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CONTENTS

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Synthesis and anticonvulsant activity of some amino acid derivatives

Part 1: Alanine derivatives[3]

R. Paruszewski[1], Grazyna Rostafinska-Suchar[1], Marzanna Strupinska[1], P. Jaworski[1] and J. P. Stables[2]

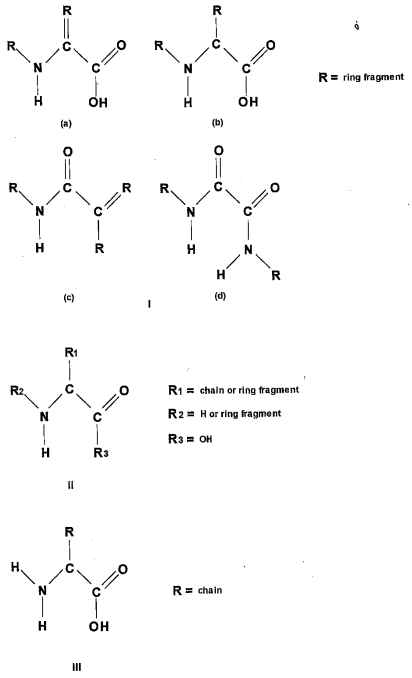
Fifteen amides of *N*-substituted D-Ala, DL-Ala and βAla have been designed and synthesized as potential anticonvulsants. All obtained amides as well as one intermediate (**8**) were evaluated in the maximal electroshock seizure (MES) test, the subcutaneous Metrazol seizure threshold (sc Met) test and the rotorod neurotoxicity (Tox) test in mice. According to the classification of the Anticonvulsant Screening Project (ASP) of the Antiepileptic Drug Development Program (ADDP) eight compounds received class I, three class II and five class III designations. Two of the most active compounds (**20**, **24**) were tested quantitatively. They exhibited, after i.p. administration in mice, a large protective index (PI) 3.2 for **20** and 4.3 for **24** and after oral administration in rat PI >18 for **20** and >14 for **24**.

Synthese and antikonvulsive Wirkung einiger Aminosäure-Derivate
Teil 1: Alanin-Derivate

Fünfzehn Amide des *N*-substituierten D-Alanins, DL-Alanins, und β-Alanins wurden als potentielle Antikonvulsiva synthetisiert. Alle Amide sowie die intermediäre Verbindung **8** wurden an Mäusen im Maximalen Elektroschock-Test (MES), im Metrazol-Minimaldosis-Test (scMet) und im Neurotoxizitätstest (Tox) geprüft. Gemäß der ASP-Klassifizierung des Programms zur Entwicklung von Antikonvulsiva (ADDP) erreichten acht Verbindungen die Klasse I, drei die Klasse II und fünf die Klasse III. Zwei der aktivsten Verbindungen, **20** und **24**, wurden quantitativ geprüft. Nach i. p. Verabreichung an Mäusen wiesen sie Schutzindices von 3,2 bzw. 4,3; nach oraler Gabe an Ratten von >18 bzw. >14 auf.

1. Introduction

Excitatory amino acids (EAAs), primarily glutamate, take part in neurotransmission. Disturbances of this transmission may be responsible for seizure disorders. EAAs exert physiological effects by the medium of distinct receptor types defined by their selective agonists: N-methyl-D-aspartic acid (NMDA), 2-carboxy-4-isopropenyl-3-pyrrolidineacetic acid (kainic acid) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) [1]. NMDA receptors received the most attention mainly on account of their importance in brain function. They have been implicated in the etiology of epilepsy [2, 3]. NMDA receptor antagonists have been shown to exhibit their anticonvulsant activity in a variety of epilepsy models [4, 5]. NMDA receptors contain several recognition sites and the compounds acting at each of the sites are numbered in various groups including glycine site antagonists and competitive NMDA antagonists.



Almost all glycine site antagonists contain structurally similar fragments I. Also, almost all compounds included in the group of competitive NMDA antagonists contain fragment II. We recognized these fragments as necessary for pharmacological activity. This structure is included in the α-amino acid molecule III. Therefore, we decided to search for anticonvulsants in the group of amino acid derivatives.

2. Investigations and results

2.1. Chemistry

N-Methylalanine amides and *N*-acetyl-*N*-methylalanine amides were prepared according to the sequence Boc-Axx-OH → Boc-MeAxx-OH → Boc-MeAxx-amide → MeAxx-amide → Ac-MeAxx-amide, *N*-acetylalanine amides as follows: Boc-Axx-OH → Boc-Axx-amide → Axx-amide → Ac-Axx-amide and *N*,*N*-dimethylalanine amide: Axx-OH → Me_2Axx-amide. *N*-ethylalanine amide was obtained in the same way as the *N*-methyl derivatives. All compounds were synthesized by procedures common for the prepa-

Table 1: Physical and analytical data and preliminary pharmacological evaluation/ASP, Phase I Identification, mice, i.p./of synthesized compounds

Compd.	Formula (m.w.)	Yield (%)	m.p. (°C)	K_D^{20} (c, MeOH)	TLC, R_f (solvent system)	Class of ASP
1 Boc-DL-Ala-BA	$C_{12}H_{24}O_3N_2$ (244.3)	84	120–122	–	0,81 (A)	–
2 Boc-D-Ala-BA	$C_{12}H_{24}O_3N_2$ (244.3)	60	74–76	+26.2 (1.9)	0.81 (A)	–
3 Boc-DL-Ala-IBA	$C_{12}H_{24}O_3N_2$ (244.3)	72	103–105	–	0.84 (A)	–
4 Boc-D-Ala-IBA	$C_{12}H_{24}O_3N_2$ (244.3)	84	102–104	+32.0 (1.5)	0.84 (A)	–
5 Boc-DL-Ala-IAA	$C_{13}H_{26}O_3N_2$ (258.4)	78	95–97	–	0.85 (A)	–
6 Boc-D-Ala-IAA	$C_{13}H_{26}O_3N_2$ (258.4)	88	73–75	+24.2 (1.6)	0.85 (A)	–
7 Boc-βAla-BZA	$C_{15}H_{22}O_3N_2$ (278.4)	77	119–120	–	0.41 (A)	–
8 Boc-DL-MeAla-BZA	$C_{16}H_{24}O_3N_2$ (292.4)	81	69–71	–	0.81 (A)	II
9 Boc-D-MeAla-BZA	$C_{16}H_{24}O_3N_2$ (292.4)	75	37–39	+23.6 (1.8)	0.81 (A)	–
10 Boc-MeβAla-OH	$C_9H_{17}O_4N$ (203.2)	89	oil	–	0.50 (E)	–
11 Boc-MeβAla-BZA	$C_{16}H_{24}O_3N_2$ (292.4)	79	64–65	–	0.57 (A)	–
12 Boc-D-Et-Ala-BZA	$C_{17}H_{26}O_3N_2$ (306.4)	75	oil	+21.6 (1.6)	0.34 (F)	–
13 Ac-DL-Ala-BA	$C_9H_{18}O_2N_2$ (186.3)	76	110	–	0.25 (B)	III
14 Ac-D-Ala-BA	$C_9H_{18}O_2N_2$ (186.3)	69	122–124	+51.3 (1.5)	0.25 (B)	III
15 Ac-DL-Ala-IBA	$C_9H_{18}O_2N_2$ (186.3)	62	119–121	–	0.30 (B)	III
16 Ac-D-Ala-IBA	$C_9H_{18}O_2N_2$ (186.3)	55	125–127	+52.1 (1.8)	0.30 (B)	III
17 Ac-DL-Ala-IAA	$C_{10}H_{20}O_2N_2$ (200.3)	63	97–99	–	0.31 (B)	III
18 Ac-D-Ala-IAA	$C_{10}H_{20}O_2N_2$ (200.3)	52	122–124	+51.3 (1.5)	0.31 (B)	I
19 Ac-βAla-BZA	$C_{12}H_{16}O_2N_2$ (220.7)	86	169–170	–	0.70 (A)	II
20 DL-MeAla-BZA	$C_{11}H_{16}ON_2$ (192.3)	72	oil	–	0.42 (D)	I
21 D-MeAla-BZA	$C_{11}H_{16}ON_2$ (192.3)	68	oil	+12.0 (1.2)	0.42 (D)	I
22 MeβAla-BZA	$C_{11}H_{16}ON_2$ (192.3)	90	oil	–	0.30 (A)	I
23 Ac-DL-MeAla-BZA	$C_{13}H_{18}O_2N_2$ (234.3)	93	oil	–	0.32 (C)	I
24 Ac-D-MeAla-BZA	$C_{13}H_{18}O_2N_2$ (234.3)	71	oil	+64.0 (1.5)	0.32 (C)	I
25 Ac-MeβAla-BZA	$C_{13}H_{18}O_2N_2$ (234.3)	91	oil	–	0.48 (A)	II
26 DL-Me_2Ala-BZA	$C_{12}H_{18}ON_2$ (206.3)	18	30–32	–	0.34 (E)	I
27 D-EtAla-BZA	$C_{12}H_{18}ON_2$ (206.3)	75	64–66	+9.9 (1.0)	0.25 (A)	I

BZA = benzylamide, BA = butylamide, IAA = usoamylamide, IBA = isobutylamide, Boc = *N*-*tert*-butoxycarbonyl, MeAla = *N*-methylalanine, Me_2Ala = *N*,*N*-dimethylalanine, EtAla = *N*-ethylalanine, MeβAla = *N*-methyl-β-alanine. HPLC purity of compounds **8** and **13**–**27** is not less than 97%. The elemental analyses were within ±0,4% of theoretical values. ^{1}H NMR data clearly confirmed the proposed structures.

ration of peptides. The pure products were characterized by TLC, HPLC, ^{1}H NMR; elemental analysis, m.p. and optical rotation (Table 1).

2.2. *Pharmacology*

Compounds **8** and **13**–**27** were evaluated in the Phase I identification of ASP in mice after i.p. administration in the MES test, sc Met test and Tox test. The results are the basis for ASP classification into one of the three classes (I, II, III, Table 1). Compounds included in class I (**18**, **20**–**24**, **26**, **27**) were tested in Phase IVa Evaluation (ASP, rat, oral). These results confirmed the classification of Phase I identification. Two compounds were considered promising and were assessed quantitatively in Phase II quantification (ASP, mice, i.p.) and in Phase VIb quantification (ASP, rat, oral). The MES ED_{50}, sc Met ED_{50} Tox TD_{50} and PI were determined in mice and in rats and are reported (Tables 2 and 3).

Table 3: Pharmacological evaluation of selected compounds (ASP, Phase VIb quantification, rat, oral)

Compd.	MES ED_{50} (mg/kg)	sc Met ED_{50} (mg/kg)	Tox TD_{50} (mg/kg)	PI
20	27.33 (16.2–48.2)	>250	>500	>18
24	34.05 (29.0–38.4)	>250	>500	>14

3. Discussion

The ability of amino acids to penetrate the blood-brain barrier is determined by their hydrophobicity. We designed a series of hydrophobic amino acid derivatives

Table 2: Pharmacological evaluation of selected compounds (ASP, Phase II quantification, mice, i.p.)

Compd.	MES ED_{50} (mg/kg)	sc Met ED_{50} (mg/kg)	Tox TD_{50} mg/kg)	PI[a]
20.	31.17 (21.36–40.84)[b]	>50	99.09 (74.75–120.93	3.2
24.	53.47 (44.66–64.52)	124.56 (100.32–144.85)	231.56 (195.32–279.71)	4.3
Phenytoin [6]	9.5 (8.1–10.4)	–	65.5 (52.5–72.1)	6.9
Phenobarbital [6]	21.8 (15.0–22.5]	–	69.0 (62.8–72.9)	3.2
Valproic acid [6]	272 (247–338)	–	426 (369–450)	1.6
Ac-DL-Ala-BZA [7]	76.54 (66.58–89.04)	–	453.86 (416.56–501.01)	5.9
Ac-DL-Ala-mFBZA[c] [7]	77.38 (62.55–91.01)	142.73 (61.53–237.97)	>500	≈6.5
Ac-DL-Mag-BZA[d] [8]	6.2 (5.4–7.2)	–	46 (38.0–56.0)	7.4
Ac-DL-Mmag-BZA[e] [8]	6.7 (5.7–7.7)	–	50.5 (40.4–59.)	7.5

a = Protective Index value, PI, Tox TD_{50}/MES ED_{50}, b = 95% confidence limits between parentheses, c: mFBZA = meta-fluorobenzylamide, d: Mag = methoxyaminoglycine, e: Mmag = methoxymethylaminoglycine. The MES or scMet ED_{50} are the estimated doses from the dose-response data to protect 50% of the animals in the MES or sc Met tests. The Tox TD_{50} is the estimated dose from the dose-response data to impair 50% of the mice.

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