Department of Pharmacobiology[1], Laboratory of Pharmacological Screening[2], Department of Chemical Technology of Drugs[3], Faculty of Pharmacy, Medical College, Jagiellonian University and Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences[4], Kraków, Poland

Evaluation of some aroxyethylamine derivatives for hypotensive properties and their affinities for adrenergic receptors

D. Maciąg[1], B. Filipek[2], T. Czekaj[3], H. Marona[3], G. Nowak[1, 4]

Received April 22, 2003, accepted July 25, 2003

Dr. Dorota Maciag, Department of Pharmacobiology, Collegium Medicum, Jagiellonian University, Medyczna 9, PL-30688 Kraków, Poland
dorota.m.farmbiol@interia.pl

Pharmazie 58: 899–906 (2003)

A series of aroxyethylamines (**1**–**10**) was synthesized and evaluated for hypotensive activity in rats after intravenous and oral administration. The 4 compounds (**4**, **7**, **8** and **10**) containing a (2-methoxy)-phenylpiperazine moiety displayed hypotensive activity and their affinities for α_1-, α_2- and β_1-adrenoreceptors were determined by radioligand binding assays. Compounds **4**, **7**, **8** and **10** were also tested for their effect on the pressor responses to epinephrine, norepinephrine, methoxamine, tyramine and DMPP. The results suggest that the hypotensive effect of these compounds is related to their α- and β-adrenolytic properties.

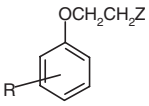
1. Introduction

Sympathetic nervous system activation plays an important role in the genesis of hypertension, coronary heart disease, cardiac arrhythmias and heart failure [1–3]. From here beta-adrenergic receptor blockers have been widely accepted in the treatment of primary hypertension, heart failure and cardiac arrhythmias. Long-term beta-blocker therapy reduced the morbidity and mortality in patients with primary hypertension and heart failure [3–5].

While the precise mechanism of the beneficial clinical and hemodynamic actions of β-adrenoceptor blockers remains unclear, several possibilities have been proposed, including heart rate reduction, modulation of systemic neurohormonal activity, antagonism of the toxic actions of catecholamines, and favourable effects on myocardial energetics [6].

In the last decade, a new generation of beta-blockers with vasodilating properties was introduced to therapy [7–11]. The nonselective third generation beta-blockers with additional α-adrenoceptor blocking activity (bucindolol, carvedilol, celiprolol), have a beneficial effect on the regional circulation in contrast to classical beta-blocker such as propranolol. It is known that most classic beta-blockers contain an 1-aryl-2-alkylaminoethanol or 1-aroxy-3-alkylamino-2-propanol group in their structure (sotalol, atenolol, metoprolol, acebutolol). Among these drugs are propranolol and its two enantiomers, for which a high anticonvulsant activity has been described [12]. On the other hand numerous investigators described phenylpiperazine derivatives with α-adrenergic blocking and hypotensive effects (urapidil, 5-methylurapidil, naftopidil), [13, 14]. It is very likely that the adrenolytic properties depend on the presence of the 1-(*o*-methoxyphenyl)-piperazine fragment in their molecule. Taking into account these facts and our own experience in the search for new compounds affecting circulation [15], we synthesized a series of aroxyethyl-

Table 1: Newly synthesized aroxyethylamines 1–10



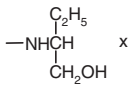
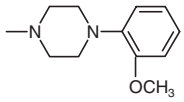
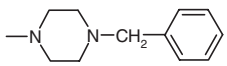
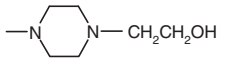
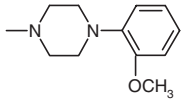
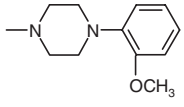
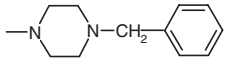
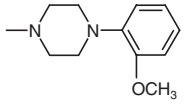
Compound	R	Z
1–3	2,6-CH_3	 x HCl (R, S), (S) and (R)
4	2,6-CH_3	 x 2 HCl
5	2,6-CH_3	 x 2 HCl
6	2,6-CH_3	 x 2 HCl
7	4-CH_3	 x 2 HCl
8	4-OCH_3	 x 2 HCl
9	4-OCH_3	 x 2 HCl
10	3-CH_3, 4-Cl	 x 2 HCl

Table 2: Hypotensive activity of tested compounds in anaesthetized normotensive rats after intravenous administration

Compd.	Dose (mg/kg)	Blood pressure (mmHg)	Time after administration (min)								
			0	1	5	10	20	30	40	50	60
4	6.5 (1/10 LD_{50})	Systolic	155 ± 6.5	102.5 ± 5.9**	117.0 ± 8.6**	113.8 ± 8.3**	112.0 ± 7.3**	110.0 ± 9.2**	113.8 ± 7.1**	111.6 ± 8.8**	112.2 ± 9.6**
		Diastolic	135.0 ± 8.1	80.0 ± 8.5**	99.3 ± 9.9*	97.7 ± 9.6*	98.0 8.1*	95.8 ± 9.6**	100.6 ± 7.3*	98.4 ± 8.8*	98.0 ± 9.6*
7	4.5 (1/10 LD_{50})	Systolic	149.6 ± 5.8	77.1 ± 1.9**	109.0 ± 5.2**	94.7 ± 1.7**	95.3 ± 4.3**	91.3 ± 1.5**	99.7 ± 6.6**	87.3 ±14.0**	94.7 ±15.0**
		Diastolic	129.0 ± 6.1	60.3 ± 3.9**	90.7 ± 4.7**	79.3 ± 5.2**	79.0 ± 1.0*	74.0 ± 2.1**	83.3 ± 3.7**	77.0 ±10.1**	77.0 ±14.5*
8	4.6 (1/10 LD_{50})	Systolic	141.5 ± 5.5	93.5 ± 5.3**	103.0 ± 5.7**	99.3 ± 4.9**	102.2 ± 5.3**	106.0 ± 4.9**	108.3 ± 4.9**	109.7 ± 4.5**	112.8 ± 4.7**
		Diastolic	121.5 ± 4.5	77.9 ± 5.8**	85.5 ± 5.8**	82.3 ± 4.6**	84.7 ± 5.1**	86.2 ± 4.5	84.2 ± 4.8**	87.3 ± 4.0**	88.2 ± 4.0**
	2.3 (1/20 LD_{50})	Systolic	156.0 ± 6.3	106.6 ± 3.8**	112.8 ± 4.7**	114.4 ± 5.2**	117.4 ± 5.0**	123.4 ± 2.7**	128.0 ± 3.5**	131.3 ± 1.4**	132.3 ± 1.4**
		Diastolic	129.0 ± 3.5	85.0 ± 4.8**	96.2 ± 2.8**	95.2 ± 4.1**	92.8 ± 4.2**	96.0 ± 3.4**	98.3 ± 2.1**	99.3 ± 4.6**	102.8 ± 1.2**

All values represent the mean from 5–6 experiments ± SEM, *$p < 0.01$, **$p < 0.001$ (ANOVA test)

amines (**1**–**10**), (Table 1) and subjected them to pharmacological screening. Among these compounds there are derivatives which contain some structural elements known from other circulatory agents e.g. the aminoalkanol (**1**–**3**) or piperazine moiety (**4**–**10**). The synthesis and properties of the examined aroxyethylaminoalkanols **1**–**3** were described earlier [16]. In a previous study we reported the anticonvulsant properties of some aminoalkanol derivatives. One of them i.e. *S*-(+)-2-*N*-[(2,6-dimethyl)-phenoxyethyl]-amino-1-butanol (**2**) potently prevents maximal electroshock (MES) seizures in mice, with an ED_{50} of 7.57 mg/kg and TD_{50} (neurotoxicity) of 34.45 mg/kg. The protective index (PI = 4.55) in the MES test in mice was higher than that of valproate (PI = 1.7), and similar to that for carbamazepine (PI = 4.9), [16, 17]. These compounds (**1**–**3**) have some structural moieties of propranolol (aminoalkanolic group), which displayed anti-MES activity in rodents [12]. This was the reason why compounds **1**–**3** were tested for their effects on the circulatory system.

The presented results deal with preliminary pharmacological studies on the expected hypotensive activity and affinity to α- and β-adrenergic receptors of some aroxethylamine derivatives (**1**–**10**), having the chiral moiety of 2-amino-1-butanol (**1**–**3**) and 4-substituted piperazine (**4**–**10**).

2. Investigations and results

2.1. Chemistry

The earlier obtained 2-*N*-[(2,6-dimethyl)-phenoxyethyl]-amino-1-butanols **1**–**3** were prepared by amination of [(2,6-dimethyl)-phenoxyethyl]-4-toluenosulfonate in 2-methoxyethanol in the presence of potassium carbonate [16]. The same method was used for the synthesis of compounds **4**–**6** (yield 57–67%). The synthesis of **7**–**10** was carried out from appropriate phenoxyethyl bromide with N-subtituted piperazine, respectively, in toluene, in the presence of potassium carbonate (yield 56–69%). Appropriate (4-methyl)-, (4-methoxy)- or [(3-methyl-4-chloro)-phenoxyethyl]-bromides were obtained according to well-known procedures [16, 18]. Compounds **1**–**10** were isolated and characterized as hydrochlorides. The IR, ^{1}H NMR, ^{13}C NMR or MS spectra of the synthesized compounds **4**–**10** were studied. Substances **4**–**10** were analysed by TLC. Finally for compounds **4**–**10** the pKa, logP (partition coefficient) and log D (distribution coefficient) values were calculated using the Pallas program.

2.2. Pharmacology

2.2.1. Influence on the blood pressure

All of the compounds listed in Table 1 were tested for hypotensive activity in normotensive Wistar rats after intravenous and oral administration (1/10–1/40 LD_{50}). The effects of the studied compounds on blood pressure in anaesthetized rats were compared with the effect of carvedilol (1/27 LD_{50} i.v. − 1 mg/kg). The baseline systolic and diastolic blood pressure before and after treatment are summarized in Tables 2, 3 and in Figs. 1 and 2. As shown in Fig. 1 and Table 2, intravenous injections of compounds **4**, **7**, **8** at doses of 1/10–1/20 LD_{50} significantly reduced the systolic (48–15%) and diastolic (53–18%) blood pressure in normotensive rats and this hypotensive effect lasted for all time of observation. Compound **8**, given at a dose of 1/40 LD_{50} decreased both systolic (28–11%) and diastolic (31–13%) blood pressure, which persisted for 50 min ($p < 0.05$), and a little weaker than reference compound − carvedilol (Fig. 1). Compound **7** given at the lowest dose (1/40 LD_{50}) initially also reduced systolic and diastolic pressure, but the pressure returned to baseline within 1–30 min after administration. Also compound **10** at a dose of 1/10 LD_{50} decreased the blood pressure but the effect lasted 10–20 min (Fig. 1). The others compounds at doses up 1/10 LD_{50} had no effect in this test (Table 3).

Compounds with a significant hypotensive effect after i.v. administration were also tested for hypotensive activity after oral administration. Compounds **4**, **7**, **8** and **10** given at doses corresponding to 1/10–1/40 LD_{50} p.o. significantly reduced the systolic and diastolic blood pressure. Compound **8** at a dose of 1/40 LD_{50} p.o. significantly decreased the systolic pressure by 23–11% and diastolic pressure by 27–15%. Compound **7** induced a reduction of blood pressure at a dose of 1/20 LD_{50} (p.o.), but compounds **4** and **10** at a dose of 1/10 LD_{50} (p.o.), (Fig. 2, Table 4). The effect of reference drug was more potent than those of compounds **8**, **7**, **4** and **10** (Fig. 2, Table 4).

Table 3: Influence of tested compounds on blood pressure in anaesthetized normotensive rats after intravenous administration (1/10 LD_{50} i.v.)

Compd.	Dose (mg/kg)	Blood pressure (mm Hg)	Time of administration (min)								
			0	1	5	10	20	30	40	50	60
1	3.0	Systolic	142.3 ± 5.1	133.7 ± 8.0	141.2 ± 5.9	140.2 ± 5.1	139.8 ± 6.3	141.7 ± 6.1	140.6 ± 7.1	142.3 ± 8.2	143.1 ± 4.2
		Diastolic	120.3 ± 2.1	116.1 ± 2.3	118.9 ± 5.1	118.4 ± 6.7	118.7 ± 6.1	119.0 ± 6.9	119.6 ± 7.3	119.4 ± 7.1	118.9 ± 5.0
2	1.8	Systolic	147.0 ± 6.0	145.7 ± 8.0	150.7 ± 6.2	146.3 ± 2.2	145.7 ± 1.5	140.0 ± 2.6	134.7 ± 2.8	133.7 ± 1.3	133.0 ± 2.0
		Diastolic	129.3 ± 3.8	125.7 ± 5.2	136.0 ± 4.0	133.0 ± 3.0	130.7 ± 4.3	125.3 ± 6.0	118.0 ± 7.8	117.7 ± 7.0	117.3 ± 7.0
3	1.3	Systolic	140.3 ± 2.7	116.3 ± 2.6	126.7 ± 11.2	129.0 ± 5.8	131.7 ± 12.8	127.0 ± 9.3	130.0 ± 16.0	127.0 ± 14.0	128.0 ± 13.5
		Diastolic	118.0 ± 4.4	104.3 ± 4.9	112.3 ± 1.2	106.8 ± 5.5	110.0 ± 3.5	107.7 ± 4.6	107.0 ± 3.5	112.3 ± 5.4	113.3 ± 4.5
5	1.5	Systolic	118.0 ± 4.4	104.3 ± 4.9	112.3 ± 1.2	106.8 ± 5.5	110.0 ± 3.5	107.7 ± 4.6	107.0 ± 3.5	112.3 ± 5.4	111.3 ± 4.5
		Diastolic	96.3 ± 4.8	85.0 ± 8.5	94.0 ± 4.0	90.7 ± 6.0	91.0 ± 4.0	89.3 ± 6.2	90.0 ± 5.0	94.3 ± 2.9	93.7 ± 2.3
6	4.0	Systolic	138.3 ± 12.0	147.7 ± 7.3	139.0 ± 10.0	138.0 ± 12.7	138.3 ± 11.9	132.7 ± 13.3	132.7 ± 14.0	132.0 ± 9.1	123.7 ± 5.8
		Diastolic	117.3 ± 9.6	122.7 ± 5.2	119.0 ± 8.9	118.7 ± 10.1	117.0 ± 9.1	113.0 ± 11.0	110.3 ± 11.6	111.7 ± 8.4	105.0 ± 4.6
9	2.4	Systolic	157.4 ± 9.3	155.6 ± 10.6	156.6 ± 8.9	156.6 ± 7.9	154.4 ± 10.1	152.2 ± 10.7	150.8 ± 10.4	148.8 ± 10.3	146.2 ± 10.4
		Diastolic	135.0 ± 6.5	132.4 ± 6.9	133.2 ± 4.1	132.8 ± 4.0	130.4 ± 5.9	128.2 ± 6.1	126.4 ± 6.4	125.6 ± 7.0	123.2 ± 6.7

All values represent the mean from 5–6 experiments ± SEM

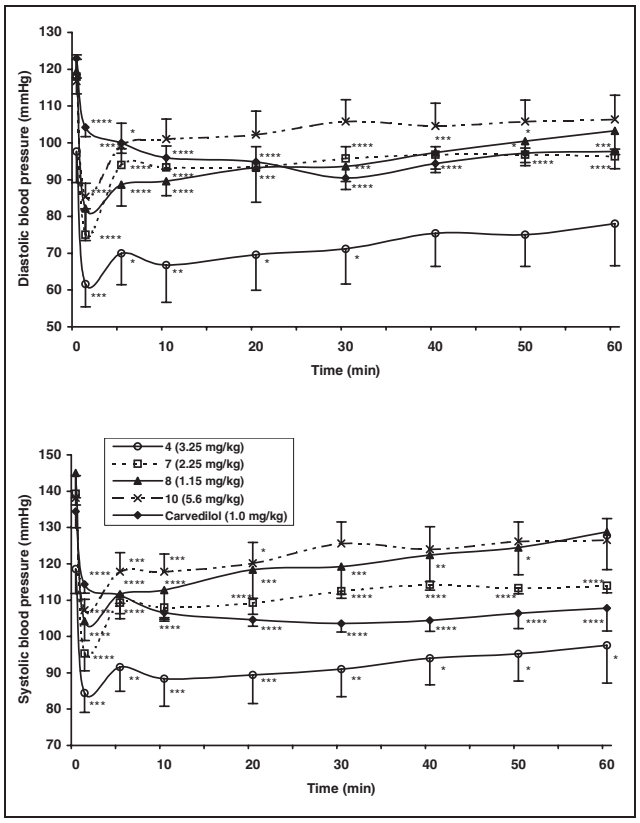


Fig. 1: Hypotensive activity of tested compounds (**4**, **7** – 1/20; **8** – 1/40; **10** – 1/10 LD_{50} i.v.) in anaesthetized normotensive rats after intravenous administration; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 (ANOVA test)

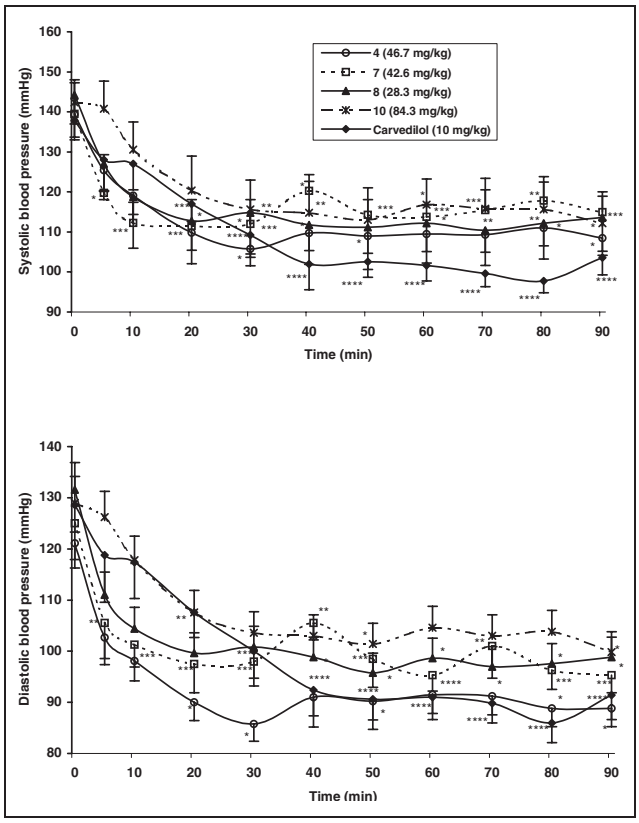


Fig. 2: Hypotensive activity of tested compounds (**4**, **10** – 1/10; **7** – 1/20; **8** – 1/40 LD_{50} p.o.) in anaesthetized normotensive rats after oral administration; *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001 (ANOVA test)

Table 4: Hypotensive activity of tested compounds in anaesthetized normotensive rats after oral administration

Compd.	Dose (mg/kg)	Blood pressure (mm Hg)	Time after administration (min) 0	5	10	20	30	40	50	60	70	80	90
7		Systolic	128.4	105.2	90.6	83.2	82.8	82.2	82.2	78.0	80.0	81.6	85.2
	85.2		± 3.4	± 4.7*	± 7.9**	± 7.2***	± 8.4***	± 8.5***	± 9.1***	± 9.2***	± 8.5***	± 7.3***	± 10.2***
	(1/10 LD_{50})	Diastolic	108.6	80.2	67.8	61.4	60.0	55.6	54.0	49.6	52.2	52.0	58.2
			± 6.0	± 8.1*	± 10.1**	± 8.8***	± 9.3***	± 8.9***	± 10.0***	± 9.9***	± 9.6***	± 7.4***	± 12.3***
8		Systolic	140.0	114.3	98.0	92.7	86.2	90.0	94.8	91.6	100.4	100.4	104.4
	113.1		± 4,7	± 6.5**	± 5.4***	± 9.4***	± 9.0***	± 8.0***	± 6.3***	± 7.5***	± 6.7***	± 5.2 ***	± 6.5***
	(1/10 LD_{50})	Diastolic	127.3	99.3	83.8	80.0	74.2	76.6	81.4	75.8	85.0	85.2	87.2
			± 5.5	± 6.9**	± 5.1***	± 8.5***	± 8.6***	± 7.9***	± 6.4***	± 8.1***	± 7.0***	± 7.0***	± 7.1***
		Systolic	143.6	118.4	110.2	87.8	82.2	83.0	89.6	93.0	96.2	97.0	101.0
	56.6		± 4.2	± 7.0**	± 7.8***	± 2.4***	± 3.0***	± 3.1***	± 3.4***	± 5.9***	± 5.8***	± 7.3***	± 8.8***
	(1/20 LD_{50})	Diastolic	130.6	104.6	98.0	71.6	68.2	71.6	78.2	81.0	83.0	83.0	86.2
			± 5.1	± 8.6**	± 9.3***	± 2.5***	± 3.1***	± 1.5***	± 3.1***	± 6.4***	± 6.5***	± 7.7***	± 9.2***

All values represent the mean from 5–6 experiments ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (ANOVA test)

Solvent (0.9% NaCl) given in a volume of 1 ml/kg, did not alter the baseline blood pressure (systolic and diastolic) after intravenous as well as oral administration.

2.2.2. Influence on the pressor responses to epinephrine, norepinephrine, methoxamine, tyramine and DMPP

Epinephrine (2 μg/kg, i.v.), norepinephrine (2 μg/kg, i.v.), methoxamine (150 μg/kg, i.v.), tyramine (200 μg/kg, i.v.) and dimethylphenylpiperazine (100 μg/kg, i.v.) caused a transient pressor response in anesthetized rats. Epinephrine (Epi), a non-selective α- and β-adrenoceptor agonist, increased the systolic pressure by about 34.0–50.3 mm Hg. Norepinephrine (NE), a non-selective α_1- and α_2-adrenoceptor agonist, increased the systolic pressure about 35.0–54.0 mm Hg, while methoxamine (Meth), a selective α_1-adrenoceptor agonist, increased the blood pressure about 46.3–60.5 mm Hg. Sympathomimetics such as tyramine (Tyr) and dimethylphenylpiperazine (DMPP), a nicotinic receptor agonist, increased the systolic pressure about 34.3–60.8 and 13.7–20.0 mm Hg, respectively (Figs. 3–6). Pretreatment of **4**, **7**, **8** and **10** with hypotensive activity caused the most potent inhibition of the pressor response to Epi, Meth, Tyr, DMPP or NE. Compound **4** and **7**, given at a dose of 1/20 LD_{50} i.v. strongly, statistically significantly inhibited the blood pressure increases elicited by Epi, Meth, Tyr and DMPP. These compounds only partially antagonized the pressor response to norepinephrine (Figs. 3, 4). While compound **8**, given at a lower dose (1/40 LD_{50} i.v.), caused the most potent inhibition of the pressor response to Epi, NE, Meth, Tyr and DMPP (Fig. 5). Compound **10**, administered intravenously at a dose of 1/10 LD_{50} (i.v.), significantly antagonized the pressor response to Epi and Tyr, but not to NE, Meth and DMPP (Fig. 6).

2.2.3. Radioligand binding assay

Compounds **4**, **7**, **8** and **10** inhibited [^{3}H]prazosin binding with K_i from 26.0 to 244.5 nM and [^{3}H]clonidine binding with K_i from 197.0 to 410.8 nM to cortical α_1- and α_2-

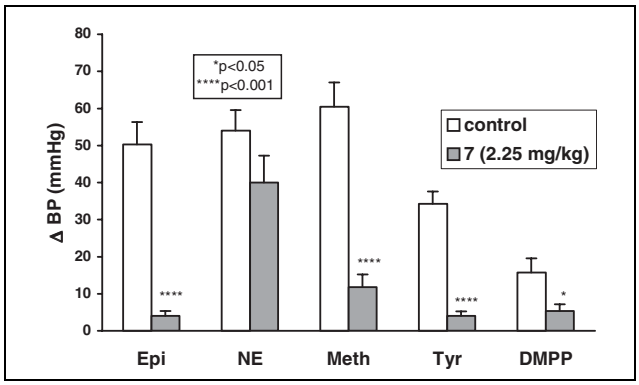


Fig. 4: Effect of **7** (1/20 LD_{50} i.v.) on the blood pressure response to epinephrine (Epi), norepinephrine (NE), methoxamine (Meth), tyramine (Tyr) and DMPP. All values represent the mean ± SEM from six rats

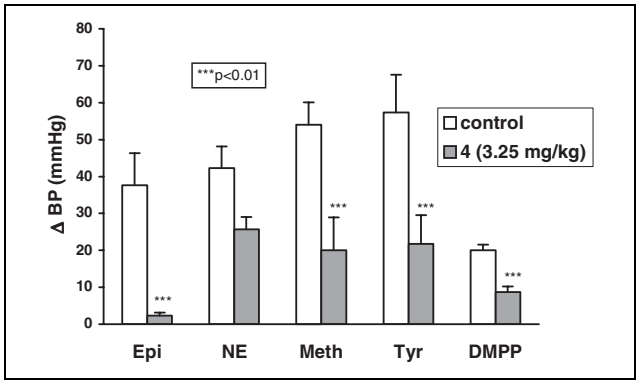


Fig. 3: Effect of **4** (1/20 LD_{50} i.v.) on the blood pressure response to epinephrine (Epi), norepinephrine (NE), methoxamine (Meth), tyramine (Tyr) and DMPP. All values represent the mean ± SEM from six rats

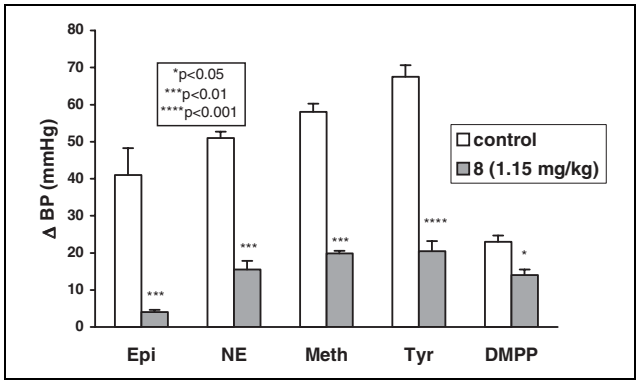


Fig. 5: Effect of **8** (1/40 LD_{50} i.v.) on the blood pressure response to epinephrine (Epi), norepinephrine (NE), methoxamine (Meth), tyramine (Tyr) and DMPP. All values represent the mean ± SEM from six rats

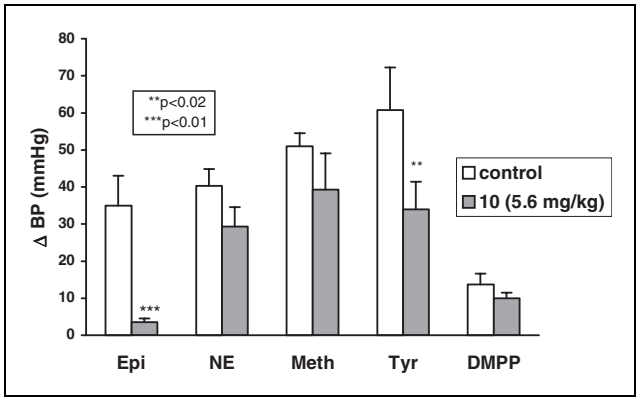


Fig. 6: The effect of **10** (1/10 LD_{50} i.v.) on the blood pressure response to epinephrine (Epi), norepinephrine (NE), methoxamine (Meth), tyramine (Tyr) and DMPP. All values represent the mean ± SEM from six rats

adrenoceptors, respectively. The affinities of tested compounds for α_1-adrenoceptors were ca 2–9-fold higher than those determined for α_2-adrenoceptors. These compounds also moderately inhibited [^{3}H]CGP-12177 binding to β_1-adrenoceptors with μM range ($K_i = 3.1–9.8$ μM). The results are summarized in Table 5.

2.2.4. Acute toxicity

The acute toxicity of the selected compounds (**4**, **7**, **8** and **10**) was determined in mice after intravenous and oral administration according to Litchfield and Wilcoxon [19]. The LD_{50} values are presented in Table 6.

3. Discussion

In the present study we evaluated the effect of 10 aroxyethylamines (**1**–**10**) on blood pressure in normotensive anaesthetized rats. For comparison we used classic nonselective β-blockers with additional α_1-adrenoceptor antagonistic activity and antioxidant properties such as carvedilol [20, 21].

The conducted preliminary studies showed that four of these compounds (**4**, **7**, **8** and **10**), which contain a (2-methoxy)-phenylpiperazine moiety, possess a significant hypotensive activity in normotensive rats, but the effect observed was weaker than that of the carvedilol. On the other hand the toxicity of the investigated compounds was about twice lower than that of the reference compound ($LD_{50} = 27$ mg/kg i.v.), [22].

To examine the mechanism of the hypotensive effect of these compounds, we studied their influence on the pressor responses to epinephrine, norepinephrine, methoxamine, tyramine and DMPP.

Compound **8** caused a significant inhibition of the vasopressor effect of epinephrine, norepinephrine, methoxamine, tyramine and DMPP, whereas compounds **4** and **7** significantly antagonized the vasopressor effect of epinephrine, methoxamine, tyramine and DMPP. Compound **10** had a significant inhibitory effect on the hypertensive response to epinephrine and tyramine.

It is generally accepted that α_1-antagonists reverse the pressor response to epinephrine, depress the pressor effect of methoxamine, tyramine and DMPP and only partially reverse the pressor response to norepinephrine, while α_2-antagonists antagonized the hypertensive effect of norepinephrine, reverse the hypertension induced by epinephrine, with no effect or enhance the pressor response to tyramine or DMPP [23–25].

These *in vivo* experiments with a non-selective agonist of α_1- and α_2-adrenoceptors such as epinephrine or norepinephrine and a selective agonist of α-adrenoceptors such as methoxamine suggested that the blockade of vascular α_1- and/or α_2-adrenoceptors is probably responsible for the hypotensive effect of these compounds. Thus, *in vitro* α_1-, α_2-, and β_1-adrenergic receptor binding affinities of the active compounds were assessed in rat brain membrane suspensions. These compounds displayed a high affinity for α_1- and α_2-adrenoceptors and a modest affinity for the β-adrenergic receptor. Our radioligand binding data demonstrated that all investigated compounds displayed ca 2–9-fold lower affinity for the α_2-adrenoceptor than for α_1-adrenoceptor. The affinity of these compounds for rat the α_1-adrenoceptor was about 12–111-times lower than that of carvedilol ($K_i = 2.2$ nM), and about 5–43-times lower than that of phentolamine ($K_i = 5.6$ nM), but affinity to β_1-adrenoceptor was much less than carvedilol ($K_i = 0.8$ nM), [26, 27].

Based on the above in vivo and in vitro experiments, we concluded that compounds **4**, **7**, **8** and **10** – contrary to carvedilol – are α_1- and α_2-blockers with additional β_1-adrenoceptor blocking activities. We found that the hypotensive effect of these compounds is related to their adrenolytic properties, and that those properties depend on the presence of the methoxyphenylpiperazine moiety.

Although the present series of experiments did not establish compounds more effective than carvedilol, they demonstrated that several new derivatives possess interesting biological activity deserving further investigation.

Table 5: K_i values for the inhibition of the binding of [^{3}H]prazosin, [^{3}H]clonidine and [^{3}H]CGP-12177 to α_1, α_2 and β_1-adrenoceptors

Compd.	K_i (nM) ± SEM		
	α_1-adrenoceptors	α_2-adrenoceptors	β_1-adrenoceptors
4	87.5 ± 19.0	252.3 ± 49.7	9800 ± 2700
7	84.6 ± 16.1	282.1 ± 39.0	3100 ± 800
8	26.0 ± 6.9	197.0 ± 40.8	8400 ± 1900
10	244.5 ± 20.0	410.8 ± 99.6	6500 ± 1800

K_i ± SEM values were derived from 2–3 experiments performed in duplicates

Table 6: Acute toxicity according to Litchfield and Wilcoxon in mice [19]

Compd.	LD_{50} (mg/kg) i.v.	LD_{50} (mg/kg) p.o.
4	65.0 (55.0–76.7)	467.0 (328.9–663.1)
7	45.0 (34.1–59.4)	852.0 (732.7–990.7)
8	45.5 (35.8–57.8)	1131.0 (1005.3–1272.4)
10	56.8 (44.3–59.3)	843.0 (733.0–969.5)

4. Experimental

4.1. Apparatus and reagents

M.p.'s are uncorrected and were determined using a Büchi SMP-20 apparatus. Analyses of C,H,N were within +/−0.4% of the theoretical values. The IR spectra were recorded on a Perkin Elmer spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer with 500.13 MHz and 125.17 MHz respectively, using signals from DMSO in DMSO-d_6 and TMS in $CDCl_3$ as internal standards. ^{1}H NMR spectra were recorded also on Gemini spectrometer at 200 MHz for solution in DMSO, with TMS as internal standard. MS at 70 eV were recorded on an AMD-604 spectrometer. Analytical TLC was carried out on precoated plates (silica gel, 60 F-254 Merck) using the solvent system and spots were visualized with UV light. Physical properties of

starting materials, viz., (2,6-dimethyl)-phenoxyethyl-4-toluenosulfonate and appropriate 1-bromo-2-aroxyethanes have been reported previously [16]. Other reagents and solvents were materials reagent-grade commercial.

4.2. Synthesized compounds

4.2.1. 1-[(2,6-Dimethyl)-phenoxyethyl]-4-[(2-methoxy)-phenyl)-piperazine dihydro-chloride (**4**)

Yield: 57%. M.p. 218–220 °C (n-propanol). IR (cm^{-1}): 3432, 2979, 2341, 1608, 1515, 1456, 1263, 1203, 1024, 763. ^{1}H NMR (200 MHz, δ, ppm): 11.95 (1 H, bs, NH^+), 7.18–6.83 (7 H, m, H-Ar), 4.25 (2 H, t, J = 5.0 Hz, $ArOCH_2$), 3.81 (3 H, s, OCH_3), 3.85–3.12 (10 H, m, $(CH_2)_5$), 2.17 (6 H, s, $2 \times CH_3$). MS (m/z): 340 (M^+, base, 26%), 223 (10%), 219 (66%), 205 (100%), 190 (36%), 162 (10%), 70 (22%). pKa: 7.33. LogP: 3.82. LogD (pH): 1.61 (5.00), 3.32 (7.00), 3.55 (7.40). $R_f = 0.63$ (toluene/acetone (7 : 3)); 0.85 (benzene/methanol (5 : 1)).
$C_{21}H_{28}N_2O_2$ 2 HCl (413.4)

4.2.2. 1-[(2,6-Dimethyl)-phenoxyethyl]-4-(benzyl)-piperazine dihydrochloride (**5**)

Yield: 67%. M.p. 258–260 °C (ethanol). IR (cm^{-1}): 3434, 2983, 2435, 2364, 2258, 1614, 1477, 1263, 1193, 1018, 754. ^{1}H NMR (500.13 MHz, δ, ppm): 12.3 (1 H, bs, NH^+), 7.67 (2 H, d, J = 3.3 Hz, H(Ar)), 7.48–7.43 (3 H, m, H(Ar), 7.03 (2 H, d, J = 7.6 Hz, H(Ar)), 6.94 (1 H, dd, J = 7.0 Hz, J = 7.1 Hz, H(Ar)), 4.16 (2 H, t, J = 5.6 Hz, CH_2O), 4.38 (2 H, s, $ArCH_2N$), 3.79 (2 H, t, J = 5.6 Hz, CH_2N), 3.75–3.38 (8 H, m, CH_2 (pip), 2.25 (6 H, s, $2 \times CH_3$). ^{13}C NMR (δ): 15.5 ($2 \times CH_3$), 18.0 ($(\underline{C}H_2)_2NCH_2Ar$), 49.0 (N-$(CH_2)_2$ (pip)), 55.1 ($OCH_2\underline{C}H_2N$), 58.7 (N–CH_2–Ar), 66.5 (Ar-OCH_2), 123.4 (C-4(Ar$(CH_3)_2$)), 128.7 [(C-2, C-6 (Ar(CH_3)), (C-2, C-3, C-5, C-6, (Ph$\underline{C}H_2$))], 129.4 (C-1 (Ph-CH_2)), 130.2 (C-3, C-5 (Ar$(CH_3)_2$)), 131.3 (C-4 (Ph–CH_2)), 154.8 (C-4 (Ar$(CH_3)_2$)). pKa: 7.45. LogP: 3.57. LogD (pH): 2.99 (7.0), 3.25 (7.4). $R_f = 0.16$ (benzene/methanol (5 : 1)).
$C_{21}H_{28}N_2O$ 2 HCl (397.4)

4.2.3. 1-[(2,6-Dimethyl)-phenoxyethyl]-4-(2-hydroxyethyl)-piperazine dihydrochloride (**6**)

Yield: 58%. Mp. 234–236 °C (ethanol). IR (cm^{-1}): 3294, 3021, 2965, 2433, 1623, 1471, 1261, 1193, 1031, 792. ^{1}H NMR (500.13 MHz, δ, ppm): 11.3 (1 H, bs, NH^+), 7.04 (2 H, d, J = 7.5 Hz, H(Ar), 6.95 (1 H, dd, J = 7.1 Hz, J = 7.2 Hz, H(Ar)), 4.15 (2 H, t, J = 5.5 Hz, CH_2OAr), 3.86 (2 H, t, J = 5.2 Hz, CH_2OH), 3.64–3.56 (4 H, m, H(pip(a))), 3.56–3.51 (4 H, m, H(pip(e))), 3.41 (2 H, t, J = 5.5 Hz, $CH_2N(Ar)$), 3.24 (2 H, t, J = 5.2 Hz, N–$C\underline{H}_2CH_2OH$), 2.28 (6 H, s, $2 \times CH_3$). ^{13}C NMR (δ): 15.6 ($2 \times CH_3$), 48.3 ($\underline{N}$–$(CH_2)_2$(pip)), 48.7 ($(CH_2)_2N$(pip)), 55.0 (CH_2OH), 55.2 ($ArOCH_2\underline{C}H_2$), 57.5 (N-$\underline{C}H_2CH_2OH$), 66.6 (OCH_2), 123.5 (C-4), 128.3 (C-2, C-6), 129.7 (C-3, C-5), 154.8 (C-1). MS (m/z): 247 (M^+_{base} − CH_2OH) (23%), 157 (66%), 143 (100%), 125 (17%), 100 (24%), 70 (32%). pKa: 7.52. LogP: 1.16. LogD (pH): − 1.00 (5.00), 0.53 (7.0), 0.80 (7.4). $R_f = 0.60$ (benzene/methanol (5 : 1)).
$C_{16}H_{26}N_2O_2$ 2 HCl (351.3)

4.2.4. 1-[(4-Methyl)-phenoxyethyl]-4-[(2-methoxy)-phenyl]-piperazine dihydrochloride (**7**)

Yield: 64%. M.p. 211–213 °C (n-propanol). IR (cm^{-1}): 3427, 3029, 2981, 2466, 2343, 2148, 1610, 1516, 1407, 1267, 1251, 1029, 1004, 750. ^{1}H NMR (200 MHz, δ, ppm): 11.85 (1 H, s, NH^+), 7.21–6.79 (8 H, m, H(Ar)), 4.45 (2 H, t, J = 4.6 Hz, $ArOCH_2$), 3.80 (3 H, s, OCH_3), 3.75–3.15 (1 OH, m, $(CH_2)_5$, 2.23 (3 H, s, CH_3). pKa: 7.33. LogP: 3.37. LogD (pH): 1.18 (5.00), 2.87 (7.00), 3.10 (7.40). $R_f = 0.59$ (toluene/acetone (7 : 3)).
$C_{20}H_{26}N_2O_2$ 2 HCl (399.3)

4.2.5. 1-[4-(Methoxy)-phenoxyethyl]-4-[2-(methoxy)-phenyl]-piperazine dihydrochloride (**8**)

Yield: 56%. M. p. 200–202 °C (n-propanol). IR (cm^{-1}): 3421, 3236, 3029, 2942, 2546, 2443, 2144, 1610, 1512, 1457, 1232, 1047, 1006, 754. ^{1}H NMR (500.13 MHz, δ, ppm): 11.55 (1 H, bs, NH^+), 11.48 (1 H, bs, NH^+), 7.07–7.01 (1 H, m, H(Ar)), 7.01–6.94 (3 H, m, H(Ar)), 6.94–6.87 (2 H, m, H(Ar)), 6.87–6.82 (1 H, m, H(Ar)), 6.75–6.71 (1 H, m, H(Ar)), 4.42 (1 H, dd, J = 4.7 Hz, J = 4.9 Hz, $C\underline{H}HOAr$), 4.37 (1 H, dd, J = 4.7 Hz, J = 4.9 Hz, $CH\underline{H}OAr$), 3.80 (3 H, s, CH_3O), 3.71 (3 H, s, CH_3O), 3.67–3.59 (2 H, m, $\underline{C}H_2$ (pip)a), 3.59–3.52 (2 H, m, CH_2N), 3.52–3.45 (2 H, m, CH_2 (pip)a), 3.39–3.25 (2 H, m, CH_2 (pip)e), 3.23–3.12 (2 H, m, CH_2 (pip)e). ^{13}C NMR δ): 46.8 ($N(CH_2)_2$ (pip)), 51.7 ($(CH_2)_2N$ (pip)), 54.5 (CH_2–N), 55.3 ($2 \times OCH_3$), 62.9 (OCH_2), 112.0 (C-6 (Ph)), 114.6 (C-3, C-5 ($ArOCH_3$)), 115.8 (C-2, C-6 ($ArOCH_3$)), 118.3 (C-3 (Ph0), 120.8 (C-4 (Ph)), 123.5 (C-5 (Ph)), 139.1 (C-1 (Ph)), 150.2 (C-1 ($ArOCH_3$)), 151.9 (C-2 (Ph)). pKa: 7.33. LogP: 2.89. LogD (pH): 0.72 (5.00), 2.39 (7.00), 2.62 (7.40). R_f = 0.27 (benzene/methanol (5 : 1)).
$C_{20}H_{26}N_2O_3$ 2 HCl (415.3)

4.2.6. 1-[4-(Methoxy)-phenoxyethyl]-4-(benzyl)-piperazine dihydrochloride (**9**)

Yield: 69%. M.p. 245–247 °C (ethanol). IR (cm^{-1}): 3434, 2948, 2389, 2295, 1597, 1512, 1442, 1305, 1234, 1108, 1033, 933, 752. ^{1}H NMR (500.13 MHz, δ, ppm): 11.8 (2 H, bs, NH^+), 7.62–7.58 (2 H, m, H(Ar)), 7.43–7.39 (3 H, m, H(Ar)), 6.96–6.92 (2 H, m, H(Ar)), 6.88–6.85 (2 H, m, H(Ar)), 4.32 (2 H, t, J = 5.3 Hz, CH_2O), 4.22 (2 H, s, $ArCH_2N$), 3.82–3.51 (4 H, m, CH_2 (pip)a), 3.71 (3 H, s, CH_3O), 3.40 (2 H, t, J = 5.0 Hz, CH_2N), 3.39–3.31 (4 H, m, CH_2 (pip)e). ^{13}C NMR (δ): 47.7 ($(CH_2)_2N$ (pip)), 48.7 (N-$(CH_2)_2$ (pip)), 54.3 (N–$\underline{C}H_2$–CH_2O), 55.4 (OCH_3), 58.6 (N-CH_2Ph), 114.6 (C-3, C-5, ($ArOCH_3$)), 115.9 (C-2, C-6 ($ArOCH_3$)), 128.8 (C-2, C-3, C-5, C-6 (Ph–CH_2)), 129.5 (C-1 (PhCH_2)), 131.4 (C-4 (PhCH_2)), 151.4 (C-1 ($ArOCH_3$)), 154.0 (C-4 ($ArOCH_3$)). pKa: 7.45. LogP: 2.65. LogD (pH): 0.41 (5.00), 2.06 (7.00), 2.32 (7.40). $R_f = 0.27$ (benzene/methanol (5 : 1)).
$C_{20}H_{26}N_2O_2$ 2 HCl (399.3)

4.2.7. 1-[3-(Methyl)-4-(chloro)-phenoxyethyl]-4-[2-(methoxy)-phenyl]-piperazine dihydrochloride (**10**)

Yield: 65%. M.p. 208–210 °C (ethanol). IR (cm^{-1}): 3434, 3029, 3010, 2966, 2935, 2613, 2304, 2165, 1608, 1514, 1485, 1460, 1415, 1313, 1265, 1242, 1174, 1122, 1010, 756. ^{1}H NMR (500.13 MHz, δ, ppm 11.49 (1 H, bs, NH^+), 7.35 (1 H, d, J = 8.7 Hz, H(Ar)), 7.07–6.87 (6 H, m, H(Ar)), 4.47 (2 H, t, J = 5.0 Hz, H(Ar)), 3.80 (3 H, s, CH_3O), 3.64–3.58 (2 H, m, CH_2N), 3.62–3.55 (2 H, m, CH_2 (pip)a), 3.51–3.47 (2 H, m, CH_2 (pip)a), 3.36–3.28 (2 H, m, CH_2 (pip)e), 3.18–3.11 (2 H, m, CH_2 (pip)e), 2.31 (3 H, s, CH_3Ar). ^{13}C NMR (δ): 19.7 (CH_3Ar), 46.8 (N–$(CH_2)_2$ (pip)), 54.3 (CH_2N), 55.3 (CH_3O), 58.2 ($(CH_2)_2N$ (pip)), 62.6 (OCH_2), 112.0 (C-6 ($ArOCH_3$)), 113.9 (C-6 (PhO)), 117.4 (C-2 (PhO)), 118.2 (C-3 ($ArOCH_3$)), 120.8 (C-4 ($ArOCH_3$)), 123.5 (C-5 ($ArOCH_3$)), 125.3 (C-4 (PhO)), 129.5 (C-5 (PhO)), 136.5 (C-3 (PhO)), 139.2 (C-1 ($ArOCH_3$)), 151.8 (C-2 ($ArOCH_3$)), 156.2 (C-1 (PhO)). pKa: 7.33. LogP: 4.25. LogD (pH): 2.03 (5.00), 3.75 (7.00), 3.98 (7.40). $R_f = 0.46$ (benzene/methanol (5 : 1)).
$C_{20}H_{25}N_2O_2Cl$ 2 HCl (433.8)

4.3. Pharmacology

4.3.1. Materials

Compounds: [^{3}H]CGP-12177 (Amersham), Carvedilol (Anpharm), [^{3}H]Clonidine (NEN), DMPP (Sigma-Aldrich Chemie Gmbh), epinephrine (Adrenalinum, Polfa), methoxamine (Sigma-Aldrich Chemie Gmbh), norepinephrine (Levonor, Polfa), [^{3}H]Prazosin (NEN), sodium heparin (Polfa), thiopental sodium (Biochemie Gmbh, Vienna), tyramine (Sigma-Aldrich Chemie Gmbh).
Animals: The experiments were carried out on male Wistar rats (180–250 g) and on male Swiss/Alb. mice (20–24 g). Animals were housed in constant temperature facilities and exposed to a 12 h light: 12 h dark cycle and maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 6–8 animals each.
Statistical analysis: The data are expressed as means ± SEM. The statistical significance was calculated using a one-way ANOVA test. Differences were considered significant when $p < 0.05$. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 — San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation [28].

4.3.2. Influence on the blood pressure

Male Wistar normotensive rats were anaesthetized with thiopental (50–75 mg/kg) by intraperitoneally injection. The right carotid artery was cannulated with polyethylene tube filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments). The studied compounds were injected in doses corresponding to 1/40–1/10 LD_{50} i.v. (mice) into the caudal vein and administered orally (1/40–1/10 LD_{50} p.o.; mice) after a 5 min stabilisation period, in a volume equivalent to 1 ml/kg.
In separate series of experiments on anaesthetised normotensive rats, the effect of studied compounds (1/20–1/10 LD_{50} i.v.; mice) on the pressor response to epinephrine (2 μg/kg), norepinephrine (2 μg/kg), methoxamine (150 μg/kg), tyramine (200 μg/kg) and dimethylphenylpiperazine (100 μg/kg) was investigated. Pressor responses of epinephrine, norepinephrine, methoxamine, tyramine and DMPP injected intravenously were obtained before and 5 min after administration of tested compounds (i.v.).

4.3.3. Radioligand binding assay

The experiment was carried out on the rat cerebral cortex. [^{3}H]Prazosin (19.5 Ci/mmol, α_1-adrenergic receptor), [^{3}H]clonidine (70.5 Ci/mmol, α_2-adrenergic receptor) and [^{3}H]CGP-12177 (48 Ci/mmol, β_1-adrenergic receptor) were used.
Rat brains were homogenised in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.6), and centrifuged at 20,000 × g for 20 min (0–4 °C). The cell pellet was resuspended in Tris-HCl buffer and centrifuged again.

Radioligand binding assays were performed in plates (MultiScreen/Millipore). The final incubation mixture (final volume 300 µl) consisted of 240 µl membrane suspension, 30 µl of a [^{3}H]prazosin (0.2 nM), [^{3}H]clonidine (2 nM) or [^{3}H]CGP-12177 (0.2 nM) solution and 30 µl buffer containing from seven to eight concentrations (10^{-11}–10^{-4} M) of investigated compounds. For measuring unspecific binding, phentolamine – 10 µM (in the case of [^{3}H]prazosin), clonidine – 10 µM (in the case of [^{3}H]clonidine) and propranolol – 1 µM (in the case of [^{3}H]CGP-12177) were applied. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed 2 times with the assay buffer and placed in scintillation vials with liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All assays were done in duplicates.

4.3.4. Acute toxicity according to Litchfield and Wilcoxon

The compounds, dissolved in 0.9% saline, were injected into the caudal vein (10 ml/kg) or administered intragastrically to mice. Each dose was given to 6 animals. The LD_{50} were calculated according to the method of Litchfield and Wilcoxon [19] after a 24 h observation period.

Acknowledgement: This work was supported by the Polish State Committee for Scientific Research; 6 P05F 046 20.

References

1 Cesario, D. A.; Fonarow, G. C.: Rev. Cardiovasc. Med. **3**, 14 (2002)
2 Kukin, M. L.: Mayo Clin. Proc. **77,** 1199 (2002)
3 Julius, S.; Nesbitt, S.: Am. J. Hypertens. **9**, 113S (1996)
4 Wehling, M.: Herz **27** (Suppl. 1), 16 (2002)
5 Prisant, L. M.: J. Clin. Hypertens. **4**, 286 (2002)
6 Kaye, D. M.; Johnston, L.; Vaddadi, G.; Brunner-LaRocca, H.; Jennings, G. L., Esler, M. D.: Hypertension **37**, 1216 (2001)
7 Hampton, J. R.: Drugs **48**, 549 (1994)
8 Ellison, K. E.; Hafley, G. E.; Hickey, K.; Kellen, J.; Coromilas, J.; Stein, K. M.; Lee, K. L.; Buxton, A. E.: Circulation **106**, 2694 (2002)
9 Hartmann, F.; Katus, H. A.: Herz **27** (Suppl. 1), 30 (2002)
10 Ruffolo Jr., R. R.; Boyle, D.; Venuit, R. P.; Lukas, M.: Drug of Today **27**, 465 (1991)
11 Hershberger, R. E.; Wynn, J. R.; Sundberg, L.; Bristow, M. R.: J. Cardiovasc. Pharmacol. **15**, 959 (1990)
12 Fischer, W.: Seizure **11**, 285 (2002)
13 Muramatsu, I.; Yamanaka, K.; Kigoshi, S.: Japan. J. Pharmacol. **55**, 391 (1991)
14 Schoetensack, W.; Bruckschen, E. G.; Zech, K.; in: Scriabine, A. (Eds): Cardiovascular Drugs., p. 19, Raven Press, New York 1983
15 Marona, H.; Pękala, E.; Filipek, B., Macigg, D.; Szneler, E.: Pharmazie **56**, 567 (2001)
16 Marona, H.; Antkiewicz-Michaluk, L.: Acta Pol. Pharm. – Drug Res. **55**, 487 (1998)
17 Mulzac, D.; Scott, K. R.: Epilepsia **34**, 1141 (1993)
18 Swain, A. P.; Naegele, S. K.: J. Am. Chem. Soc. **76**, 5091 (1954)
19 Litchfield, J. T.; Wilcoxon, F.: J. Pharmacol. Exp. Ther. **96**, 99 (1949)
20 Nichols, A. J.; Sulpizio, A. C.; Ashton, D. J.; Hieble, J. P.; Ruffolo Jr., R. R.: Pharmacology **39**, 327 (1989)
21 Yue, T. L.; McKenna, P. J.; Lysko, P. G.; Ruffolo Jr., R. R.; Feuerstein, G. Z.: Atherosclerosis **97**, 209 (1992)
22 Kleemann, A.; Engel, J.; Kutscher, B.; Reichert, D.; in: Pharmaceutical Substances: Syntheses, Patents, Applications, 3.Ed.; p. 339, Thieme, Stuttgart-New York 1999
23 Mouillé, P.; Dabiré, H.; Joly, G.; Schmitt, H.: J. Pharmacol. (Paris) **14**, 325 (1983)
24 Mouillé, P.; Huchet, A. M.; Chelly, J.; Schmitt, H.; in: Les alpha-bloquants. Pharmacologie expérimentale at clinique, p. 14, Masson, Paris-New York 1981
25 Koss, M. C.: Pharmacol. Commun. **4**, 11 (1993)
26 Pönicke, K.; Heinroth-Hoffmann, I.; Brodde, O.: J. Pharmacol. Exp. Ther. **301**, 71 (2002)
27 Asano, M.; Uchida, W.; Shibasaki, K.; Terai, M.; Inagaki, O.; Takenaka, T.; Matsumoto, Y.; Fujikura, T.: J. Pharmacol. Exp. Ther. **254**, 204 (1990)
28 Cheng, Y. C.; Prusoff, W. H.: Biochem. Pharmacol. **22**, 3099 (1973)