_{18}H_{19}O_4N_2$ (313.3)	85	semisolid	+15.8 (1.5)	0.85 (B)	III	3.84
9 (*RS*)-Ac-Arg(NO)$_2$-BZA	$C_{15}H_{22}O_6N_6$ (350.4)	79	32–36	–	0.37 (C)	III	0.45
10 (*RS*)-Me-4-Tzl-BZA	$C_{12}H_{16}ON_2S$ (236.3)	17	semisolid	–	0.40 (A)	I	1.85
11 (*RS*)-Ac-4-Tzl-BZA	$C_{13}H_{16}O_2N_2S$ (264.3)	65	semisolid	–	0.41 (C)	I	1.49
12 (*RS*)-Cbz-Gly(OH)-OH	$C_{10}H_{11}O_5N$ (225.2)	65	196–198	–	0.72 (B)	II	1.63
13 (*RS*)-Cbz-Gly(OChe)-OChe	$C_{26}H_{39}O_5N$ (445.6)	75	oil	–	0.87 (H)	III	6.39
14 (*RS*)-Cbz-Gly(OBz)-OBz	$C_{24}H_{23}O_5N$ (405.4)	14	74–77	–	0.70 (H)	III	5.50
15 Ac-χAbu-BZA	$C_{13}H_{18}O_2N_2$ (234.3)	86	123–124	–	0.48 (D)	III	1.01
16 MeχAbu-BZA × HCl	$C_{12}H_{19}ON_2Cl$ (242.7)	96	136–138	–	0.27 (G)	III	1.26

Boc = N-tert-butoxycarbonyl group, Cbz = N-benzyloxycarbonyl group, BZA = benzylamide group, Che = cyclohexylethyl group, Tzl = thiazolidine-4-carboxylic acid, Gly(OH)–OH = hydroxyglycine.
HPLC purity of compounds **7**–**16** is not less than 98%. ^{1}H NMR data clearly confirmed the proposed structure. The elemental analyses were within ±0.4% of theoretical value.
[a] I = anticonvulsant activity at dose 100 mg/kg or less, II = anticonvulsant activity at dose greater than 100 mg/kg, III = no anticonvulsanactivity at dose up to including 300 mg/kg.

Table 3: ^{1}H NMR spectra of the synthesized compounds

Compd.	Chemical shifts δ (ppm), $CDCl_3$
1	1.37 (s, 9 H, C_4H_9), 168 (s br, 4 H, 2 HC_3, 2 HC_4), 3.27 (s br, 2 HC_5), 4.22 (s br, HC_2), 4.37 (d, J = 2 Hz, 2 H, CH_2BZA), 5.63 (s br, 1 H, NH), 7.26 (s, 5 H, C_6H_5), 7.38 (s br, 1 H, NH).
2	2.70 (s, 3 H, N−CH_3), 3.25–3.47 (m, 2 HC_5), 3.88–4.12 (m, 2 HC_2), 4.37–4.52 (m, HC_4).
3	1.38 (s, 9 H, C_4H_9), 3.08–3.28 (m, 2 HC_5), 4.25–4.82 (m, 5 H, HC_4, 2 HC_2 BZA), 6.62 (s br, 1 H, NH), 7.29 (s, 5 H, C_6H_5).
4	1.45 (s, 9 H, C_4H_9), 1.87 (p, J = 7 Hz, 2 HC_3), 2.34 (t, J = 5 Hz, 2 HC_2), 2.83 (s, 3 H, N−CH_3), 2.29 (t, J = 5 Hz, 2 HC_4).
5	1.44 (s, 9 H, C_4H_9), 1.80 (p, J = 5 Hz, 2 HC_3), 2.23 (t, J = 3 Hz, 2 HC_2), 3.12 (q, J = 5 Hz, 2 HC_4), 4.40 (d, J = 2.5 Hz, CH_2 BZA), 4.95 (s br, 1 H, NH), 6.77 (s br, 1 H, NH), 7.28 (s, 5 H, C_6H_5).
6	1.45 (s br, 9 H, C_4H_9), 1.87 (p, J = 7 Hz, 2 HC_3), 2.20 (t, J = 5 Hz, 2 HC_2), 2.82 (s, 3 H, N−CH_3), 2.26 (t, J = 5 Hz, 2 HC_4), 4.44 (d, J = 4 Hz, 2 H, CH_2 BZA), 7.28 (s, 5 H, C_6H_5).
7	1.48 (d, J = 5 Hz, CH_3 Ala), 4.32 (d, J = 2 Hz, HC_2), 4.52 (d, J = 5 Hz, 2 H, CH_2 BZA), 5.02 (s, 2 H, CH_2 Cbz), 5.63 (d, J = 4 Hz, 1 H, NH Ala), 6.82 (s br, 1 H NH amid), 7.26 (s, 10 H, 2 · C_6H_5).
8	1.42 (d, J = 5 Hz, CH_3 Ala), 4.38 (d, J = 2 Hz, HC_2), 5.13 (s, 2 H, CH_2), 5.18 (s, 2 H CH_2), 5.64 (d, J = 2 Hz, 1 H, NH), 7.35 (s, 10 H, 2 · C_6H_5).
9	1.20–1.93 (m, 4 H, 2 HC_3, 2 HC_4), 2.03 (s, 3H, CH_3 Ac), 3.25 (q, J = 3 Hz, 2 HC_5), 4.32 (d, J = 2 Hz, 2 H, CH_2 BZA), 7.28 (s, 5 H, C_6H_5), 8.10 (s br, 1 H, NH).
10	2.45 (s, 3 H, N−CH_3), 3.00–4.12 (m, 4 H, HC_4, 2 HC_2, NH), 4.42 (d, J = 5 Hz, 2 H, CH_2 BZA), 7.28 (s, 5 H, C_6H_5).
11	2.12 (s, 2 H; CH_3 Ac), 2.98–3.63 (m, 2 HC_5), 4.26–4.71 (m, 5 H, HC_4, 2 HC_2, CH_2 BZA), 4.88–5.06 (m, 1 H, NH), 7.25 (s, 5 H, C_6H_5).
12	5.13 (s, 2 H, CH_2 Cbz), 5.47 (s, HC_2), 7.33 (s, 5 H, C_6H_5).
13	0.75–1.87 (m, 30 H, 14 · CH_2 Che, 2 · CH Che), 5.14 (s, 2 H, CH_2 Cbz), 5.35 (d, J = 7 Hz, 1 H, HC_2), 5.87 (d, J = 7 Hz, 1 H, NH), 7.35 (s, 5 H, C_6H_5
14	4.70 (s, 2 H, CH_2 Cbz), 5.06 (s, 2 H, CH_2 OBz), 5.09 (s, 2 H, CH_2 OBz), 5.55 (d, J = 7 Hz, 1 H, HC_2), 5.98 (d, J = 7 Hz, 1 H, NH), 7.25–7.45 (m, 15 H, 3 · C_6H_5).
15	1.82 (p, J = 7 Hz, 2 HC_3), 1.92 (s, 3 H, CH_3 Ac), 2.26 (t, J = 5 Hz, 2HC_2), 3.25 (q, J = 2.5 Hz, 2 HC_4), 4.00 (d, J = 2.5 Hz, 2 H, CH_2 BZA), 6.47 (s br, 1 H, NH), 6.84 (s br, 1 H, NH), 7.38 (s, 5 H, C_6H_5).
16	1.37 (p, J = 5 Hz, 2 HC_3), 2.19 (t, J = 5 Hz, 2 HC_2), 2.82 (s, 3 H, N−CH_3), 3.15–3.30 (m, 2 HC_4), 4.48 (d, J = 5 Hz, 2 H, CH_2 BZA), 7.00 (s br, 1 H, NH), 7.30 (s, 5 H, C_6H_5).

Table 4: Pharmacological evaluation of the selected compound (ASP, Phase II quantification mice, i.p.)

Compound	Time of test[a] (h)	MES ED_{50}[b] (mg/kg)	Tox TD_{50}[b] (mg/kg)	PI[c]
10	6.0	29.05 (14.65–40.97)	109.61 (83.06–147.16)	3.77
Phenytoin [10]	2.0	9.5 (8.1–10.4)	65.5 (52.5–72.1)	6.90
Phenobarbital [10]	1.0/0.5	21.8 (15.2–22.5	69.0 (62.8–72.9)	3.20
Valproic acid [10]	0.25	272 (247–338)	426 (369–450)	1.60

[a] Time of MES test/Tox test. Single number indicates all tests performed at the same time.
[b] 95% Confidence limits between parentheses. The MES ED_{50} are the estimated doses from the dose-response data to protect 50% of the animals in the MES test. The TD_{50} is the estimated dose from the dose-response data to impaire 50% of the mice.
[c] Protective index value PI = Tox TD_{50}/MES ED_{50}.

pound **7** with a log P value of 3.52 was also strongly active. Simultaneously, **8** which is a benzyl ester, not an amide as **7**, is deprived of activity. It confirms our finding that EAA receptor antagonists are required to be amides [4]. On the other hand **15** and **16** expected to be active because of log P values of 1.01 and 1.26, as a matter of fact are inactive. We consider that this is due to a long distance between both nitrogen atoms (four carbon atoms, not two as in α-amino acid amides). Considering the relationships between EAA receptor antagonistic activity, structure and physico chemical properties we are able to formulate some dependencies. Strong antagonistic potency of amino acid derivatives demands an α-amino acid structure, a N-acyl- or N-alkyl group, a small substituent at Cα, an aromatic amide substituent and a mean hydrophobicity.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were recorded on a Varian Unity 200 or 500 or a Tesla 100 spectrometer. Chemical shifts were measured as δ units (ppm) relative to tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer Microanalyser. M.p.'s were determined with a Böetius apparatus. 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 Typ 302 apparatus equipped with an UV detector LCD 2040 (190–250 nm) (Laboratorni Pristroje, Praha) and a computer registrator/recorder CHROMA. The Peaks were recorded at 210 nm. The solvent systems used in TLC and CC were: $CHCl_3$ (A), $CHCl_3$/MeOH (97 : 5) (B), (97 : 5) (C), (90 : 10) (D), CH_2Cl_2/MeOH (99 : 1) (E), (99 : 5) (F), (80 : 20) (G), hexane/EtOAc (80 : 20) (H), butanol/AcOH/H_2O (4 : 1 : 5; organic phase) (I). Carbobenzyloxy derivatives of amino acids were obtained according to the method of Bergmann et al. [5] and *N-tert*-butyloxycarbonyl derivatives according to the method of Schwyzer et al. [6]. The benzyl ester group was introduced using the procedure of Wang et al. [7]. Syntheses of acetyl derivatives and benzyl amides are described elsewhere [1]. N-methylation of Boc-derivatives of amino acids was carried out according to Cheung et al. [8]. Condensation of glyoxal with benzyl carbonate was performed according to Zoller et al. [9]. Removal of the Boc group was performed in a typical way, as reported previously [1].

4.2. Pharmacology

4.2.1. Preliminary evaluation

All the synthesized compounds **7**–**16** were tested for anticonvulsant activity and neurotoxicity in Phase I Identification (ASP) in mice after i.p. administration using MES test, sc Met test and Tox test according to the method given by Krall et al. [10] and described in a previous paper [1].

4.2.2. Quantitative assessment

Compound **10** categorized into class I and considered as the most promising was assessed quantitatively in phase II quantification (ASP) in mice after i.p. administration. The anticonvulsant test (MES) as well as the neurotoxicity test (Tox) were performed using the method of Krall et al. [10]. The procedure was described in detail previously [2]. The data obtained were subjected to statistical analysis and the ED_{50}, 95% confidence interval and the slope of the regression line were calculated. The data received in the toxicity determination were statistically analyzed and the results recorded. The results of both determinations are given in Table 4.

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