Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 5 pp. 571–578, 2009
ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACOLOGY

PRELIMINARY EVALUATION OF ANTICONVULSANT ACTIVITY AND NEUROTOXICITY OF SOME 1,4-SUBSTITUTED PIPERAZINE DERIVATIVES

HENRYK MARONA[1*], AGNIESZKA GUNIA[1], KAROLINA SŁOCZYŃSKA[1], ANNA RAPACZ[2], BARBARA FILIPEK[2], MAREK CEGŁA[3] and WŁODZIMIERZ OPOKA[4]

[1]Department of Technology and Biotechnology of Drugs, [2] Department of Pharmacodynamics, [3]Department of Organic Chemistry, [4]Department of Inorganic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, 9 Medyczna St., 30-688 Kraków, Poland

Abstract: A series of 1,4-piperazine derivatives was synthesized and evaluated for anticonvulsant activity in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole seizure threshold (ScMet) assays and for neurotoxicity (TOX). The compounds were only moderately effective. The anticonvulsant activity was accompanied by neurotoxicity. 1-[(4-Chlor-3-methylphenoxy)-acetyl]-4-(2-methoxyphenyl)-piperazine was also evaluated in six hertz seizure test (6-Hz) and showed good activity. At the dose of 100 mg/kg b. w. the compound produced 100% protection after 0.5 h without neurotoxic effect.

Keywords: anticonvulsant activity, neurotoxicity, 1,4-piperazine derivatives

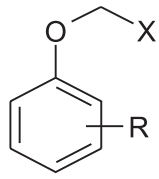
Despite considerable progress in the pharmacotherapy of epilepsy during the last decades, up to 30% of all patients are still poorly treated with the available antiepileptic drugs. Although the new generation of antiepileptic drugs like lamotrigine, vigabatrin, gabapentin, oxcarbazepine, topiramate, levetiracetam, pregabalin is a real progress in the treatment of refractory patients, the problem of intractable seizures has not disappeared. Just as epilepsy itself is a heterogeneous neurological condition with multiple etiologies, the pathogenesis of resistance is considered to be multifactorial and variable. Moreover, recently developed drugs exhibit significant side effects, especially in long term therapy, such as ataxia, double vision, dizziness, gastrointestinal disturbances, hepatotoxicity and megaloblastic anemia which limit their clinical use. Therefore, the continued search for more effective and safer antiepileptic drugs is very important (1-6). Wide range of action to central nervous system of newer antiepileptic drugs may serve not only for clinical seizure suppression but also for neuroprotection, what might be taken into consideration while searching for new active structures (7). Among antiepileptic drugs, there could be found different chemical classes of compounds: hydantoins, barbiturates, iminostilbenes, benzodiazepines, valproate, imides, oxazolidinediones and miscellaneous agents. The drugs exert their action by different mechanisms and, what is more, the majority of antiepileptic drugs act by more than one mechanism (8).

Searching for new compounds with potential anticonvulsant activity, we noticed that several of piperazine derivatives showed anticonvulsant properties in several models of seizures. In previous studies we noticed that some piperazine derivatives displayed protection against electroshock (MES) induced seizures, low neurotoxicity (TOX) and little protection in subcutaneous pentylenetetrazole induced seizures (ScMet). Some of them, i.e. 1,4-bis[(4-chloro-3-methyl)-phenoxyethyl]-piperazine dihydrochloride prevent maximal electroshock seizures in mice with an ED_{50} of 115.9 mg/kg and protective index PI = 2.05 in the MES test in mice which is higher than that of valproate (PI = 1.7) (9).

We previously reported on the synthesis and cardiovascular activity of some 1,4-piperazine derivatives [**I**-**VI**] (10, 11). Antiarrhythmic and hypotensive properties as well as affinity for adrenergic receptors were evaluated. The most active compounds [**I**, **II**, **IV**, **V**] have the 1-(2-methoxyphenyl)-piperazine moiety, which seems to

* Corresponding author: Tel /fax: + 48 12 657 04 88; e-mail: hen.mar@interia.pl

Table 1. The structure of the tested compounds.



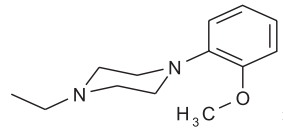
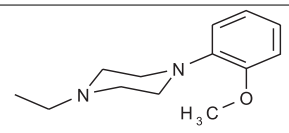
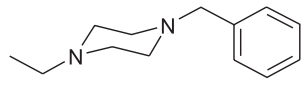
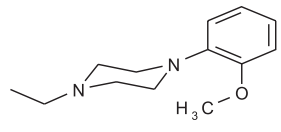
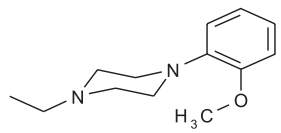
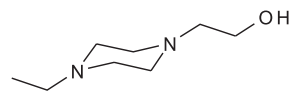
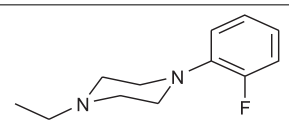
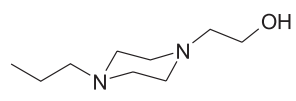
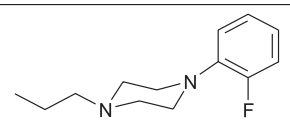
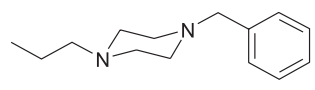
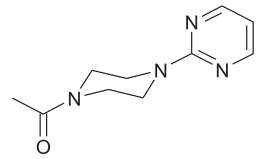
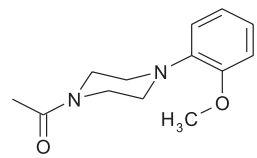
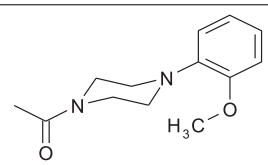
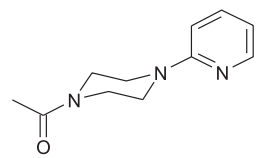
Compd.	R	X
I	2,6-$(CH_3)_2$	 x 2HCl
II	4-Cl, 3-CH_3	 x 2HCl
III	2,6-$(CH_3)_2$	 x 2HCl
IV	4-(OCH_3)	 x 2HCl
V	4-CH_3	 x 2HCl
VI	2,6-$(CH_3)_2$	 x 2HCl
VII	2,4,6-$(CH_3)_3$	 x 2HCl
VIII	2,6-$(CH_3)_2$	 x 2HCl
IX	2,6-$(CH_3)_2$	 x 2HCl
X	2,6-$(CH_3)_2$	 x 2HCl

Table 1. Cont.

Compd.	R	X
XI	2,6-$(CH_3)_2$	
XII	2,6-$(CH_3)_2$	
XIII	4-Cl, 3-CH_3	
XIV	4-Cl, 3-CH_3	

be required for their pharmacological activity. They were active in adrenaline-induced arrhytmia in anesthetized rats following both intravenous and oral administration. In other tests, they significantly decreased systolic and diastolic blood pressure in normotensive rats after both *i.v.* and *p.o.* administration. The observed hypotensive effect of the tested compounds was weaker but at the same time the toxicity (LD_{50} = 27 mg/kg) was about twice lower than that of the reference compound – carvedilol. The tested compounds showed to possess affinity for α_1-, α_2- and β_1-adrenoceptors (10, 11).

Considering the results of evaluated activity of compounds **I**-**VI** in circulatory system, compounds **VII**-**XIV** (Table 1), which are their analogues or homologues, were synthesized. They are currently being tested for cardiovascular activity. Taking into account the fact that several circulatory drugs (e.g. propranolol) have also antiepileptic properties (12), as well as previously reported anticonvulsant properties of some piperazine derivatives, the neurologic profile of the tested compounds [**I**-**XIV**] was checked. The present paper reports on both anticonvulsant activity and neurotoxicity of compounds **I**-**XIV** which were evaluated in preliminary pharmacological testing according to the Antiepileptic Drug Development Program (ADD) at the National Institute of Neurological Disorders and Stroke (NINDS, Rockville, MD 20852, USA). All of them completed phase I testing, which included: maximal electroshock-induced seizures (MES; mice, *i.p.*), subcutaneous pentylenetetrazole-induced seizures (ScMet; mice, *i.p.*), and neurological toxicity (TOX), which was measured by the rotarod test. Compound **XIII** was also evaluated in the six hertz seizure test (6-Hz test).

EXPERIMENTAL

Chemistry

Apparatus and reagents

Melting points were determined using a Büchi SMP-20 apparatus and are uncorrected. Values of calculated and found percentage content of carbon, hydrogen and nitrogen in the tested compounds are listed below. The IR spectra were recorded on a Jasco FT/IR 410 spectrometer. The ^{1}H-NMR and ^{19}F-NMR spectra were recorded on Varian –VX 300 NMR or Bruker AMX spectrometers in $CDCl_3$ or DMSO-d_6. Analytical TLC was performed on precoated aluminium plates (silica gel, 60 F_{254}, Merck) using as a mobile phase solvent systems described below. The results were visualized by means of UV light. The theoretical values of the partition coeffi-

Table 2. Anticonvulsant screening project: phase I. The results in mice (*i.p.*).

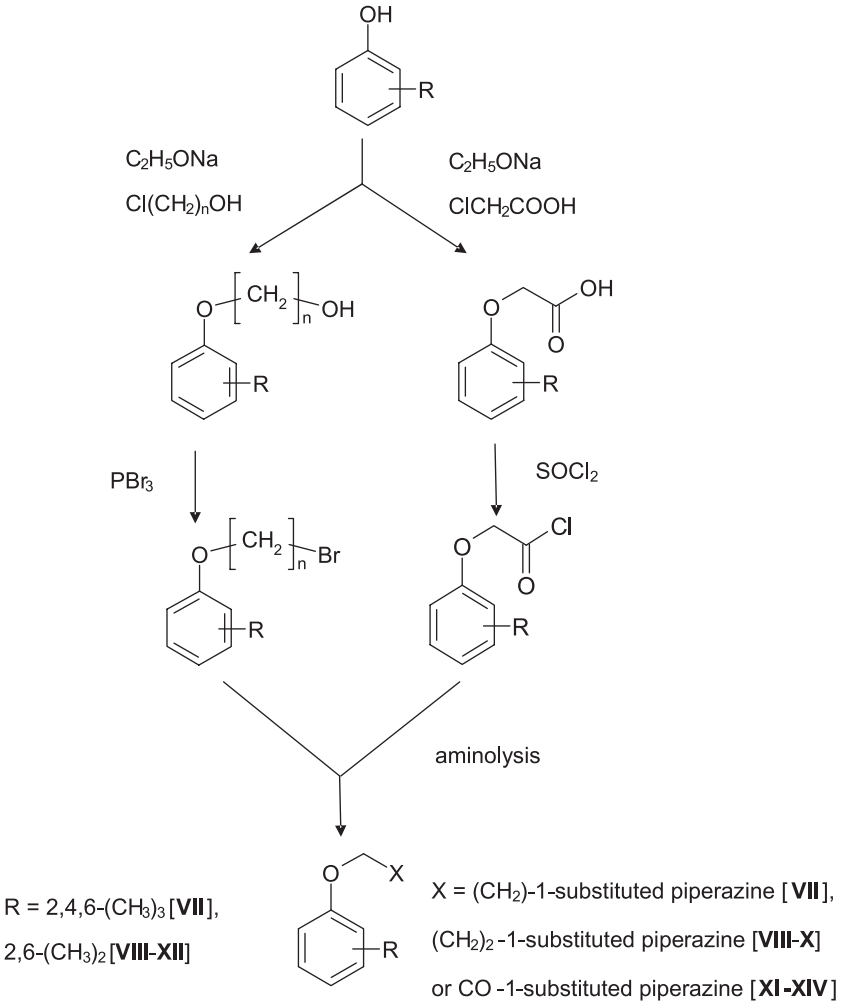
Compd.	Dose	MES[a]		ScMet[a]		Neurotoxicity[b]	
	mg/kg	0.5h	4h	0.5h	4h	0.5h	4h
I	30	0/1	0/	0/1	0/1	0/4	0/2
	100	3/3	0/3	0/1	0/1	6/8	1/4
	300	1/1	1-	0/1	0/1	4/4	1/1
II	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	2/5	0/1	0/4	0/2
III	30	0/4	-	0/1	0/1	0/4	0/2
	100	0/4	-	-	-	8/8	-
	300	1/1	0/1	-	-	4/4	-
IV	30	0/1	0/1	0/1	0/1	2/4	0/2
	100	0/3	0/3	0/1	0/1	7/8	0/4
	300	-	-	-	0/1	4/4	1/1
V	30	0/1	0/1	2/5	0/1	1/4	0/2
	100	0/3	0/3	0/1	3/5	7/8	1/4
	300	-	0/1	0/1	0/1	4/4	2/2
VI	30	1/1	0/1	0/1	0/1	1/4	0/2
	100	3/3	-	0/1	0/1	8/8	1/1
	300	-	-	-	-	4/4	-
VII	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	1/1	0/1	0/1	1/4	0/2
VIII	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	0/3	0/1	0/1	8/8	0/4
	300	-	-	-	-	4/4	-
IX	30	0/1	0/1	1/1	0/1	3/4	0/2
	100	0/3	0/3	1/1	0/1	8/8	1/4
	300	1/1	0/1	0/1	0/1	4/4	2/2
X	30	1/1	0/1	0/1	0/1	0/4	0/2
	100	3/3	3/3	0/1	0/1	7/8	2/4
	300	0/1	-	-	-	3/4	-
XI	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	1/8	0/4
	300	1/1	0/1	1/1	0/1	4/4	0/2
XII	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	2/5	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
XIII	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	0/1	0/1	0/1	0/1	0/4	0/2
XIV	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	3/8	0/4
	300	0/1	0/1	0/1	0/1	3/4	0/2

[a]Number of animals protected/number of animals tested in the MES and ScMet tests; [b]Number of animals displaying motor impairment/number of animals used in the rotarod test; - the compound was not tested in the particular case.

Table 3. Anticonvulsant evaluation, psychomotor seizure test (6-Hz) after *i.p.* injection into mice.

Compound	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
XIII	4	4	3	0	0

[a]Dose of 100 mg/kg was administrated. The data indicate the number of mice of four that were protected.



Scheme 1. Synthesis of compounds **VII-XIV**

cient (Log $P_{comb.}$), Log D and pKa of the tested compounds were calculated with PALLAS 3.1 program. Reagents and solvents were commercially available materials of reagent grade.

The synthesis and physicochemical properties of compounds **I-VI** were previously described (11).

Physicochemical properties of compounds VII-XIV

1-[2-(2,4,6-Trimethylphenoxy)-ethyl]-4-(2-fluorophenyl)-piperazine hydrochloride [VII]

$C_{21}H_{27}N_2OF \times HCl$ (378.90), yield: 22%, m. p. 207-209°C. C_{calc}/C_{found} 66.56/66.52; N_{calc}/N_{found}

7.39/7.29; H_{calc}/H_{found} 7.45/7.42. IR (KBr, cm^{-1}): 3436, 2950, 2418, 1502, 1449, 1210. ^1H NMR: (300 MHz, DMSO-d_6, δ ppm): 2.17 (s, 3H, Ar-CH_3); 2.21 (s, 6H, Ar-$(CH_3)_2$); 3.22-3.72 (m, 10H, -CH_2-pip.); 4.16 (t, *J* = 4.6 Hz, 2H, O-$\underline{CH_2}$); 6.83 (s, 2H, Ar-H); 6.99-7.21 (m, 4H, Ar-H); 11.53 (bs, 1H, NH^+). ^{19}F NMR: (300 MHz, DMSO-d_6, δ ppm): -118.16. pKa: 8.02. Log P_{comb}. 4.53. Log D (pH): 1.59 (5.0), 3.48 (7.0), 3.82 (7.40). R_f = 0.88, methanol : ethyl acetate (2:1, v/v), R_f = 0.83, methanol : ethyl acetate : toluene (0.5:1:2, v/v/v).

1-[3-(2,6-Dimethylphenoxy)-propyl]-4-(2-hydroxyethyl)-piperazine dihydrochloride [VIII]

$C_{17}H_{28}N_2O_2 \times 2$ HCl (365.47), m. p. 248-250°C (base m. p. 40-42°C). C_{calc}/C_{found} 55.89/55.96; N_{calc}/N_{found} 7.67/7.55; H_{calc}/H_{found} 8.28/8.41. IR (KBr, cm^{-1}): 3345, 2914, 2364, 1464, 1201, 1089. ^1H NMR (base): (300 MHz, $CDCl_3$, δ ppm): 1.93-1.99 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2); 2.27 (s, 6H, Ar-$(CH_3)_2$); 2.48-2.61 (m, 12H, =N-CH_2); 2.95 (bs, 1H, -OH); 3.62 (t, *J* = 5.4 Hz, 2H, HO-$\underline{CH_2}$-); 3.80 (t, *J* = 6.4 Hz, 2H, Ar-O-CH_2-); 6.90 (t, *J* = 7.3 Hz, 1H, 4H-Ar), 7.00 (t, *J* = 7.3 Hz, 2H, 3,5H-Ar). pKa: 15.03. Log P_{comb} 1.83. Log D (pH): -0.87 (5.0), 0.9 (7.0), 1.23 (7.40). R_f = 0.31, methanol : ethyl acetate (1:1, v/v).

1-[3-(2,6-Dimethylphenoxy)-propyl]-4-(2-fluorophenyl)-piperazine hydrochloride [IX]

$C_{21}H_{27}N_2OF \times$ HCl (378.97), yield: 24%, m. p. 200-202°C. C_{calc}/C_{found} 66.56/66.31; N_{calc}/N_{found} 7.39/7.52; H_{calc}/H_{found} 7.45/7.54. IR (KBr, cm^{-1}): 3437, 2925, 2446, 2848, 1503, 1445, 1261. ^1H NMR: (300 MHz, DMSO-d_6, δ ppm): 2.21 (s, 6H, Ar-$(CH_3)_2$); 3.17-3.62 (m, 10H, -CH_2-piper.); 3.79 (t, *J* = 5.9 Hz, 2H, O-CH_2); 6.88-7.21 (m, 7H, Ar-H); 11.43 (b s, 1H, NH^+). ^{19}F NMR: (300 MHz, DMSO-d_6, δ ppm): -118.14. pKa: 8.71. Log P_{comb} 4.15. Log D (pH): 0.75 (5.0), 2.44 (7.0), 2.82 (7.40). R_f = 0.76, methanol : ethyl acetate (1:1, v/v).

1-[3-(2,6-Dimethylphenoxy)-propyl]-4-benzylpiperazine dihydrochloride [X]

$C_{22}H_{30}N_2O \times 2$ HCl (411.48), m. p. 276-278 °C. C_{calc}/C_{found} 64.22/63.85; N_{calc}/N_{found} 6.81/6.75; H_{calc}/H_{found} 7.84/7.57. IR (KBr, cm^{-1}): 2986, 2910, 2318, 1455, 1374, 1198, 956, 781, 754. ^1H NMR (base): (300 MHz, $CDCl_3$); δ [ppm]: 1.98-2.07 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); 2.31 (s, 6H, Ar-$(CH_3)_2$); 2.54-2.65 (m, 10H, O-$(CH_2)_3$N + 4H, piper.); 3.55 (s, 2H, N-CH_2-Ar); 3.84 (t, *J* = 6.2 Hz, 2H, Ar-O-$\underline{CH_2}$); 6.91-7.38 (m, 8H, Ar-H). pKa: 7.88. Log P_{comb} 3.92. Log D (pH): 1.10 (5.0), 2.98 (7.0), 3.31 (7.40). R_f = 0.79 (methanol : ethyl acetate (1:1, v/v).

1-[(2,6-Dimethylphenoxy)-acetyl]-4-(2-pyrimidyl)-piperazine [XI]

$C_{18}H_{22}N_4O_2$ (326.44), yield: 57%, m. p. 128-130 °C. C_{calc}/C_{found} 66.18/65.80; N_{calc}/N_{found} 17.24/17.34; H_{calc}/H_{found} 6.79/7.19. IR (KBr cm^{-1}) 2907, 2855, 1640, 1592. ^1H NMR: (500.13 Hz, DMSO-d_6, δ ppm): 2.24 (d, *J* = 0.6 Hz, 6H, Ar-CH_3); 3.52-3.57 (m, 2H, CH_2 (piper. e); 3.57-3.62 (m, 2H, CH_2 (piper. e)); 3.74-3.82 (m, 4H, CH_2, piper. a); 4.55 (s, 2H, Ar-O-CH_2); 6.94 (d, *J* = 4.7 Hz, 1H, 5-H-pyrim.); 6,94 (d,d,d, *J* = 7.5, *J* = 1.2, *J* = 0.3 Hz, 1H, 4H-Ar); 7.03 (d,d,d,d, *J* = 7.5, *J* = 1.2, *J* = 0.6, *J* = 0.6 Hz, 2H, 3,5-H-Ar); 8.39 (d, *J* = 4.7 Hz, 2H, 4,6-H-pyrim.). pKa: 0.94. Log P_{comb} 1.62. Log D (pH): 1.62 (5.0), 1.62 (7.0), 1.62 (7.40). R_f = 0.59 (toluene : acetone (1:1, v/v).

1-[(2,6-Dimethylphenoxy)-acetyl]-4-(2-methoxyphenyl)-piperazine [XII]

$C_{21}H_{26}N_2O_3$ (354.49), m. p. 102-104 °C. C_{calc}/C_{found} 71.16/70.80; N_{calc}/N_{found} 7.90/8.00; H_{calc}/H_{found} 7.39/7.25. IR (KBr, cm^{-1}): 2970, 2837, 1453, 1280. ^1H NMR: (300 MHz, DMSO-d_6, δ ppm): 2.24 (s, 6H, Ar-$(CH_3)_2$); 2.85-3.15 (m, 4H, N-$(CH_2)_2$); 3.56-3.72 (m, 4H, N-$(CH_2)_2$); 3.87 (s, 3H, O-CH_3); 4.53 (s, 2H, O-CH_2); 6.82-7.15 (m, 7H, Ar-H). pKa: 3.29. Log P_{comb} 3.13. Log D (pH): 3.12 (5.0), 3.13 (7.0), 3.13 (7.40). R_f = 0.60 (toluene : methanol (5:1, v/v).

1-[(4-Chlor-3-methylphenoxy)-acetyl]-4-(2-methoxyphenyl)-piperazine [XIII]

$C_{20}H_{23}N_2O_3Cl$ (374.90), yield: 65%, m. p. 132-134°C. C_{calc}/C_{found} 64.07/63.97; N_{calc}/N_{found} 7.47/7.42; H_{calc}/H_{found} 6.18/6.23. IR (KBr, cm^{-1}): 3446, 1666, 1440, 1238, 1029. ^1H NMR: (500.13 MHz, DMSO-d_6, δ ppm): 2.29 (s, 3H, Ar-CH_3); 2.85-3.05 (m, 4H, piper. e); 3.54-3.68 (m, 4H, piper. a); 3.79 (s, 3H, O-CH_3); 4.86 (s, 2H, Ar-O-CH_2); 6.80 (d,d, *J* = 3.2, *J* = 8.8 Hz, 1H, H-6); 6.88 (d, 1H, Ar-H); 6.89 (d, 1H, 2H-Ar); 6.94-7.01 (m, 3H, Ar-H); 7.29 (d, 1H, 5H-Ar). pKa: 3.29. Log P_{comb} 4.28. Log D (pH): 4.27 (5.0), 4.28 (7.0), 4.28 (7.40). R_f = 0.50, toluene : methanol (5:1, v/v).

1-[(4-Chlor-3-methylphenoxy)-acetyl]-4-(2-pyridyl)-piperazine [XIV]

$C_{18}H_{20}N_3O_2Cl$ (345.86), yield: 63%, m. p. 102-104°C. C_{calc}/C_{found} 62.52/61.97; N_{calc}/N_{found} 12.15/12.46; H_{calc}/H_{found} 5.83/5.61. IR (KBr, cm^{-1}): 2859, 1680, 1478, 1437. ^1H NMR: (500.13 MHz, DMSO-d_6, δ ppm): 2.29 (s, 2H, CH_3Ar); 3.47-3.62 (m, 8H, piper.); 4.87 (s, 2H, Ar-OCH_2); 6.67 (d,d, *J* = 4.9, *J* = 6.8 Hz, 1H, pyrid.); 6.8 (d,d, *J* = 8.8, *J* = 3.1 Hz, 1H, 6H-Ar); 6.86 (d, *J* = 8.7 Hz, 1H, pyrid.); 6.97 (d,

J = 3.1 Hz, 1H, 2H-Ar); 7.29 (d, *J* = 8.7 Hz, 1H, 5H-Ar); 7.56 (d,d,d, *J* = 8.7, *J* = 6.8, *J* = 1.9 Hz, 1H, pyrid.); 8.13 (d,d,d, *J* = 0.7, *J* = 1.9, *J* = 4.9 Hz, 1H, pyrid.). pKa: 4.90. Log P_{comb} 3.55. Log D (pH): 3.29 (5.0), 3.54 (7.0), 3.54 (7.40). R_f = 0.40, toluene : methanol (5:1, v/v).

General procedure for synthesis of compounds VII-XIV

Compounds **VII – X** were synthesized in reaction between 2-(2,4,6-trimethylphenoxy)-ethyl- [**VII**] or 3-(2,6-dimethylphenoxy)-propyl-bromide [**VIII**-**X**] and appropriate 1-substituted piperazine. The reactions were carried out in toluene in the presence of anhydrous K_2CO_3. The bromides used in the reactions were obtained according to earlier published procedures (Scheme 1). Respective bases were converted into hydrochlorides using an excess of ethanol saturated with HCl (9, 11-13).

Compounds **XI – XIV** were obtained through N-acylation of corresponding amines which were 1-(2-pyrimidin)- [**XI**], 1-(2-pyridin)- [**XIV**] or 1-(2-methoxyphenyl)-piperazines. [**XII**, **XIII**] using (2,6-dimethylphenoxy)- [**XI**, **XII**] or (4-chlor-3-methylphenoxy)-acetylchlorides [**XII**, **XIV**] in toluene in the presence of anhydrous K_2CO_3. Appropriate phenoxyacetyl chlorides were obtained according to earlier published procedures (Scheme 1) (6).

Pharmacology

Initial evaluations for anticonvulsant activity were performed within the ADD program Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Rockville, MD 20852, USA. The program performed initial evaluations for anticonvulsant activity and included Phase I tests procedures. The screens were performed in male Carworth Farms no. 1 (CF 1) mice (18-25 g). In the phase I studies which deal with qualitative assay, all the compounds were tested for activity in the MES and ScMet tests as well as in the rotarod screen for TOX. The examined compounds were dissolved or suspended in 0.5% aq. methylcellulose and then administered at three dosage levels (30, 100 and 300 mg/kg) with anticonvulsant activity observed 0.5 and 4 h after *i.p.* administration in mice. The details of these procedures were published earlier (15). The results of phase I test are listed in Table 2. The 6-Hz model test was carried out according to the protocol originally described by Brown et al. (16) and more recently by Barton at al. (17) and Kaminski et al. (18). Corneal stimulation (0.2 ms duration monopolar rectangular pulses at 6 Hz for 3 s) was delivered by a constant-current device. During the stimulation, mice were manually restrained and released into the observation cage immediately after the current application. The seizures were often preceded by a brief period of intense locomotor agitation. The animals then exhibited a "stunned" posture associated with rearing, forelimb automatic movements and clonus, twitching of the vibrissae, and Straub-tail. The duration of the seizure activity ranged from 60 to 120 s in untreated animals. At the end of seizure, the animals resumed their normal exploratory behavior. The experimental end point was protection against the seizure. The animal was considered to be protected if it resumed its normal exploratory behavior within 10 s from the stimulation (18).

RESULTS AND DISCUSSION

The MES and ScMet tests have become the most widely employed seizure models for early identification of candidate anticonvulsants. The protective activity in the MES test in mice after *i.p.* administration was found for compounds [**I**], [**III**], [**VI**], [**VIII**], [**IX**], [**X**] and [**XI**]. The most potent activity in MES screen in mice showed compounds **VI**, **VIII** and **X**. Compounds **VI** and **X,** at the dose 30 mg/kg, demonstrated anticonvulsant protection without neurotoxic effects at 0.5 h for **X**, low neurotoxicity at 0.5 h for **VI** and with no neurotoxic effects at 4 h for **VI** and **X**. At the dose 100 mg/kg, these compounds were more active but neurotoxic. Compound **VIII** demonstrated anticonvulsant protection at the dose 100 mg/kg with neurotoxicity at 0.5 h and with no neurotoxic effects at 4 h. Compound **I** was active at the dose 100 mg/kg with low neurotoxic effects. Compounds **III** and **IX** were active at the dose 300 mg/kg but also neurotoxic. Compound **XI** was active at the dose 300 mg/kg with neurotoxicity at 0.5 h and with no neurotoxic effects at 4 h (Table 2).

In the ScMet test on mice protective activity was seen for compounds [**II**], [**V**]**,** [**IX**] and [**XII**]. Compound **XII** was active at the dose of 100 mg/kg and compound **II** at the dose of 300 mg/kg, in which they protected 2/5 of animals without toxicity at the doses studied. Compound **V** was active at the dose of 30 mg/kg at 0.5 h (2/5 of protected animals) with low neurotoxicity and at the dose of 100 mg/kg at 4 h (3/5 protected animals) but also was neurotoxic. Compound **IX** protected 1/1 of animals at the doses of 30 and 100 mg/kg but was neurotoxic (Table 2). 1-[(4-Chlor-3-methylphenoxy)-acetyl]-4-(2-methoxyphenyl)-piperazine [**XIII**] was also evaluat-

ed in 6-Hz test. It is alternative electroshock paradigm that uses low-frequency (6-Hz), long duration (3 s) electrical stimulation. The 6-Hz screen has been validated as a model of therapy-resistant epilepsy, recently. It was not used widely because of its lack of clinical validity since the hydantoins such as phenytoin failed to show protective activity. Nevertheless, the clinically effective antiepileptic drug levetiracetam, which is not active in the conventional MES and scMet tests, does exhibit protective activity in the 6-Hz model. This suggested that the 6-Hz model might be capable for identifying antiseizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action (18). At the dose of 100 mg/kg the compound produced 100% protection after 0.5 h without neurotoxic effect (Table 3).

From the results obtained, there is no possibility to find a clear structure/activity relationship. The values of calculated partition coefficient do not correspond with the activity of the tested compounds. Anyway, it is worth to mention that presented procedures of synthesis and evaluation of new compounds can lead to finding of some active structures. Research of that kind among compounds with potentially anticonvulsant activity is reasonable as, what should be emphasized, antiepileptic drugs belong to different chemical groups and have different mechanisms of action.

In conclusion, the obtained results revealed that a number of novel 1,4-piperazine derivatives were moderately effective, especially in the MES test.

Acknowledgments

The authors would like to anknowledge Professor P. Stables for providing the results of pharmacological assays through the ADD program at the National Institute of Neurological Disorders and Stroke (NINDS, Rockville, MD 20852, USA).

REFERENCES

1. Löscher W., Schmidt D.: Epilepsia 47, 1 (2006).
2. Bialer M., Johannessen S.I., Kupferberg H.J, Levy R.H., Perucca E, Tomson T.: Epilepsy Res. 73, 1 (2007).
3. Aldenkamp A.P.: Seizure 15, 219 (2006).
4. Vingerhoets G.: Seizure 15, 221 (2006).
5. Sander J. W.: Curr. Opin. Neurol. 16, 165 (2003).
6. Marona H., Waszkielewicz A.M., Szneler E.: Acta Pol. Pharm Drug Res. 62, 345 (2005).
7. Stępień K., Tomaszewski M., Czuczuwar S.J: Pharmacol. Rep. 57, 719 (2005).
8. Jastrzębska-Więsek M., Czarnecki R., Marona H.: Acta Pol. Pharm Drug Res. 65, 591 (2008).
9. Marona H., Korona R., Szneler E.: Boll. Chim. Farmac. 143, 329 (2004).
10. Maciąg D., Filipek B., Czekaj T., Marona H., Nowak G.: Pharmazie 58, 899 (2003).
11. Maciąg D., Bednarski M., Filipek B., Pociecha T., Szkaradek N., Marona H.: Pharmazie 63, 480 (2008).
12. Fischer W.: Seizure 11, 285 (2002).
13. Marona H., Antkiewicz-Michaluk L.: Acta Pol. Pharm. Drug Res. 55, 487 (1998).
14. Swain A.P., Naegele S.K.: J. Am. Chem. Soc. 76, 509 (1954).
15. Stables J.P., Kupferberg H.J.: The NIH anticonvulsant drug development (ADD) program: preclinical anticonvulsant screening project. in Molecular and Cellular Targets for Antiepileptic Drugs. Avanzini G., Tanganelli P., Avoli M. Eds., John Libbey, London 1997.
16. Brown W.C., Schiffman D.O., Swinyard E.A., Goodman L.S.: J. Pharmacol. Exp. Ther. 107, 273 (1953).
17. Barton M.E., Klein B.D., Wolf H.H., White H.S.: Epilepsy Res. 47, 217 (2001).
18. Kaminski R.F., Livingood M.R., Rogawski M.A.: Epilepsia 45, 864 (2004).

Received: 19. 02. 2009