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Synthesis, α-adrenoceptors affinity and α_1-adrenoceptor antagonistic properties of some 1,4-substituted piperazine derivatives

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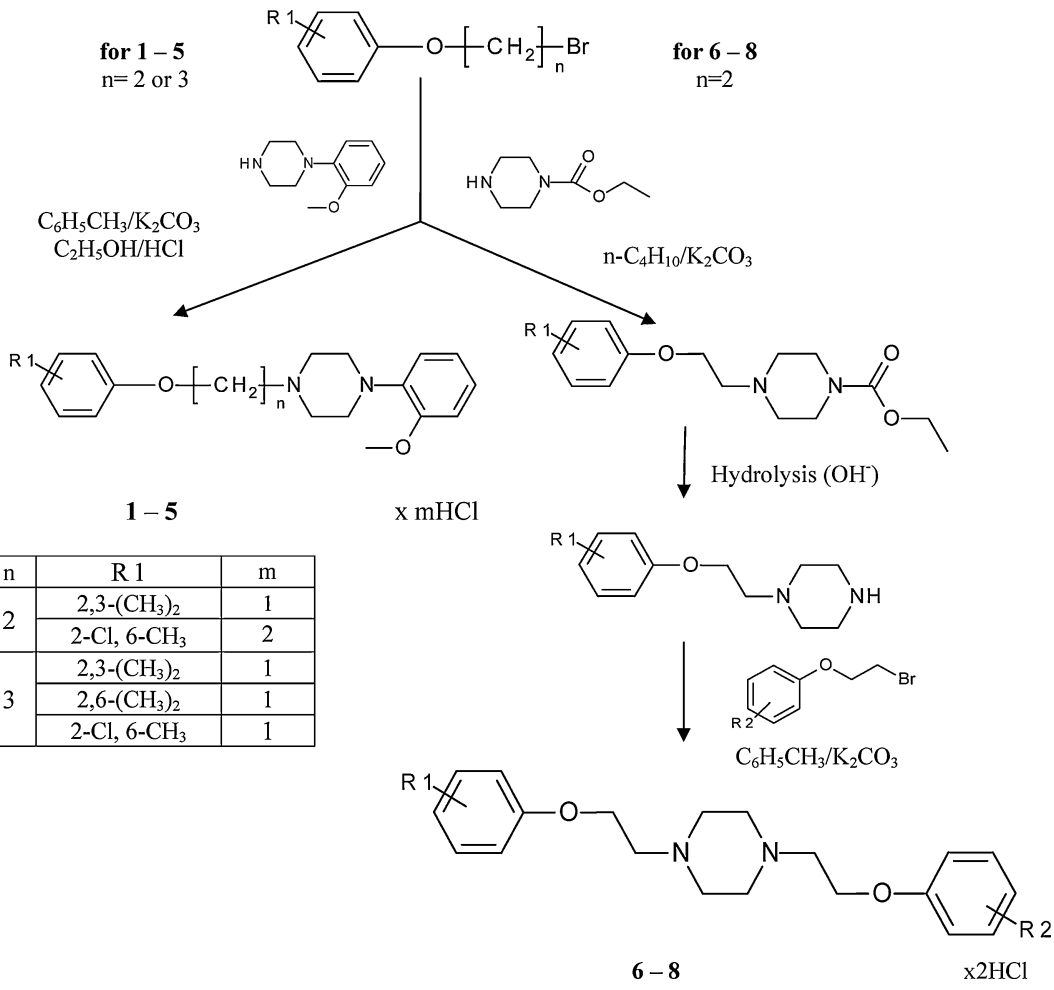
A series of different 1,4-substituted piperazine derivatives (**1–11**) was synthesized. It comprised 1-(substituted-phenoxyalkyl)-4-(2-methoxyphenyl)piperazine derivatives (**1**–**5**); 1,4-bis(substituted-phenoxy-ethyl)piperazine derivatives (**6**–**8**) and 1-(substituted-phenoxy)-3-(substituted-phenoxyalkylpiperazin-1-yl)propan-2-ol derivatives (**9**–**11**). All compounds were evaluated for affinity toward α_1- and α_2-receptors by radioligand binding assays on rat cerebral cortex using [^{3}H]prazosin and [^{3}H]clonidine as specific radioligand, respectively. Furthermore α_1-antagonistic properties were checked for most promising compounds (**1–5** and **10**) by means of inhibition of phenylephrine induced contraction in isolated rat aorta. Antagonistic potency stayed in agreement with radioligand binding results. The most active compounds (**1–5**) displaced [^{3}H]prazosin from cortical binding sites in low nanomolar range ($K_i = 2.1 - 13.1$ nM). Compound **10** showed slightly lower affinity for α_1-adrenoceptor ($K_i = 781$ nM). Compounds **2–5** displayed the strongest antagonistic activity with pA_2 values ranging from 8.441 to 8.807. Compound **1** gave a pA_2 value of 7.868, while compound **10** showed the weakest antagonistic potency, giving a pA_2 value of 6.374. 1-[3-(2-Chloro-6-methylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (**5**) showed the best α_1- affinity properties with a $K_i(\alpha_1)$ value of 2.1 nM and it was 61.05 fold more selective toward α_1 than α_2-receptors. The best properties showed 1-[3-(2,6-dimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (**4**) with a $K_i(\alpha_1)$ value of 2.4 nM, a 142.13 fold better selectivity to α_1- over α_2-adrenoceptors and the best antagonistic potency ($pA_2 = 8.807$). It is worth to emphasized that all most promising compounds possessed an 1-(*o*-methoxyphenyl)piperazine moiety which probably plays an important role in the affinity to α-adrenoceptors.

1. Introduction

The adrenergic receptors (AR) belong to the family of G-protein coupled seven-transmembrane spanning receptors. They are divided into three subclasses: α_1, α_2 and β, and further into several subtypes (i.e. α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3). The classification is based on structural similarity, localization and pharmacology as well as on cloning techniques (Bishop 2007; Docherty 1998; Gauthier et al. 2007). The α-adrenoceptors are involved in central and peripheral nervous system functions (Gyires et al. 2009) while β-adrenoceptors are mainly responsible for the physiological function of the cardiovascular system, respiratory tract and of fat tissue (Johnson 2006). More detailed α_1 receptors roles are connected with vascular smooth muscle contraction, increasing blood pressure, dilation of the pupil, the human prostate smooth muscle contraction (Jain et al. 2008) as well as regulation of cerebral microcirculation (Yokoo et al. 2000). On the other hand α_2 receptors are responsible for lowering blood pressure, sedation, analgesia, anesthesia, inhibition of intestinal secretion, aggregation of the platelets and inhibition of neurotransmitters release (Jain et al. 2008; Giovannoni et al. 2009; Gyires et al. 2009).

Considering the physiological function of α-adrenoceptors ligands both agonist and antagonists of those receptors were introduced to the pharmacotherapy and are currently used as medications. α_1-Antagonists have been used with considerable success in the treatment of hypertension over the past two decades. It can be expected that their well established status will not change as overactivity of the symphatetic nervous system constitutes a common phenomenon in resistant forms of hypertension. Additively α_1-antagonists are currently first-line therapy for lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH). They improve both symptom score and urinary flow in that condition. It is worth to mention that selective urinary α_1-blockers (α_{1A}/α_{1D}) constitute important treatment tools for hypertensive patients with BPH since such patients are best treated by the separate management of each condition (Schwinn and Roehrborn 2008; Sica 2005). Substances with α_2-adrenoceptor agonist activity are used as hypotensive agents but this role is currently not important. However new fields of their application have occurred, particularly in the management of pain and anesthesia (Crassous et al. 2007; Gyires et al. 2009).
In this context, the search for α-adrenoceptor antagonists constitutes an important area in medicinal chemistry. Furthermore,



	n	R 1	m
1	2	2,3-$(CH_3)_2$	1
2		2-Cl, 6-CH_3	2
3	3	2,3-$(CH_3)_2$	1
4		2,6-$(CH_3)_2$	1
5		2-Cl, 6-CH_3	1

	R 1	R 2
6	2-Cl, 5-CH_3	H
7	4-Cl, 3-CH_3	H
8	2-Cl, 6-CH_3	4-Cl, 3-CH_3

Scheme 1: Synthesis of compounds 1–8

new applications are under investigation, so the clinical utility of both antagonist and agonists may expand in the future (Crassous et al. 2007).

In recent years various new adrenergic receptor ligands have been synthesized. A large group of them contains an arylpiperazine moiety and more detailed – a phenylpiperazine moiety (Betti et al. 2004; Bremner et al. 2000; Ismail et al. 2006; Pittalá et al. 2006). The encouraging fact is that potent α-receptors antagonists – prazosin, terazosin and doxazosin, which have a well established position in pharmacotherapy, posses a structural fragment of piperazine (Heran et al. 2009). It is also worth to mention other α-receptors blockers urapidil and naftopidil are containing an 1-(*o*-methoxyphenyl)piperazine moiety (Cherney and Straus 2002; Muramatsu et al. 1991).

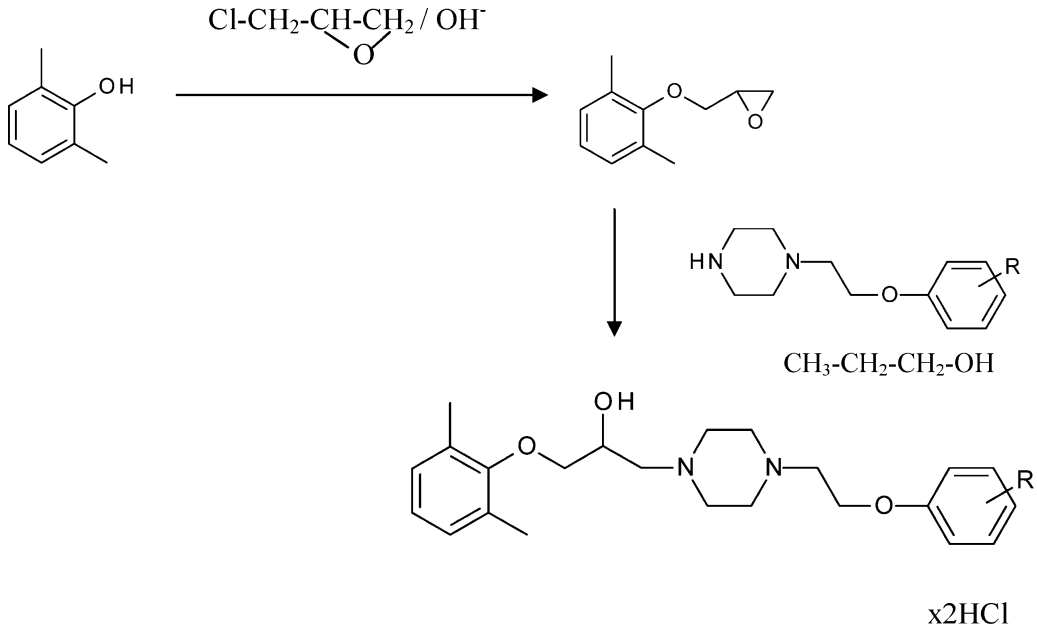
Our previous research has dealt with the synthesis and biological activity of compounds in the group of 1,4-substituted piperazine derivatives that proved α_1- and α_2-adrenoceptors binding properties (Maciąg et al. 2003, 2008; Marona et al. 2008). The best results showed aroxyethylpiperazine derivatives e.i. 1-(4-methoxyphenoxyethyl)-, 1-(2,6-dimethylphenoxyethyl)- and 1-(4-methylphenoxyethy)-4-(2-methoxyphenyl)piperazine dihydrochlorides with K_i values related to inhibiting *in vitro* [^{3}H]prazosin binding to cortical α_1-receptors of 26.0 ± 6.9, 87.5 ± 19.0, 84.6 ± 16.1 nM and K_i values related to inhibiting *in vitro* [^{3}H]clonidine binding to cortical α_2-receptor of 197.9 ± 40.8, 252.3 ± 49.7, 282.1 ± 39.0 nM, respectively. At the same time all substances proved α_1 antagonistic properties by showing hypotensive activity in normotensive rats. The substances showed also β_1-receptors binding properties as well as antiarrhythmic activity (Maciąg et al. 2003).

Taking into consideration those facts and our former experience we synthesized a series of 1,4-substituted piperazine derivatives. Among them there were both aroxyethyl and aroxypropyl derivatives of piperazine, in addition compounds **9**, **10** and **11** contained a structural moiety of propranolol while compounds **1–5** and **10** possessed a 1-(*o*-methoxyphenyl)piperazine fragment in their molecule. All compounds were subjected to preliminary pharmacological screening. The present paper reports on the results of their *in vitro* affinity to α_1 and α_2-adrenoceptors as well as their antagonistic properties at α_1-adrenoceptors on isolated rat aorta.

2. Investigations and results

2.1. Chemical synthesis

Synthesis of compounds **1–8** is shown in Scheme 1. Compounds **1–5** were obtained in reaction between 1-(2-methoxyphenyl)piperazine and appropriate phenoxyalkyl bromides carried out in toluene in the presence of anhydrous



	R
9	H
10	2-OCH_3
11	4-OCH_3

Scheme 2: Synthesis of compounds 9–11

potassium carbonate according to well known procedures (Maciąg et al. 2003; Marona et al. 2004). Compounds **6–8** were synthesized in multistep reaction according to earlier published methodology (Marona et al. 2004, 2009). At first appropriate phenoxyethyl bromides were used in the reaction with 1-ethoxycarbonylpiperazine in n-butanol in the presence of potassium carbonate, intermediate products were not separated. Hydrolysis in 85% potassium hydroxide of derivatives gave the corresponding 1-[2-(phenoxyethyl)piperazine]. The raw products were used in further reaction with another appropriate phenoxyethyl bromides. Bases received as oils were converted into hydrochlorides using an excess of ethanol saturated with gas hydrochloride. Raw salts were recrystallized from ethanol. Compounds **9–11** were obtained according to well known procedures via epoxy derivatives (Hlaváčová et al. 1995; Marona et al. 1997, 1998; Mlynárova et al. 1996). The way of synthesis is presented in Scheme 2. At first 2,6-dimethylphenol was used in the reaction with (+/-)-epichlorohydrin in alkaline medium. Then the crude oil product i.e. (+/-)-1,2-epoxy-3-(2,6-dimethylphenoxy)propane was used in the reaction of aminolyzis by means of an appropriate 2-(phenoxyethyl)piperazine carried out in *n*-propanol. Received crude oil bases were converted into hydrochlorides using an excess of ethanol saturated with HCl. Raw salts were recrystallized from ethanol/acetone mixture (1:1).

2.2. Pharmacology

2.2.1. Radioligand binding results

Compounds **1–12** were tested for their *in vitro* affinity toward α_1- and α_2-adrenoceptors on rat cerebral cortex by radioligand binding assays using [^{3}H]prazosin and [^{3}H]clonidine as specific radioligands (Maj et al. 1985). The affinities described by K_i values [nM] are shown in Table 1. Selectivity toward α_1-AR with respect to α_2-AR was calculated as $K_{i\alpha2}/K_{i\alpha1}$. Compounds **1–5** displaced [^{3}H]prazosin from cortical binding sites in low nanomolar range ($K_i = 2.1-13.1$ nM). Compound **10** showed a slightly lower affinity for the α_1-adrenoceptor ($K_i = 781$ nM), whereas other compounds (**6–9**, **11**, **12**) had a low affinity for both α_1- and α_2-adrenoceptors, moderately inhibiting radioligands binding in the μM-range. The most selective compounds (**4** and **5**) showed a 142.1- and 61-fold higher affinity for α_1- than for α_2-adrenoceptors.

2.2.2. Functional bioassays results

The antagonist activity of compounds **1–5** and **10** toward α_1-adrenoceptors present in rat aorta from adult Wistar rats was assessed by inhibition of phenylephrine induced contractions. The investigated compounds, concentration-dependently, shifted the phenylephrine response to the right. For all test compounds Schild slopes did not differ significantly from unity, indicating a competitive interactions with the α_1-adrenoceptors,

Table 1: Binding properties of compounds 1–11

Compd.	Affinity K_i (nM) α_1-AR	α_2-AR	α_2/α_1
1	13.1 ± 4.5	171.6 ± 7.1	13.10
2	5.1 ± 0.3	6.4	1.25
3	2.8 ± 0.6	37.3 ± 1.2	13.32
4	2.4 ± 0.1	341.1 ± 70.5	142.13
5	2.1 ± 0.2	128.2 ± 0.3	61.05
6	9200 ± 1700	141.8 ± 45.4	0.02
7	4700 ± 2300	13400 ± 1800	2.85
8	5500 ± 1300	1600 ± 400	0.29
9	4500 ± 1400	1850 ± 300	0.41
10	781.0 ± 79.0	95.0 ± 3.2	0.12
11	9600 ± 2100	610 ± 100	0.06
Urapidil	127.9 ± 5.3	–	–

Inhibition constants (Ki) were calculated according to the equation of Cheng and Prusoff. Radioligands binding assays to rats cortex membrane using [^{3}H]-prazosin (α_1) and [^{3}H]-clonidine (α_2), respectively

Table 2: Functional bioassays results for selected compounds 1–5, 10

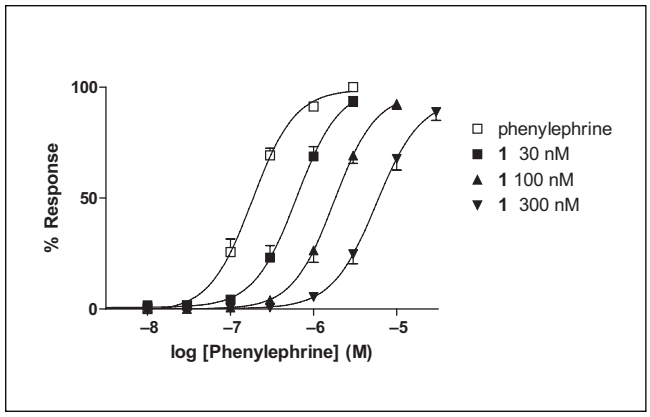
Compd.	$pA_2 \pm SEM$	(Slope ± SEM)
1	7.868 ± 0.02	(1.09 ± 0.01)
2	8.441 ± 0.02	(0.99 ± 0.02)
3	8.534 ± 0.08	(1.06 ± 0.04)
4	8.807 ± 0.02	(0.96 ± 0.01)
5	8.603 ± 0.07	(1.02 ± 0.01)
10	6.374 ± 0.09	(1.09 ± 0.05)
Urapidil	7.334 ± 0.04	(0.90 ± 0.02)

Antagonist potency of selected compounds, expressed as $pA_2 \pm SEM$ values, in isolated rat thoracic aorta (α_1-AR) pA_2 values were obtained from the linear regression of Schild plot
Each value was the mean ± SEM of 5–8 experimental results

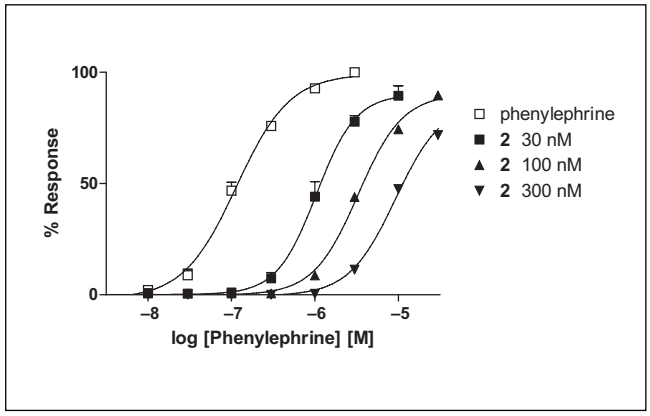
and thus allowing for the determination of the pA_2 values. The obtained results are shown in Table 2 as well as Figs. 1 and 2.
The strongest antagonistic activity occurred with compounds **2–5** with pA_2 values ranging from 8.441 to 8.807. Compound **1** gave a pA_2 value of 7.868 which was comparable with the pA_2 value obtained for the reference compound, urapidil ($pA_2 = 7.334$). Compound **10** showed the weakest antagonistic potency, giving a pA_2 value of 6.374. It is worth to note that the affinity from the functional test for all test compounds and urapidil was in the same concentration range as determined in radioligand binding assay.

3. Discussion

The aim of this study was to evaluate α_1 and α_2-adrenoceptors affinity of 11 different 1,4-substituted piperazine derivatives. We focused on piperazine derivatives bearing in mind literature data (Betti et al. 2004; Bremner et al. 2000; Ismail et al. 2006; Pittalá et al. 2006) and our former research (Maciąg et al. 2003, 2008; Marona et al. 2008). Compounds which are presented in this work can be divided into three chemically different groups namely: **I**-1-(substituted-phenoxyalkyl)-4-(2-methoxyphenyl)piperazine derivatives (compounds **1–5**); **II**-1,4-bis(substituted-phenoxyethyl)piperazine derivatives (compounds **6–8**) and **III**-1-(substituted-phenoxy)-3-(substitutedphenoxyalkylpiperazin-1-yl)propan-2-ol derivatives (compounds **9–11**).
Among the tested compounds the values of inhibition constants (K_i) to α_1-adrenergic receptors ranged from 2.1 to 9600 nM. The best affinity was characteristic for the group **I**, where all compounds possessed K_i values at the level of a few nM. All of them had an *o*-methoxyphenyl piperazine moiety. Our research is consistent with the notion that this structural fragment exerts beneficial influence on α_1-adrenoceptor antagonistic properties. Taking into consideration the structure of the compounds **1 – 5** it could be stated that the substitution in phenyl ring with either 2 methyl groups or with 1 methyl group and the chlorine atom did not play an important role in the activity. On the other hand there was a slight difference in the affinity of the compounds possessing either a 2 or a 3 carbon atoms linker wherein the latter seemed to be favorable.
Compound **10** is the only one in group **III** which had an *o*-methoxyphenyl moiety but it was not directly connected with piperazine. Its $K_i(\alpha_1)$ value was in the middle between those for most and least active substances. Considering results for the structures **9 – 11** it was once again proved that the substitution in a phenyl ring in *ortho* position is beneficial as compared to *meta* as well as no substitution.
In terms of selectivity, the compounds were differently selective towards α_1-AR compared to α_2-AR (0.02 to 142.13-fold). The most interesting results are characteristic for group **I**, where all substances possessed very good affinity to α_1-adrenoceptors. Only compound **4** displayed noticeable selectivity. Considering the chemical structure it was the only one from the group which possessed a 2,6-dimethyl substituted phenyl ring. The results could suggest that this kind of substitution is favourable in case of α_1 as compared to α_2 selectivity. Comparing compounds **2** and **5**, the first showed almost the same affinity to both kind of adrenergic receptors while the second was more selective towards α_1-AR. The only difference in their structures was a linker consisting of 2 or 3 carbon atoms, respectively. The affinities of compounds **1** and **3** did not confirm the relationship between the number of carbon atoms in the linker and selec-



(a)



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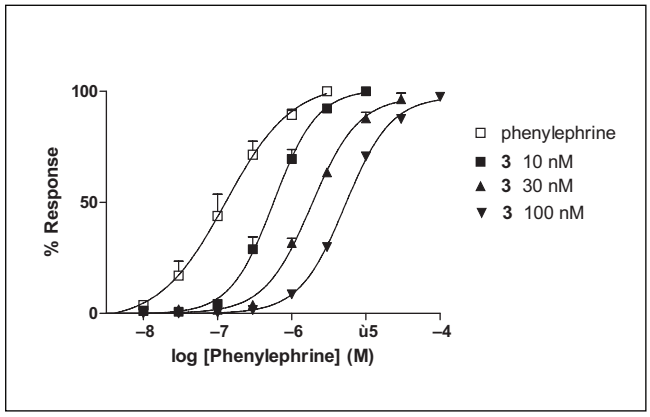
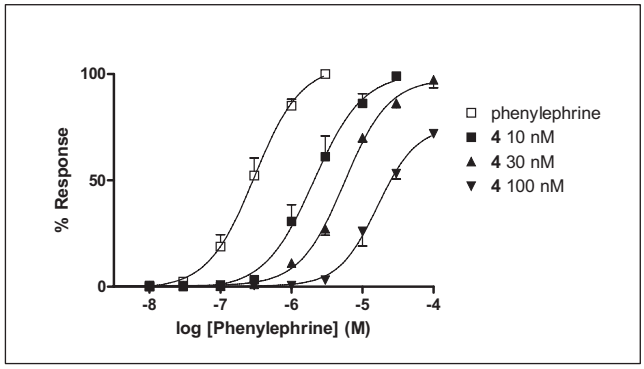
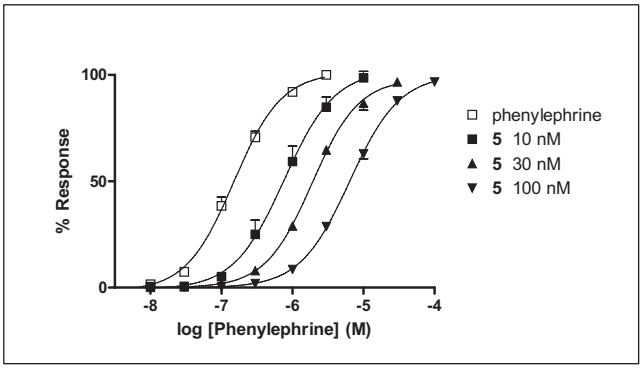


(c)

Fig. 1: Concentration-response curves to phenylephrine in the rat aorta in the absence (□) or presence of a) **1** (■ 30, ▲ 100 and ▼ 300 nM); b) **2** (■ 30, ▲100 and ▼300 nM); c) **3** (■ 10, ▲ 30 and ▼ 100 nM). Results are expressed as percentage of the maximal response to phenylephrine in the first concentration-respose curve. Each point represents the mean ± SEM (n = 4–8).



(a)



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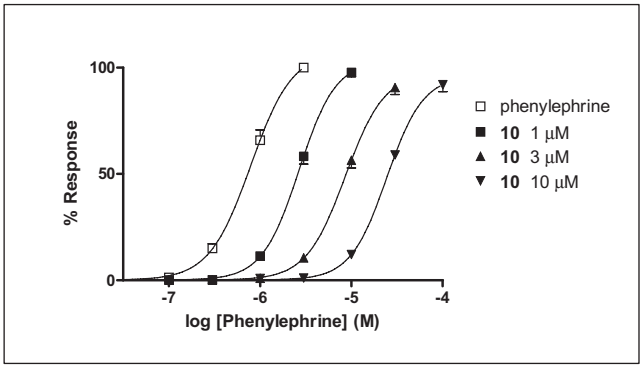

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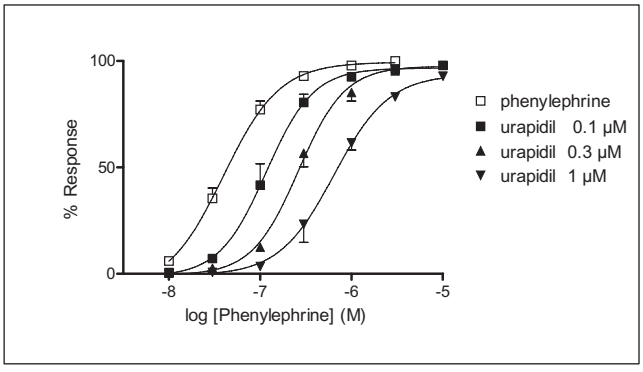

(d)

Fig. 2: Concentration-response curves to phenylephrine in the rat aorta in the absence (□) or presence of a) **4** (■ 10, ▲ 30 and ▼ 100 nM); b) **5** (■ 10, ▲ 30 and ▼ 100 nM); c) **10** (■ 1, ▲ 3 and ▼ 10 μM); d) **urapidil** (■ 0.1, ▲ 0.3 and ▼ 1 μM). Results are expressed as percentage of the maximal response to phenylephrine in the first concentration-respose curve. Each point represents the mean ± SEM (n = 4–8).

tivity. In group **III** all compounds were highly more selective towards α_2-adrenoceptors. In group **II** surprisingly compound **6** displayed significant selectivity toward α_2 compared to α_1 but there was no noticeable relationship between structure and affinity.

Based on the radioligand binding results, the 6 most promising compounds were evaluated for their interaction with α_1-adrenoceptors in functional bioassays. The results showed α_1-AR antagonistic activity for all the tested compounds. All substances displayed competitive interaction with α_1-adrenoceptors in rat aorta. The best antagonistic properties were characteristic for compound **4** which at the same time showed the highest α_1/α_2 selectivity.

The outcome of pharmacological tests confirmed that among 1-(*o*-methoxyphenyl)piperazine derivatives the affinity to α-adrenoceptors could be expected, however, the proposed chemical modifications of that moiety (compounds **6–11**) did not improve the affinities. In cases where the methoxyphenyl moiety was left unchanged (compounds **1–5**), the values of affinity to α_1-adrenergic receptors (K_i) were at the level of a few nanomol (2.1 – 5.1 nM). The affinity was even better than that of the reference compound – urapidil. It was also proved that compounds **2–5** possessed good antagonistic activity to the receptors. The results from the study are very encouraging and incentivizes further research regarding pharmacological properties of the most promising compounds.

4. Experimental

4.1. Chemistry

Melting points (m.p.) were determined using a Büchi SMP-20 apparatus and were uncorrected. Analyses of percentage content of carbon, hydrogen and nitrogen were within 0.4% of the theoretical values. Thin-layer chromatography was performed on precoated aluminium sheets (silica gel 60 F_{254}, Merck) using as a mobile phase a mixture of methanol and ethyl acetate in volume ratio 1:1. The results were visualized by means of ultraviolet light. The proton magnetic resonance spectra were recorded on Bruker Avance II 500, Bruker Avance II 300 or Bruker AMX 500 spectrometer using TMS as an internal standard. Compounds were dissolved in DMSO-d_6 99.8% in 300K. The results were presented in the following format: chemical shift δ (ppm), multiplicity, coupling constants (*J*) values in Hertz (Hz), number of protons, proton's position (where "pip" indicates piperazin, "a"-axial, "e"-equatorial). Multiplicities were shown as the abbreviations: s (singlet), bs (broad singlet), d (doublet), ddq (double doublet of quintets), m (multiplet). The IR spectra were recorded on a Jasco FT/IR 410 apparatus using KBr pellets and are reported in cm^{-1}. The theoretical values of the partition coefficient (LogP) of the tested compounds were calculated by means of ACDLABS 12.0 program. Reagents were purchased from Alfa Aesar GmbH&Co KG (Karlsruhe, Germany) or from SigmaAldrich, solvents were commercially available materials of reagent grade.

*4.1.1. 1-[2-(2,3-Dimethylphenoxy)ethyl]-4-(2-methoxyphenyl) piperazine hydrochloride (**1**)*

Yield: 62%. M.p. = 206–208 °C; ^{1}H NMR 2.13 (s, 3H, Ar-C$\underline{H}_3$), 2.23 (s, 3H, Ar-C$\underline{H}_3$), 3.28−3.12 (m, 2H, pip(e)), 3.30−3.45 (m, 2H, pip(e)), 3.45−3.55 (m, 2H, pip(a)), 3.57−3.62 (m, 2H, -C$\underline{H}_2$-NH$^+$), 3.62−3.68 (m, 2H, pip(a)), 3.90 (s, 3H, Ar-O-C$\underline{H}_3$), 4.45 (t, *J* = 5.0 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.78−7.11 (m, 7H, Ar), 11.71 (bs, 1H, N$\underline{H}^+$); IR 3434.60, 2976.59, 2327.66, 1460.81, 1262.18. 756.92; LogP = 4.55+/− 0.44; R_f = 0.84
$C_{21}H_{29}N_2O_2Cl$ (376.96).

*4.1.2. 1-[2-(2-Chloro-6-methylphenoxy)ethyl]-4-(2-methoxyphenyl) piperazine dihydrochloride (**2**)*

Yield: 58%. M.p. = 188−190 °C; ^{1}H NMR 2.35 (s, 3H, Ar-C$\underline{H}_3$), 3.14−3.48 (m, 4H, pip(e)), 3.49−3.77 (m, 6H, pip(a) + -C$\underline{H}_2$-NH$^+$), 3.81 (s, 3H, Ar-O-C$\underline{H}_3$), 4.38 (t, *J* = 5.2 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.87−7.38 (m, 7H, Ar), 11.73 (bs, 1H, N$\underline{H}^+$); IR 3433.64, 2980.45, 2335.37, 1458.89, 1262.18, 1024.98, 767.53; LogP = 4.64+/− 0.51; R_f = 0.84
$C_{20}H_{27}N_2O_2Cl_3$, (433.83).

*4.1.3. 1-[3-(2,3-Dimethylphenoxy)propyl]-4-(2-methoxyphenyl) piperazine hydrochloride (**3**)*

Yield: 59%. M.p. = 222–224 °C; ^{1}H NMR 2.10 (s, 3H, Ar-C$\underline{H}_3$), 2.22 (s, 3H, Ar-C$\underline{H}_3$), 2.23−2.32 (m, 2H, -CH$_2$-C$\underline{H}_2$-CH$_2$-), 3.02−3.33 (m, 6H, pip(e)) + -C$\underline{H}_2$-NH$^+$), 3.44−3.69 (m, 4H, pip(a)), 3.80 (s, 3H, Ar-O-C$\underline{H}_3$), 4.03 (t, *J* = 5.9 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.75−7.08 (m, 7H, Ar), 11.03 (bs, 1H, N$\underline{H}^{\pm}$); IR 3433.64, 2360.44, 1465.63, 1264.11, 759.82; LogP = 4.99+/− 0.44; R_f = 0.84
$C_{22}H_{31}N_2O_2Cl$; (390.99).

*4.1.4. 1-[3-(2,6-Dimethylphenoxy)propyl]-4-(2-methoxyphenyl) piperazine hydrochloride (**4**)*

Yield: 65%. M.p. = 216–218 °C; ^{1}H NMR 2.24 (s, 6H, Ar-C$\underline{H}_3$,Ar-C$\underline{H}_3$), 2.25−2.33 (m, 2H, -CH$_2$-C$\underline{H}_2$-CH$_2$-), 3.14−3.30 (m, 4H, pip(e)), 3.33−3.42 (m, 2H, -C$\underline{H}_2$-NH$^+$), 3.47−3.65 (m, 4H, pip(a)), 3.81 (s, 3H, Ar-O-C$\underline{H}_3$), 3.82 (t, *J* = 6.0 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.89−7.08 (m, 7H, Ar), 11.48 (bs, 1H, N$\underline{H}^{\pm}$); IR 3409.53, 2959.23, 2349.84, 1461.78, 1263.15, 1200.47, 778.14; LogP = 4.99+/− 0.44; R_f = 0.84
$C_{22}H_{31}N_2O_2Cl$(390.99).

*4.1.5. 1-[3-(2-Chloro-6-methylphenoxy)propyl]-4-(2-methoxyphenyl)-piperazine hydrochloride (**5**)*

Yield: 58%. M.p. = 199–201 °C; ^{1}H NMR 2.30 (dd, *J* = 0.8 Hz, *J* = 0.6 Hz, 3H, Ar-C$\underline{H}_3$), 2.24−2.39 (m, 2H, -CH$_2$-C$\underline{H}_2$-CH$_2$-), 3.19−3.67 (m, 10H, pip + -C$\underline{H}_2$-NH$^+$), 3.81 (s, 3H, Ar-O-C$\underline{H}_3$), 3.96 (t, *J* = 5.9 Hz, 2H, Ar-O-C$\underline{H}_2$), 6.89−7.05 (m, 4H, Ar), 7.06 (dd, *J* = 7.8 Hz, *J* = 7.7 Hz, 1H, Ar(5), 7.21 (ddq *J* = 7.6 Hz, *J* = 1.7 Hz, *J* = 0.8 Hz, 1H, Ar(4)), 7.31 (ddq, *J* = 7.9 Hz, *J* = 1.7 Hz, *J* = 0.6 Hz, 1H, Ar(6)), 11.64 (bs, 1H, N$\underline{H}^{\pm}$); IR 3472.20, 2459.76, 1463.71, 1264.11, 772.35; LogP = 5.07+/− 0.51; R_f = 0.77
$C_{21}H_{28}N_2O_2Cl_2$(411.40).

*4.1.6. 1-[2-(2-Chloro-5-methyl-phenoxy)ethyl]-4-(2-phenoxyethyl) piperazine dihydrochloride (**6**)*

Yield: 60%. M.p. = 218–220 °C; ^{1}H NMR 2.31 (s, 3H, Ar-C$\underline{H}_3$), 3.14−4.21 (m, 12H, N-C$\underline{H}_2$), 4.43 (t, *J* = 4.9 Hz, 2H, Ar-O-C$\underline{H}_2$-), 4.52 (t, *J* = 4.5 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.77−7.40 (m, 8H, Ar), 12.34 (bs, 1H, N$\underline{H}^{\pm}$); IR 3433.64, 2974.66, 2244.74, 1490.70, 1459.85, 1246.75, 759.82; LogP = 4.03+/− 0.45; R_f = 0.60
$C_{21}H_{29}N_2O_2Cl_3$ (447.86) (447.86).

*4.1.7. 1-[2-(4-Chloro-3-methylphenoxy)ethyl]-4-(2-phenoxyethyl) piperazine dihydrochloride (**7**)*

Yield: 63%. M.p. = 226–228 °C; ^{1}H NMR 2.31 (s, 3H, Ar-C$\underline{H}_3$), 3.30−3.97 (m, 12H, N-C$\underline{H}_2$), 4.40 (t, *J* = 5.0 Hz, 2H, Ar-O-C$\underline{H}_2$-), 4.41 (t, *J* = 5.0 Hz, 2H, Ar-O-C$\underline{H}_2$-), 6.83−7.40 (m, 8H, Ar), 12.17 (bs, 1H, N$\underline{H}^{\pm}$); IR 3433.64, 2380.48, 1242.90, 750.17; LogP = 4.17+/− 0.40; R_f = 0.61; $C_{21}H_{29}N_2O_2Cl_3$, M = (447.86).

*4.1.8. 1-[2-(2-Chloro-6-methylphenoxy)ethyl]-4-[2-(4-chloro-3 methylphenoxy)ethyl]piperazine dihydrochloride (**8**)*

Yield: 59%. M.p. = 224–226 °C; ^{1}H NMR 2.31 (s, 3H, Ar-C$\underline{H}_3$), 2.34 (s, 3H, Ar-C$\underline{H}_3$), 3.47−3.91 (m, 12H, N-C$\underline{H}_2$-), 4.31 (t, *J* = 5.0 Hz, 2H, Ar-O-CH$_2$-), 4.42 (t, *J* = 4.7 Hz, 2H, Ar-O-CH$_2$-), 7.39−6.82 (m, 6H, Ar), 12.32 (bs, 1H, -N$\underline{H}^{\pm}$); IR 3432.67, 2360.44, 1479.13, 1174.44, 762.71; LogP = 5.18+/− 0.47; R_f = 0.76
$C_{22}H_{30}N_2O_2Cl_4$(496.33).

*4.1.9. (+/-)-1-(2,6-Dimethylphenoxy)-3-[4-(2-phenoxyethyl)-piperazin-1-yl]propan-2-ol dihydrochloride (**9**)*

Yield: 64%. M.p. = 214–216 °C; ^{1}H NMR 2.25 (s, 6H, Ar-(C$\underline{H}_3$)$_2$), 3.23−4.10 (m, 14H, -O-C$\underline{H}_2$ + = N-C$\underline{H}_2$, = N-C$\underline{H}_2$, pip), 4.44 (bs, 3H, -O-C$\underline{H}_2$, = C$\underline{H}$-OH), 5.90 (bs, 1H, -O$\underline{H}$), 6.88−6.96 (m, 1H, Ar), 6.96−7.10 (m, 5H, Ar), 7.29−7.38 (m, 2H, Ar), 11.53 (bs, 1H, N$\underline{H}^{\pm}$), 12.37 (bs, 1H, N$\underline{H}^{\pm}$); IR 3374.82, 2928.28, 2375.87, 1476.24, 1200.47. 752.10; LogP = 3.89+/− 0.44; R_f = 0.75
$C_{23}H_{34}N_2O_3Cl_2$(457.46).

*4.1.10. (+/-)-1-(2,6-Dimethylphenoxy)-3-{4-[2-(2-methoxyphenoxy) ethyl]-piperazin-1-yl}propan-2-ol dihydrochloride (**10**)*

Yield: 61%. M.p. = 215–217 °C; ^{1}H NMR 2.25 (s, 6H, Ar-(C$\underline{H}_3$)$_2$, 3.27−4.11 (m, 14H, (-C$\underline{H}_2$-NH$^+$) + C$\underline{H}_2$-O-), 3.79 (s, 3H, Ar-O-CH$_3$), 4.38−4.50 (m, 3H, Ar-O-CH$_2$-, C$\underline{H}$-OH), 6.03 (bs, 1H, OH), 7.09−6.89 (m, 7H, Ar), 11.64 (bs, 1H, N$\underline{H}^{+}$), 12.37 (bs, 1H, N$\underline{H}^{+}$); IR 3239.82, 2957.30, 2308.37, 1508.06, 1254.47, 1023.05, 764.64; LogP = 3.72+/− 0.45; R_f = 0.69; $C_{24}H_{36}N_2O_4Cl_2$, M = 487.48.

*4.1.11. (+/−)-1-(2,6-Dimethylphenoxy)-3-{4-[2-(4-methoxy-phenoxy)-ethyl]piperazin-1-yl}-propan-2-ol dihydrochloride (**11**)*

Yield: 54%.; M.p. = 216–218 °C; ^{1}H NMR 2.23 (s, 6H, Ar-(C$\underline{H}_3$)$_2$, 3.10−4.00 (m, 12H, NH$^+$-C$\underline{H}_2$), 3.71 (s, 3H, Ar-O-C$\underline{H}_3$), 3.70−3.77 (m, 2H, Ar-O-C$\underline{H}_2$-), 4.36 (t, *J* = 4.7 Hz, 2H, C$\underline{H}_2$-O-Ar), 4.40−4.48 (m, 1H, CH$_2$-C$\underline{H}$(OH)-CH$_2$), 5.90 (bs, 1H, OH), 6.87−7.05 (m, 7H, Ar), 11.43 (bs, 1H, N$\underline{H}^{\pm}$), 12.81 (bs, 1H, N$\underline{H}^{\pm}$); IR 3261.04, 2406.73, 1509.03, 1224.58, 1035.59, 832.13; LogP = 3.86 +/− 0.47; R_f = 0.73
$C_{24}H_{36}N_2O_4Cl_2$(487.51).

4.2. Pharmacology

The pharmacological studies were carried out on male Wistar rats ((KRF.(WI).WU), Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Cracow) weighing 170 −350 g. Treatment of laboratory animals in the present study was in full accordance with the respective Polish regulations. All procedures were conducted according to Animal Care and Use Committee guidelines, and approved by the Ethical Committee of Jagiellonian University, Krakow.
Source of compounds: phenylephrine hydrochloride, acetylcholine hydrochloride, (±)-noradrenaline hydrochloride (Sigma, Aldrich Chemie Gmbh); thiopental sodium (Biochemie Gmbh, Vienna); [^{3}H]prazosin, [^{3}H]clonidine (Amersham). Other reagents were of analytical grade from local sources.

4.2.1. Radioligand binding test

The compounds were evaluated on their affinity for α_1- and α_2-adrenoceptors by determining for each compound its ability to displace [^{3}H]prazosin or [^{3}H]clonidine from specific binding sites on rat cerebral cortex. [^{3}H]prazosin (19.5 Ci/mmol, α_1-adrenergic receptor) and [^{3}H]clonidine (70.5 Ci/mmol, α_2-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 x 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 [^{3}H]prazosin (0.2 nM) or [^{3}H]clonidine (2 nM) solution and 30 µl buffer containing from seven to eight concentrations (10^{-11} – 10^{-4} M) of tested compounds. For measuring unspecific binding, phentolamine −10 µM (in the case of [^{3}H]prazosin) and clonidine −10 µM (in the case of [^{3}H]clonidine) were applied. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/C) using a vacum manifold (Millipore). The filters were then washed 2 times with the assay buffer and placed in scintillation vials with liquid scintillation coctail. Radioactivity was measured in WALLAC 1409 DSA – liquid scintillation counter. All assays were done in duplicates.
Radioligand binding data were analyzed (Maj et al. 1985) using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). Ki values were calculated from the Cheng and Prusoff equation.

4.2.2. Functional bioassay

Isolated rat aorta was used in order to test the antagonistic activity of investigated compounds for α_1-adrenoceptors. The male Wistar rats weighing 200–350 g were anaesthetized with thiopental sodium (75 mg/kg ip) and the aorta was dissected and placed in a Krebs-Henseleit solution and cleaned of surrounding fat tissues. The thoracic aorta was denuded of endothelium and cut into approximately 4 mm long rings. The aorta rings were incubated in 30 ml chambers filled with a Krebs-Henseleit solution (NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.25 mM, $MgSO_4$ 1.64 mM, KH_2PO_4 1.18 mM, $NaHCO_3$ 24.88 mM, glucose 10 mM, $C_3H_3O_3Na$ 2.2 mM, EDTA 0.05 mM) at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1). Two stainless steel pins were inserted through the lumen of each arterial segment: one pin was attached to the bottom of the chamber and the other to an isometric FDT10-A force displacement transducer (BIOPAC Systems, Inc., COMMAT Ltd., Turkey). The aortae rings were stretched and maintained at optimal tension of 2 g and allowed to equilibrate for 2 h. The lack of endothelium was confirmed by the absence of acetylocholine (1 µM) vasorelaxant action in aortic rings precontracted by noradrenaline (0.1 µM).
Cumulative concentration-response curves to phenylephrine (0.003 to 3 µM) were obtained by the method of van Rossum (1963). Following the first phenylephrine curve, aortae rings were incubated with one of three concentrations of tested compounds (one concentration of the antagonist was used in each arterial ring in every experiment) for 20 min and the next

cumulative concentration curve to phenylephrine was constructed. In order to avoid fatigue of the aortae preparation, a 60 min recovery period was allowed between phenylephrine curves.

Concentration-response curves were analysed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). Contractile responses to vasoconstrictor (in the presence or absence of tested compounds) are expressed as a percentage of the maximal phenylephrine effect (E_{max} = 100%), reached in the concentration-response curves obtained before incubation with the tested compounds. Data are the means ± SEM of at least 4 separate experiments. From the EC_{50} values of the agonist in the presence and absence of different antagonist concentrations, concentration-ratios were calculated. Schild plots were constructed, and where the slope was not significantly different from unity, the pA_2 values were determined (Arunlakshana and Schild 1959).

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