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PHARMACOLOGY

PRELIMINARY EVALUATION OF ANTICONVULSANT ACTIVITY OF SOME 4–(BENZYLOXY)–BENZAMIDES

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Abstract: A series of alkanolamides have been tested for anticonvulsant activity in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole seizure treshold (ScMet) assays and for neurotoxicity (TOX) in rodents. Most interesting were the anticonvulsant results of 2N–methylaminoethanol derivative **II**, which displayed anti– MES activity (mice) with a protective index (TD_{50}/ED_{50}) of 2.536 higher than that for valproate.

Keywords: 4–(benzyloxy)–benzamides, ^{1}H NMR spectroscopy, anticonvulsant activity

Epilepsy is one of the oldest and major neurological disorder in the world. The most clinically used antiepileptic drugs act by more than one mechanism, e.g. the drugs can influence the inhibitory or exicitatory neurotransmitter systems (γ–aminobutyric or glutamic and aspartic acids, respectively or the ion transport across cell membranes (1, 2). Many patients with epilepsy fail to experience adequate control of their seizures despite the optimal use of available antiepileptic drugs. Other patients do so only at the expense of significant toxic side effects (3).

In the course of our investigations of compounds with anticonvulsant properties (4–7), we have directed our attention to the appropriate aminoalkanol derivatives (amides) **I–VIII** (Table 1) as potential anticonvulsant agents. The physicochemical and biological properties of some respective alkanolamides, that exhibited a diversified action on CNS have been reported (8, 9). Amides **I–VIII** contain some structural moieties of known drugs which act on CNS in particular of antiepileptic drugs derivatives of hydrantoin and succinimide (phenytoin, ethosuximide, etc.) valproic acid and its amides are also used in epilepsy.

EXPERIMENTAL

Chemistry

Melting points: a Büchi SMP–20 instrument, uncorrected, ^{1}H NMR were recorded on a Bruker spectrometer (500.13 MHz) in DMSO–d_6 or $CDCl_3$, chemical shifts are reported relative to internal TMS in the following format: chemical shift δ (ppm), multiplicity, number of protons. IR (KBr) spectra were recorded on a Perkin Elmer spectrometer. All the results of elemental analysis (C, H, N) were in acceptable range. Log P (combined) values prediction were taken from the PALLAS program. Thin–layer chromatography was carried out on Merck silica gel GF_{254} plates. The physical properties of **I**, **II**, **IV**, **V** and **VII** (without ^{1}H NMR data) were described earlier (10). New compounds **III**, **VI** and **VIII** were obtained by N–acylation of appropriate aminoalkanol with 4–(benzyloxy)–benzoyl chloride in two phase–system (toluene/water) (10).

(R,S)–2–[4–(Benzyloxy)–benzoyl]–amino–1–propanol III

Yield (67%), m.p. 150–152°C (toluene/heptane, 4/1), $C_{17}H_{19}NO_3$ (285.3), R_f = 0.59 (toluene/acetone, 1/1). IR (KBr, cm^{-1}) ν: 3375, 3064, 3033, 2967, 2932, 1630, 1606, 1547, 1504, 1454, 1247, 1186.

4–[4–(Benzyloxy)–benzoyl]–amino–1–butanol VI

Yield (60%), m.p. 126–128°C (toluene), $C_{18}H_{21}NO_3$ (299.4), R_f = 0.48 (toluene/acetone, 1/1). IR (KBr, cm^{-1}) ν: 3387, 3302, 3033, 2950, 1630, 1610, 1537, 1506, 1462, 1255.

1–[4–(Benzyloxy)–benzoyl]–4–[2–(hydroxy)–ethyl]–piperazine VIII

Yield (62%), m.p. 106–108°C (toluene), $C_{20}H_{24}N_2O_3$ (340.4), R_f = 0.45 (toluene/methanol, 5/1). IR (KBr, cm^{-1}) ν: 3390, 3035, 2941, 2800, 1626, 1606, 1514, 1454, 1244.

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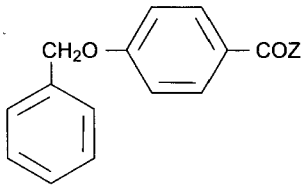
Table 1. The structure and ^{1}H NMR data of tested compounds **I–VIII**



Compd.	Z	^{1}H NMR (δd (ppm), J(H_2)
I	$NHCH_2CH_2OH$	7.74 (dm, J = 8.8 Hz, 2H (H–3, H–5)); 7.44–7.29 (m, 5H, (C_6H_5)); 6.99 (dm, J = 8.8 Hz, 2H, (H–2, H–6)); 6.42 (s, 1H, NH); 5.12 (s, 2H, OCH_2Ar); 3.82 (dt, J = 4.8 Hz, J = 4.8 Hz, 2H, $C\underline{H}_2OH$); 3.61 (dt, J = 4.8 Hz, J = 5.5 Hz, 2H, NCH_2); 2.37 (s, 1H, OH), ($CDCl_3$)
II	NCH_2CH_2OH (N-CH$_3$)	7.46–7.31 (m, 7H, (C_6H_{25}, H–3, H–5)); 6.98 (dm, J = 8.7 Hz, 2H, (H–2, H–6)); 5.09 (s, 2H, $ArCH_2O$); 3.86 (bb, 2H, CH_2OH); 3.68 (bs, 2H, NCH_2); 3.08 (s, 3H, NCH_3); 2.30 (bb, 1H, OH), ($CDCl_3$)
III	$NHCHCH_2OH$ (CH$_3$) (R,S)	7.71 (dm, J = 8.9 Hz, 2H (H–3, H–5)); 7.40–7.28 (m, 5H, (C_6H_5)); 6.97 (dm, J = 8.8 Hz, 2H, (H–2, H–6)); 6.08 (bs, 1H, NH); 5.10 (s, 2H, CH_2Ar); 4.28–4.19 (m, 1H, CH); 3.75 (dd, J = 10.8 Hz, J = 3.8 Hz, 1H, $H\underline{C}HOH$); 3.63 (dd, J = 10.8 Hz, J = 5.7 Hz, 1H, $HC\underline{H}OH$); 2.45 (s, 1H, OH); 1.27 (d, J = 6.9 Hz, 3H, CH_3), ($CDCl_3$)
IV	$NHCHCH_2OH$ (C_2H_5) (R,S)	7.73 (dm, J = 8.8 Hz, 2H (H–3, H–5)); 7.43–7.30 (m, 5H, (C_6H_5)); 6.96 (dm, J = 8.8 Hz, 2H, (H–2, H–6)); 6.35 (d, J = 8.0 Hz, 1H, NH); 5.08 (s, 2H, CH_2OAr); 4.07–3.99 (m, 1H, CH); 3.76 (dd, J = 11.0 Hz, J = 3.5 Hz, 1H, $H\underline{C}HOH$); 3.66 (dd, J = 11.0 Hz, J = 5.5 Hz, 1H, $HC\underline{H}OH$); 3.19 (s, 1H, OH); 1.73–1.53 (m, 2H, $\underline{CH_2}$–CH_3); 0.98 (t, J = 7.4 Hz, 3H, CH_3), ($CDCl_3$)
V	$NHCH_2CHOH$ (C_2H_5) (R,S)	8.25 (t, J = 5.7 Hz, 1H, NH); 7.84 (dm, J = 8.9 Hz, 2H (H–3, H–5)); 7.48–7.31 (m, 5H, (C_6H_5)); 7.07 (dm, J = 8.9 Hz, 2H, (H–2, H–6)); 5.17 (s, 2H, OCH_2Ar); 4.72 (d, J = 5.1 Hz, 1H, OH); 3.57–3.49 (m, 1H, CH); 3.32–3.25 (m, 1H, $N\underline{H}CH$); 3.19–3.12 (m, 1H, $NHC\underline{H}$); 1.51–1.41 (m, 2H, $\underline{CH_2}$–CH_3); 0.89 (t, J = 7.4 Hz, 3H, $CH_3\underline{CH_3}$); (DMSO–d_6)
VI	$NH(CH_2)_3CH_2OH$	7.72 (dm, J = 8.8 Hz, 2H (H–3, H–5)); 7.43–7.29 (m, 5H, (C_6H_5)); 6.98 (dm, J = 8.8 Hz, 2H, (H–2, H–6)); 6.22 (bs, 1H, NH); 5.11 (s, 2H, $ArOCH_2$); 3.72 (q, J = 5.5 Hz, 2H, $C\underline{H}_2OH$); 3.49 (q, J = 6.5 Hz, 2H, CH_2N); 1.77–1.70 (m, 2H, CH_2); 1.70–1.64 (m, 2H, CH_2); 1.54 (bb, 1H, OH), (CDCl3)
VII	$NHCH_2CHOH$ (C_6H_5) (R,S)	8.44 (t, J = 5.6 Hz, 1H, NH); 7.86 (dm, J = 8.9 Hz, 2H (H–3, H–5)); 7.53–7.25 (m, 10H, (C_6H_5)); 7.10 (dm, J = 8.9 Hz, 2H, (H–2, H–6)); 5.57 (bs, 1H, OH); 4.84–4.78 (m, 1H, CH); 3.54–3.47 (m, 1H, $HC\underline{H}$); 3.36–3.29 (m, 1H, $H\underline{C}H$); (DMSO–d_6)
VIII		7.44–7.31 (m, 7H, (C_6H_5), H–3, H–5)); 6.98 (dm, J = 8.8 Hz, 2H, (H–2, H–6)); 5.09 (s, 2H, $ArOCH_2$); 3.80–3.45 (m, 4H, (pip.(a)); 3.64 (t, J = 5.3 Hz, 2H, CH_2OH); 2.59 (t, J = 5.3 Hz, 2H, N–CH_2); 2.70–2.40 (m, 4H, (pip.(e)); 2.30 (bb, 1H, OH), ($CDCl_3$)

Table 2. Anticonvulsant activity of 4-(benzyloxy)-benzamides **I–VIII** in mice after intraperitoneal injection and log P (combined)

Compd.	Dose mg/kg	MES[a]		ScMet[a]		Toxicity[b]		Log P
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
I	30	–[c]	–	–	–	–	–	2.00
	100	–	–	1/5	–	1/8	–	
	300	–	1/1	–	–	3/4	2/2	
II	30	1/1	–	–	–	–	–	2.03
	100	3/3	–	–	–	–	–	
	300	1/1	–	1/1	–	4/4	–	
III	30	–	–	–	–	1/4	–	2.53
	100	1/3	–	–	–	1/8	–	
	300	1/1	–	–	–	3/4	1/2	
IV	30	–	–	–	–	–	–	3.04
	100	1/3	–	–	–	–	–	
	300	1/1	1/1	3/4	1/2	3/4	1/2	
V	30	–	–	–	–	–	–	3.04
	100	–	–	–	–	1/8	–	
	300	–	–	–	–	2/4	1/2	
VI	30	–	–	–	–	–	–	2.86
	100	–	–	–	–	1/8	–	
	300	–	–	3/5	1/1	4/4	2/2	
VII	30	–	–	–	–	–	–	3.68
	100	–	–	–	–	–	–	
	300	–	–	–	–	–	1/2	
VIII	30	–	–	–	–	–	–	1.31
	100	–	–	–	–	–	–	
	300	1/1	–	–	–	–	–	

[a] number of animals protected/number of animals tested in the MES and ScMet test; [b] number of animals exhibiting toxicity/number of animals tested in the rotorod test; [c] – denotes no activity nor toxicity

Pharmacology

Preliminary pharmacological testing

The Antiepileptic Drug Development (ADD) Program Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological Disorders and Stroke (NINDS) (Bethesda, MD, USA), have provided preliminary pharmacological testing of the reported compounds. These testing procedures have been described (11–12). Phase I studies of the amides **I–VIII** involved three tests: maximal electroshock–induced seizures (MES), subcutaneous pentylenetetrazol seizures (ScMet), and neurological toxicity (TOX). The TOX was measured by the rotorod test. Active compounds in the Phase I evaluation were tested either for an ED_{50} quantitation in mice (Phase II), or qualitatively in rats (Phase Via). The result from anticonvulsant assays for **I–VIII** (Table 1) are summarized in Tables 2–4.

RESULTS AND DISCUSSION

Table 2 presents results of the Phase I test according to the ADD Program. Table 3 shows data for compounds **II–IV** and **VIII**, which were advanced to phase VIa according to the ADD Program and were evaluated in the rats after oral administration. The protective activity in the MES and/or rats without neurotoxicity was shown for compounds **II–IV** and **VIII** (Tables 2 and 3). In the ScMet test in mice, the protective activity was seen for **I**, **–II, IV** and **VI** in doses up to 100(**I**) and 300 mg/kg. Neurotoxicity was not observed for all tested compounds in doses up to 30 mg/kg and for **II**, **IV** and **VII** in doses up to 100 mg/kg, and for **VIII** in a dose up to 300 mg/kg, 0.5 h and 4 h after administration. At this stage, it seems that **II** and **IV** are the most promising compounds, active in both the anticonvulsant screens (MES and ScMet) indicating its potential antiepileptic action in both the grand mal and petit mal attacks.

Table 4 compares the effects of **II** with those of valproate (13). The protective index in the MES test in mice is higher but in the ScMet test is lower than that for valproate. Modifications in the chemical structures of most active compounds **II** and **IV**, for example, by introduction of isomeric aminoalkanols (1–amino–2–butanol or 4–amino–1–buta-

Table 3. Anticonvulsant screening project: phase VIa. Test results in rats (dose 30 mg/kg, *p.o.*)

Compd.	Test	Time in hours				
		0.25	0.5	1.0	2.0	4.0
II	MES*	1/4	2/4	3/4	1/4	2/4
	TOX*	–	–	–	–	–
III	MES	1/4	1/4	–	1/4	–
	TOX	–	–	–	–	–
IV	MES	3/4	2/4	1/4	1/4	2/4
	TOX	–	–	–	–	–
VIII	MES	1/4	1/4	–	–	–
	TOX	–	–	–	–	–

* see Table 2

Table 4. Quantitative anticonvulsant activity and neurotoxicity of **II** in mice

Compd.	TPE[a] (h)	TD_{50}[b]	ED_{50}[c] MES	ED_{50} ScMet[c]
II	1/4, 1/4	131.37 (103.65–163.71) [6.59]	51.8 (46.39–61.74) [11.52] PI 2.536	142.98 (124.38–200.34) [15.43] PI 0.919
VPA[d]	1/4, 1/4 (2.85)[e]	483 (412–571) [12.3]	287 (237–359) [7.31] PI 1.7	290 (176–249) [8.51] PI 2.3

[a] Time to peak effect. The first value is for the rotorod test; the second is for the anticonvulsant tests. [b] Dose (mg/kg) eliciting evidence of minimal neurotoxicity in 50% of animals; [c] Dose (mg/kg) eliciting the MES and in the ScMet protection in 50% of animals. The 95% confidence interval is shown in parentheses; the slope regression line is shown in brackets. [d] VPA – valproate. [e] Calculated value of log P. PI – protection index (TD_{50}/ED_{50}).

nol) or piperazine moiety (**VIII**), did not increase the anticonvulsant activity in mice or rats, however the neurotoxicity in the case of **VIII** was diminished. To explain these differences, the synthesis and evaluation of further, especially chiral derivatives, will be necessary.

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