Pharmacological Reports
2010, 62, 68–85
ISSN 1734-1140

Synthesis and pharmacological evaluation of pyrrolidin-2-one derivatives as antiarrhythmic, antihypertensive and α-adrenolytic agents

Katarzyna Kulig[1], Cindy Spieces[1], Jacek Sapa[2], Christa Caspers[2], Barbara Filipek[2], Barbara Malawska[1]

[1]Department of Physicochemical Drug Analysis, [2]Department of Pharmacodynamic, Jagiellonian University Medical College, Medyczna 9, PL 30-688 Kraków, Poland

Correspondence: Katarzyna Kulig, e-mail: mfkkulig@cyf-kr.edu.pl

Abstract:
A series of novel arylpiperazines bearing a pyrrolidin-2-one fragment were synthesized and evaluated for their binding affinity for α_1- and α_2-adrenoceptors (ARs) as well as their antiarrhythmic and antihypertensive activities. The highest affinity for the α_1-AR was displayed by 1-{3-[4-(2-chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one **7**, which binds with a $pK_i = 7.13$. The highest affinity for the α_2-AR was shown by 1-{3-[4-(4-chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one **18,** which binds with a $pK_i = 7.29$. Among the compounds tested, 1-{3-[4-(2-ethoxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one **13** had the highest prophylactic antiarrhythmic activity in epinephrine-induced arrhythmia in anesthetized rats. Its ED_{50} value was 1.0 mg/kg intravenously (*iv*). The compounds with a hydroxy group in the 4-position of the phenyl ring or two substituents such as fluorine atoms in the 2 and 4 positions of the phenyl ring significantly decreased systolic and diastolic pressure in normotensive anesthetized rats at a dose of 2.5 mg/ kg *iv*, and their hypotensive effects lasted for longer than an hour.

Key words:
α-adrenolytic, antihypertensive, antiarrhythmics, pyrrolidin-2-one derivatives

Introduction

Adrenoreceptors (ARs), belonging to the superfamily of G-protein-coupled receptors, are grouped into three classes, α_1-, α_2-, and β-ARs, and are considered attractive therapeutic targets for the treatment of various diseases [11].

α_1-Adrenergic receptors mediate the effects of the sympathetic nervous system by binding the catecholamines, epinephrine and norepinephrine [9, 23]. Initially, α_1-AR antagonists were used in the therapy of hypertension (prazosin, doxazosin) [12], and then they became common in the treatment of benign prostatic hyperplasia (alfuzosin, tamsulosin) [4], in which they reduce the 'dynamic' component of bladder outlet obstruction and appear to have additional actions in reducing the irritative symptoms of this disease. α_1-AR antagonists are also used in the treatment of lower urinary tract symptoms and cardiac arrhythmia [4, 6, 12].

The α_2-ARs are located in the central nervous system. It has also been shown that α_2-ARs may be localized presynaptically, and act as negative modula-

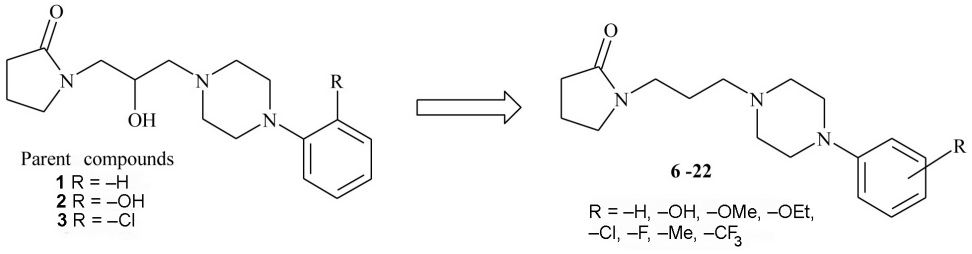

Fig. 1. Structures of parent and synthesized compounds

tors in the release of catecholamines and other neurotransmitters such as acetylcholine, γ-aminobutyric acid and nitrous oxide [7, 10]. α_2-AR antagonists are mainly used for the treatment of depression and diabetes (mirtazapine, yohimbine, idazoxan). Other potential clinical applications include cardiovascular disease, obesity, Raynaud's disease, male sexual dysfunction, Alzheimer's disease and Parkinson's disease [3, 22].

Non-selective α-AR antagonists such as phenoxybenzamine are effective in reducing radial artery spasm in coronary artery surgery [24].

We have previously reported that a series of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]- or 1-[3-(4-arylpiperazin-1-yl)-2-acetoxy]-propyl-pyrrolidin-2-one derivatives possess affinity for α_1- and α_2-ARs and show marked hypotensive and antiarrhythmic activities. Among the compounds tested, the most active were 1-[2-hydroxy-3(4-phenylpiperazin-1-yl)-propyl]-pyrrolidin-2-one **1** and those containing the hydroxyl- (**2**) or chloro- (**3**) substituent in the 2nd position of the phenyl ring [8, 15, 19, 20]. In this context, the goal of our research was the development of derivatives of arylpiperazine propyl-pyrrolidin-2-one as novel α-ARs antagonists. In this work, we report on the synthesis and *in vitro* and *in vivo* pharmacological studies of a series of new analogues of compounds **1**–**3**. For these compounds, the following were studied: the influence of the replacement of the 2-hydroxy-propyl fragment by a propyl one, modifications in the arylpiperazinyl moiety on their α_1- and α_2-AR affinity and their antiarrhythmic and hypotensive properties. Knowing that a hydrophobic group larger than a methoxy substituent may be accommodated by a hydrophobic pocket [1, 2], phenylpiperazine compounds with alkoxy moieties larger than a methoxy group at the 2nd position of the phenyl ring were prepared. The modifications in the arylpiperazinyl moiety also included an introduction of one (2-fluoro-, 2-, 3-, or 4-hydroxy-, 2-methyl; 2-trifluoromethyl-, 3-trifluoromethyl-, 2-, 3-, or 4-chloro-, 2-, 3-, or 4-methoxy-) or two (2,4-difluoro- and 2-methoxy-5-chlorophenyl-) different substituents into the phenyl ring. Finally, a more rigid analog of **1** was designed (Fig. 1). The newly synthesized compounds (as water-soluble hydrochlorides) were tested for α_1- and α_2-AR binding affinity in addition to their antiarrhythmic and hypotensive activities.

Materials and Methods

CHEMICAL PART

Unless otherwise noted, the starting materials were obtained from commercial suppliers (arylpiperazines from Chess chemicals GmbH, 1-bromo-3-chloropropane and 1-benzhydrylpiperazine from Sigma-Aldrich Chemie GmbH) and used without purification. All experiments in which air-sensitive materials were used were carried out in oven-dried glassware under a dry nitrogen atmosphere. Standard vacuum techniques were used for handling air-sensitive materials. Tetrahydrofuran (THF) was dried, kept under nitrogen and freshly distilled over sodium-benzophenone before use. Uncorrected melting points were determined in open glass capillaries on the Büchi 353 melting point apparatus. Elemental analyses (C, H, N) were carried out within 0.4% of the theoretical values and were performed on an Elementar Vario EL III (Elementar Analysensystem, Hanau, Germany). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury VX 300 MHz instrument in [d_6]-DMSO or $CDCl_3$ at ambient temperature using the solvent signal as an internal standard. Thin layer chromatography (TLC)

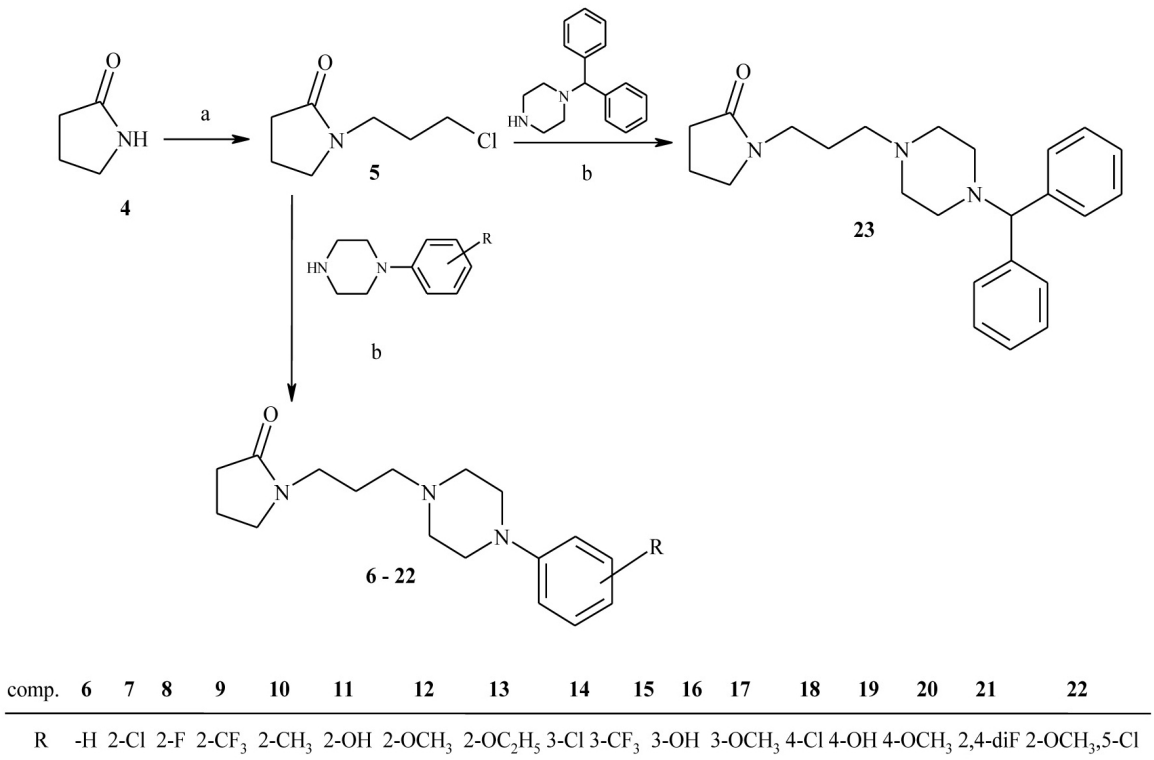

Fig. 2. Reagents and conditions: (a) NaH, THF, 1-bromo-3-chloro-propane, reflux, 12 h; (b) alloy, 150 C, 6 h. All compounds were isolated as hydrochloride salts using HCl_{gas} in anhydrous EtOH

was carried out on Merck silica gel pre-coated 60 F_{254} plates (0.2 mm) using S_1 – chloroform-acetone (1:1) as a developing system. The plates were visualized with UV light or iodine solution (0.05 M in a 10% HCl_{aq} (v/v)). Column chromatography was performed using Merck silica gel 60 and a chloroform-acetone (1:1) mixture as a solvent system.

Synthetic routes leading to the new compounds are presented in Figure 2.

1-(3-Chloro-propyl)-pyrrolidin-2-one **5**

To a suspension of NaH (0.2 mol, 6.0 g) in THF (30 mL), a solution of pyrrolidin-2-one **4** (0.2 mol, 15.2 mL) in THF (20 mL) was added dropwise and the resulting mixture was stirred at room temperature for an hour. Then a solution of 1-bromo-3-chloropropane (0.4 mol, 62.8 g) in THF (50 mL) was slowly added, and the reaction mixture was refluxed for 24 h. The precipitated inorganic salt was filtered through celite, and the filtrate concentrated under vacuum. The obtained oil was distilled under reduced pressure giving 25.4 g (yield 76%) 1-(3-chloro-propyl)-pyrrolidin-2-one. Anal. Calc. for $C_7H_{12}ClON$, calc. C 52.02%, H 7.48%, N 8.67%, found C 52.13%, H 7.59%, N 8.81%; b.p. 140°C/12 mbar, ^{1}H-NMR ($CDCl_3$): δ 1.72–1.83 (m, $CH_2CH_2CH_2Cl$ (propyl chain), 2H), 1.90–2.00 (m, CH_2-CH_2CH_2 (pyrrolidin-2-one), 2H), 2.23 (t, CH_2CH_2CO (pyrrolidin-2-one), 10.2 Hz, 2H), 3.20 (t, $CH_2CH_2CH_2Cl$ (propyl chain), 6.7 Hz, 2H), 2.35–2.49 (m, CH_2CH_2-CH_2Cl (propyl chain), NCH_2 CH_2CH_2 (pyrrolidin-2-one), 4H); ^{13}C-NMR ($CDCl_3$): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one)), 31.2 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 32.6 ($CH_2CH_2CH_2$ (propyl chain)), 42.2 (CH_2Cl (propyl chain)), 43.7 ($CH_2CH_2CH_2Cl$ (propyl chain)), 46.4 (NCH_2CH_2 (pyrrolidin-2-one)), 173.4 (carbonyl).

General procedure for the synthesis of 1-(3-substituted aminopropyl)pyrrolidin-2-one **6–23**

1.6 g (10 mmol) of 1-(3-chloro-propyl)-pyrrolidin-2-one **5** and 10 mmol of the corresponding amine were heated in an autoclave at 150°C (pressure 10 bar) for 6 h. The progress of the reaction was monitored by TLC. The obtained oily residue was purified by col-

umn chromatography using a mixture of chloroform-acetone (1:1) as a solvent. Then the obtained oil was dissolved in EtOH and HCl gas was bubbled through the solution until the mixture become acidic. The obtained precipitate was crystallized from EtOH.

1-[3-(4-Phenyl-piperazin-1-yl)-propyl]-pyrrolidin-2-one dihydrochloride **6**

Yield: 45.4%. Anal. for $C_{17}H_{25}N_3O$ 2HCl, calc. C 56.67%, H 7.55%, N 11.66%, found C 56.79%, H 7.62%, N 11.69%; M_r 360.32; m.p. 191.0–192.6°C; TLC $R_f = S_1(0.69)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.20–2.29 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.06–3.30 (m, piperazine, 8H), 3.53 –3.60 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3,73–3,79 (m, NCH_2CH_2 (propyl chain), CH_2CH_2N (pyrrolidin2-one), 4H), 6.82–7.27 (m, arom., 5H); ^{13}C-NMR ([d_6]-DMSO): δ 17.9 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 114.3, 118.3, 129.7 (arom.), 173.3 (carbonyl).

1-{3-[4-(2-Chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **7**

Yield: 42.8%. Anal. for $C_{17}H_{24}ClN_3O$ 2HCl, calc. C 51.72%, H 6.64%, N 10.64%, found C 51.92%, H 6.69%, N 10.80%; M_r 394.77; m.p. 197.5°C (dec.); TLC $R_f = S_1(0.68)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.19–2.28 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.10–3.28 (m, piperazine, 8H), 3.34-3.40 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.55 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.6 Hz), 3.75 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 4.1 Hz), 7.06–7.45 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 25.2 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 49.5 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 115.7, 119.7, 127.8, 123.2, 150.7 (arom.), 173.4 (carbonyl).

1-{3-[4-(2-Fluoro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **8**

Yield: 39.4%. Anal. for $C_{17}H_{24}FN_3O$ 2HCl, calc. C 53.97%, H 6.93%, N 11.11%, found C 54.00%, H 6.04%, N 11.20%; M_r 378.31; m.p. 175.2–176.9°C; TLC $R_f = S_1(0.75)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.20–2.30 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.12–3.29 (m, piperazine, 8H), 3.38–3.45 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.56 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.3 Hz), 3.74 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 4.8 Hz), 7.68–7.19 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 25.0 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 115.9, 116.4, 119.9, 125.3, 137.3, 155.7 (arom.), 173.4 (carbonyl).

1-{3-[4-(2-Trifluoromethyl-phenyl)-piperazin-1--yl]-propyl}-pyrrolidin-2-one dihydrochloride **9**

Yield: 47.8%. Anal. for $C_{18}H_{24}F_3N_3O$ 2HCl, calc. C 50.47%, H 6.12%, N 9.81%, found C 50.57%, H 6.32%, N 9.98%; M_r 428.32; m.p. 189.4–190.9°C; TLC $R_f = S_1(0.62)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.15–2.26 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.20–3.35 (m, piperazine, 8H), 3.40-3.54 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.56 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.4 Hz), 3.83 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 4.2 Hz), 7.54–7.69 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 113.3, 114.6, 118.9, 126.1, 133.0, 143.4 (arom.), 115.7 (CF_3), 173.4 (carbonyl).

1-[3-(4-o-Tolyl-piperazin-1-yl)-propyl]-pyrrolidin-2-one dihydrochloride **10**

Yield: 35.1%. Anal. for $C_{18}H_{27}N_3O$ 2HCl, calc. C 57.75%, H 7.81%, N 11.22%, found C 57.93%, H 7.83%, N 11.26%; M_r 374.35; m.p. 233°C (dec); TLC $R_f = S_1(0.71)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.18 (s, CH_3, 3H), 2.20-2.30 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.15–3.26 (m,

piperazine, 8H), 3.40–3.54 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.57 (t, NCH_2CH_2 (propyl chain), 2H, J = 6.0 Hz), 3.75 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 3.29 Hz), 6.96 –7.18 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 15.8 (CH_3), 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 114.2, 118.2, 127.0, 126.7, 127.0, 147.7 (arom.), 173.4 (carbonyl).

1-{3-[4-(2-Hydroxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **11**

Yield: 57.5%. Anal. for $C_{17}H_{25}N_3O_2$ 2 HCl, calc. C 54.26%, H 7.23%, N 11.17%, found C 54.30%, H. 7.30%, N 11.20%; M_r 376.32; m.p. 209.8–211.3°C; TLC R_f = S_1(0.76), ^{1}H-NMR ([d_6]-DMSO): δ 1.90–2.10 (m, $CH_2CH_2CH_2$, $CH_2CH_2CH_2$ (pyrrolidin-2-one), 4H), 2.21–2.35 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.69–2.74 (m, CH_2 piper., 4H), 2.85–2.99 (m, CH_2 piper., 4H), 3.25 (dd, CH_2CH_2N (propyl chain), 2H), 3.26 (s, O*H*, 1H), 3.43–3.58 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 6.42–6.64 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 115.7, 116.8, 119.7, 122.3, 142.4, 145.9 (arom.), 173.4 (carbonyl).

1-{3-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **12**

Yield: 35.1%. Anal. for $C_{18}H_{27}N_3O_2$ 2HCl, calc. C 55.38%, H 7.49%, N 10.76%, found C 55.42%, H 7.56%, N 10.76%; M_r 390.35; m.p. 195 °C (dec); TLC R_f = S_1(0.65), ^{1}H-NMR ([d_6]-DMSO): δ 2.20–2.29 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.08–3.21 (m, piperazine, 8H), 3.43–3.54 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.62 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.8 Hz), 3.70 (s, OCH_3, 3H), 3.75 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 3.3 Hz), 6.86 –7.03 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 55.9 (CH_3), 115.2, 115.3, 119.3, 122.0, 144.3, 145.5 (arom.), 173.4 (carbonyl).

1-{3-[4-(2-Ethoxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **13**

Yield: 39.4%. Anal. for $C_{19}H_{29}N_3O_2$ 2HCl, calc. C 56.43%, H 7.73%, N 10.39%, found 56.46%, H 7.80%, N 10.40%; M_r 404.37; m.p. 169.4–171.0°C; TLC R_f = S_1(0.71), ^{1}H-NMR ([d_6]-DMSO): δ 1.32 (t, CH_3CH_2, 3H, J = 6.3 Hz), 2.18–2.28 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.01–3.26 (m, piperazine, 8H), 3.49–3.56 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.54 (t, NCH_2CH_2 (propyl chain), 2H, J = 6.7 Hz), 3.64 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 3.7 Hz), 4.02 (s, OCH_2, 2H, J = 6.3 Hz), 6.96–7.18 (m, arom., 4H), ^{13}C-NMR ([d_6]-DMSO): δ 14.8 (CH_3), 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 64.7 (CH_2), 114.9, 115.3, 118.9, 121.3, 142.3, 144.4 (arom.), 173.4 (carbonyl).

1-{3-[4-(3-Chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **14**

Yield: 37.6%. Anal. for $C_{17}H_{24}ClN_3O$ 2HCl, calc. C 51.72%, H 6.64%, N 10.64%, found C 52.00%, H 6.68%, N 10.67%; M_r 394.77; m.p. 201.4°C (dec); TLC R_f = S_1(0.81), ^{1}H-NMR ([d_6]-DMSO): δ 2.20–2.32 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.10–3.28 (m, piperazine, 8H), 3.38-3.48 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.61 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.3 Hz), 3.89 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 4.9 Hz), 7.12-7.30 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 25.0 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 112.4, 114.7, 118.4, 131.1, 135.2, 151.0 (arom.), 173.4 (carbonyl).

1-{3-[4-(3-Trifluoromethyl-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **15**

Yield: 57.2%. Anal. for $C_{18}H_{24}F_3N_3O$ 2HCl, calc. C 50.47%, H 6.12%, N 9.81%, found C 50.50%, H 6.13%, N 9.85%; M_r 428.32; m.p. 192.3–193.6°C; TLC R_f = S_1(0.48), ^{1}H-NMR ([d_6]-DMSO): δ 2.22–2.34 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.17–3.37 (m, piperazine, 8H), 3.43-3.57 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.60 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.9 Hz), 3.92 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 4.2 Hz), 7.65–7.72 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.2 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 110.1, 114.7, 117.6, 130.0, 131.9, 149.9 (arom.), 124.2 (CF_3), 173.4 (carbonyl).

1-{3-[4-(3-Hydroxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **16**

Yield: 57.5%. Anal. for $C_{17}H_{25}N_3O_3$ 2 HCl, calc. C 54.26%, H 7.23%, N 11.17%, found C 54.30%, H 7.0%, N 11.20%; M_r 376.32; m.p. 132.3–133.8°C; TLC R_f = S_1(0.55), ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.26–2.38 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.70– 2.79 (m, CH_2 piper., 4H), 2.79–2.85 (m, CH_2 piper., 4H), 3.13 (dd, CH_2CH_2N (propyl chain), 2H), 3.46 (s, O*H*, 1H), 3.58–3.72 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 6.06–6.15 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 25.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 99.1, 105.4, 106.9, 131.1, 151.0, 159.4 (arom.), 173.4 (carbonyl).

1-{3-[4-(3-Methoxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **17**

Yield: 53.1%. Anal. for $C_{18}H_{27}N_3O_2$ 2HCl, calc. C 55.38%, H 7.49%, N 10.76%, found C 55.43%, H 7.50%, N 10.80%; M_r 390.35; m.p. 190.2–191.6°C; TLC R_f = S_1(0.58), ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.36–2.48 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.65–2.71 (m, CH_2 piper., 4H), 2.82–2.97 (m, CH_2 piper., 4H), 3.21 (dd, CH_2CH_2N (propyl chain), 2H), 3.52–3.68 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 3.73 (s, CH_3, 3H), 5.99–6.43 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (propyl chain)), 52.6 (piperazine), 55.9 (CH_3), 97.5, 103.8, 106.6, 130.7, 150.6, 161.6 (arom.), 173.4 (carbonyl).

1-{3-[4-(4-Chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **18**

Yield: 43.2%. Anal. for $C_{17}H_{24}ClN_3O$ 2HCl, calc. C 51.72%, H 6.64%, N 10.64%, found C 52.00%, H 6.70%, N 10.60%; M_r 394.77; m.p. 213.0°C (dec.); TLC R_f = S_1(0.71), ^{1}H-NMR ([d_6]-DMSO): δ 2.17–2.32 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 3.11–3.30 (m, piperazine, 8H), 3.40–3.52 (m, CH_2CH_2N (propyl chain), CH_2CH_2CO (pyrrolidin-2-one), 4H), 3.68 (t, NCH_2CH_2 (propyl chain), 2H, J = 5.7 Hz), 3.92 (t, CH_2CH_2N (pyrrolidin-2-one), 2H, J = 5.4 Hz), 7.12–7.26 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (pyrrolidin-2-one)), 52.6 (piperazine), 115.7, 123.8, 129.8, 147.7 (arom.), 173.4 (carbonyl).

1-{3-[4-(4-Hydroxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **19**

Yield: 57.5%. Anal. for $C_{17}H_{25}N_3O_2$ 2 HCl, calc. C 54.26%, H 7.23%, N 11.17%, found C 54.30%, H 7.30%, N 11.20%; M_r 376.32; m.p. 189.2–190.2°C; TLC R_f = S_1(0.54), ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.26–2.38 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.70–2.79 (m, CH_2 piper., 4H), 2.79–2.85 (m, CH_2 piper., 4H), 3.13 (dd, CH_2CH_2N (propyl chain), 2H), 3.46 (s, O*H*, 1H), 3.58–3.72 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 6.42–6.55 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)),

44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (pyrrolidin-2-one)), 52.6 (piperazine), 115.7, 116.8, 142.2, 148.0 (arom.), 173.4 (carbonyl).

1-{3-[4-(4-Methoxy-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **20**

Yield: 56.1%. Anal. for $C_{18}H_{27}N_3O_2$ 2HCl, calc. C 55.38%, H 7.49%, N 10.76%, found C 55.40%, H 7.54%, N 10.85%; M_r 390.35; m.p. 165.2–166.9°C; TLC $R_f = S_1(0.56)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.36–2.48 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.65–2.71 (m, CH_2 piper., 4H), 2.82–2.97 (m, CH_2 piper., 4H), 3.21 (dd, CH_2CH_2N (propyl chain), 2H), 3.52–3.68 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 3.83 (s, CH_3, 3H), 6.32–6.55 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 (CH_2 CH_2CH_2 (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO(pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (pyrrolidin-2-one)), 52.6 (piperazine), 55.9 (CH_3), 115.2, 115.3, 141.9, 150.2 (arom.), 173.4 (carbonyl).

1-{3-[4-(2,4-Difluorophenyl)piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **21**

Yield: 65.2%. Anal. for $C_{17}H_{23}F_2N_3O$ 2HCl, calc. C 51.52%, H 6.36%, N 10.60%, found C 51.59%, H 6.47%, N 10.76%; M_r 396.30; m.p. 184.3–185.8°C; TLC $R_f = S_1(0.42$ ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.46–2.68 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.75–2.77 (m, CH_2 piper., 4H), 2.92–3.01 (m, CH_2 piper., 4H), 3.11 (dd, CH_2CH_2N (propyl chain), 2H), 3.45–3.51 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 6.39–6.52 (m, arom., 3H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 (CH_2 CH_2CH_2 (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (pyrrolidin-2-one)), 52.6 (piperazine), 105.6, 112.0, 117.5, 132.9, 154.0, 157.3 (arom.), 173.4 (carbonyl).

1-{3-[4-(4-Chloro-2-methoxyphenyl)piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **22**

Yield: 58.3%. Anal. for $C_{18}H_{26}ClN_3O_2$ 2HCl, calc. C 50.89%, H 6.64%, N 9.89%, found C 50.93%, H 6.74%, N 9.96%; M_r 424.79; m.p. 195.2–196.8°C; TLC $R_f = S_1(0.24)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.32–2.48 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.65–2.79 (m, CH_2 piper., 4H), 2.82–2.99 (m, CH_2 piper., 4H), 3.07 (dd, CH_2CH_2N (propyl chain), 2H), 3.57–3.68 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 3.79 (s, CH_3, 3H), 6.29–6.58 (m, arom., 3H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$), 32.6 (CH_2CO), 44.3 (NCH_2CH_2), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.3 (piperazine), 51.5 (CH_2CH_2N), 52.6 (piperazine), 55.9 (CH_3), 116.7, 122.1, 124.6, 142.4, 146.9 (arom.), 173.4 (carbonyl).

1-[3-(4-Benzhydryl-piperazin-1-yl)-propyl]-pyrrolidin-2-one dihydrochloride **23**

Yield: 58.3%. Anal. for $C_{24}H_{31}N_3O$ 2HCl, calc. C 63.99%, H 7.38%, N 9.33%, found C 64.32%, H 7.43%, N 10.05%; M_r 450.44; m.p. 204.2–204.8°C; TLC $R_f = S_1(0.35)$, ^{1}H-NMR ([d_6]-DMSO): δ 2.00–2.20 (m, $CH_2CH_2CH_2$ (pyrrolidin-2-one), $CH_2CH_2CH_2$ (propyl chain), 4H), 2.37–2.49 (m, CH_2CO (pyrrolidin-2-one), NCH_2CH_2 (propyl chain), 4H), 2.59–2.69 (m, CH_2 piper., 4H), 2.82–3.00 (m, CH_2 piper., 4H), 3.07 (dd, CH_2CH_2N (propyl chain), 2H), 3.57–3.68 (m, CH_2CH_2N (pyrrolidin-2-one), 2H), 4.21 (s, CH, 1H), 6.29–6.58 (m, arom., 10H); ^{13}C-NMR ([d_6]-DMSO): δ 17.7 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 24.9 ($CH_2CH_2CH_2$ (propyl chain)), 32.6 (CH_2CO (pyrrolidin-2-one)), 44.3 (NCH_2CH_2 (propyl chain)), 46.4 ($CH_2CH_2CH_2$ (pyrrolidin-2-one), 50.0 (piperazine), 51.5 (CH_2CH_2N (pyrrolidin-2-one)), 53.3 (piperazine), 73.6 (CH), 126.3, 128.3, 129.3, 142.8 (arom.), 173.4 (carbonyl).

PHARMACOLOGICAL PART

Chemicals

[^{3}H]Clonidine (Amersham, Germany), epinephrine (Adrenalinum hydrochloricum, Polfa, Poland), norepinephrine (Levonor, Polfa, Poland), methoxamine (Sigma-Aldrich Chemie GmbH, Germany), [^{3}H]pra-

zosin (Amersham, Germany), tyramine (Sigma-Aldrich Chemie GmbH, Germany), sodium heparin (Polfa, Poland), thiopental sodium (Biochemie GmbH, Vienna, Austria), Urapidil (Sigma-Aldrich Chemie GmbH, Germany).

α-Adrenoceptor radioligand binding assay

These experiments were carried out in rat cerebral cortex. [^{3}H]Prazosin (19.5 Ci/mmol, an α_1-adrenergic receptor) and [^{3}H]clonidine (70.5 Ci/mmol, an α_2-adrenergic receptor) were used. Rat brains were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.6) and were centrifuged at 20,000 × g for 20 min (0–4 °C). The cell pellet was resuspended in the Tris-HCl buffer and centrifuged again. Radioligand binding assays were performed in plates (MultiScreen/Millipore, USA). The final incubation mixture (final volume 300 μL) consisted of 240 μL of the membrane suspension, 30 μL of [^{3}H]prazosin (0.2 nM) or [^{3}H]clonidine (2 nM) solution and 30 μL of the buffer containing seven to eight concentrations (10^{-11}–10^{-4}M) of the tested compounds [18]. To measure the unspecific binding, 10 μM phentolamine (in case of [^{3}H]prazosin) or 10 μM clonidine (in case of [^{3}H]clonidine) was applied. The incubation was terminated by rapid filtration over glass fiber and placed in scintillation vials with a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter (Perkin Elmer USA). All assays were carried out in duplicate. Radioligand binding was analyzed using an iterative curve-fitting routine (GraphPad/Prism, Version 3.0 – San Diego, CA, USA). K_i values were calculated from methods described by Cheng and Prusoff [5].

Animals

The experiments were carried out on male Wistar rats (180–250 g). Animals were housed in constant temperature facilities under 12/12 h light-dark cycle, maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 8–10 animals each. All procedures were done according to the Animal Care and Use Committee Guidelines and approved by the Ethical Committee of the Jagiellonian University, Kraków, Poland (registration number 17/OP/2005 and ZI/UJ/217/2005).

Prophylactic antiarrhythmic activity in a model of epinephrine-induced arrhythmia according to Szekeres and Papp [25]

Arrhythmia was evoked in thiopental (60 mg/kg, *ip*) anesthetized rats by *iv* injection of adrenaline (20 μg/kg). The tested compounds were administered intravenously 15 min before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and the inhibition of rhythm disturbances in comparison with the control group (ventricular bradycardia, atrioventricular block, ventricular tachycardia or ventricular fibrillation). The cardiac rhythm disturbances were recorded for 15 min after adrenaline injection. ECGs were analyzed according to the guidelines of the Lambeth Convention [26] on ventricular premature beats (VBs), bigeminy, salvos (less than four successive VBs), ventricular tachycardia (VT, four or more successive VBs) and ventricular fibrillation (VF). The ED_{50} value was calculated according to the method of Lichfield and Wilcoxon [17].

The influence on blood pressure

Male Wistar normotensive rats were anesthetized with thiopental (50–75 mg/kg, *ip*). The right carotid was cannulated with a polyethylene tube filled with heparin in saline to facilitate pressure measurement using the Datamax apparatus (Columbus Instruments, USA). The studied compounds were injected in a single dose of 2.5 or 5.0 mg/kg into the caudal vein after a 5 min stabilization period at a volume equivalent to 1 ml/kg.

Compound (**1**) and urapidil were used as references.

Statistical analysis

The data are expressed as the mean ± SEM. The statistical significance was calculated using a one-way ANOVA. Differences were considered significant when $p < 0.05$.

Results

In the present study, several pharmacological tests were carried out to assess α_1- and α_2-AR affinity, as well as antiarrhythmic and hypotensive activities of the novel pyrrolidin-2-one derivatives **6–23**.

Tab. 1. Affinity of compounds 1–3 and 6–23 for different α-AR subtypes in rat cerebral cortex

Compound	pK_i [^{3}H] prazosin (α_1 rec.)	pK_i [^{3}H] clonidine (α_2 rec.)
1	5.72 [7]	4.54 [7]
2	6.71 [18]	5.64 [18]
3	6.57 [18]	4.79 [18]
6	6.25 ± 0.36	6.98 ± 0.01
7	7.13 ± 0.02	6.69 ± 0.01
8	6.73 ± 0.07	7.14 ± 0.07
9	5.33 ± 0.02	5.52 ± 0.01
10	7.05 ± 0.04	6.82 ± 0.09
11	6.79 ± 0.02	6.54 ± 0.05
12	6.81 ± 0.02	6.94 ± 0.06
13	7.09 ± 0.01	6.98 ± 0.08
14	6.61 ± 0.07	6.79 ± 0.09
15	6.06 ± 0.04	6.67 ± 0.12
16	6.37 ± 0.09	5.89 ± 0.01
17	6.30 ± 0.04	6.87 ± 0.03
18	6.21 ± 0.04	7.29 ± 0.07
19	5.59 ± 0.08	5.57 ± 0.04
20	5.29 ± 0.02	6.08 ± 0.07
21	6.30 ± 0.13	6.20 ± 0.06
22	6.94 ± 0.10	6.85 ± 0.05
23	6.10 ± 0.04	7.03 ± 0.03
Urapidil	6.89 ± 0.05	–
Tolazoline	5.79 ± 0.02	6.50 ± 0.03

The mean pK_i ± SEM values were obtained from three experiments. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff [4]

The pharmacological profile of the new compounds was evaluated by radioligand binding assays (the ability to displace [^{3}H]prazosin or [^{3}H]clonidine from α_1- or α_2-ARs, respectively) in rat cerebral cortex [5, 16]. All tested compounds displaced [^{3}H]prazosin (pK_i α_1-AR 5.33–7.13) and [^{3}H]clonidine (pK_i α_2-AR 5.52–7.29) from cortical binding sites. The results obtained are presented in Table 1.

Compounds **6**–**23** were tested for their prophylactic antiarrhythmic activity in an epinephrine-induced arrhythmia rat model [25]. Intravenous (*iv*) injections of adrenaline at a dose of 20 μg/kg caused reflex bradycardia (100%), supraventricular and ventricular extrasystoles (100%), bigeminy (75%) and ventricular tachycardia (50%) in rats, which led to the death of approximately 50% of animals within 10 ± 5 min. Compounds **6**–**8**, **11**, **13** and **17** injected intravenously 15 min before adrenaline administration diminished the occurrence of extrasystoles, bigeminy and reduced mortality. The ED_{50} values are presented in Table 2. The highest activity in this model of arrhythmia was displayed by compound **13**, which had an ED_{50} value of 1.0 mg/kg.

Tab. 2. The prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia in anesthetized rats. Compounds were administered intravenously

Compound	ED_{50} (mg/kg)
1	7.6 (6.9–8.4) [7]
6	2.8 (2.1–3.6)
7	5.1 (3.7–6.1)
8	8.9 (6.4–10.5)
11	13.1 (9.9–15.4)
12	5.2 (4.1–6.4)
13	1.0 (0.8–1.2)
17	12.7 (8.7–14.7)
Tolazoline	3.4 (2.6–4.4)
Propranolol	1.05 (0.64–1.73)
Urapidil	1.26 (0.97–1.64)

Each value was obtained from three experimental groups. Each group consisted of six animals. The ED_{50} values and their confidence limits were calculated according to the methods of Litchfield and Wilcoxon [16]

The hypotensive activity of compounds **6**–**23** was determined after *iv* administration to normotensive anesthetized rats at doses of 2.5–10 mg/kg. The results are presented in Table 3. Compounds **6**, **7**, **11**–**13**, **15** and **19**–**23** significantly decreased systolic and diastolic pressure. The observed effect lasted for more than 60 min. Compounds **8**–**10**, **14** and **16**–**19** were found to be inactive.

The influence of compounds **6**–**23** on pressor responses to epinephrine, norepinephrine, methoxamine and tyramine was studied. Intravenous epinephrine, norepinephrine, methoxamine and tyramine were given to rats at a dose of 2 μg/kg, 2 μg/kg, 150 μg/kg and 200 μg/kg, respectively, to induce pressor response. Compounds **7**, **8**, **10**, **13** and **21** given *iv* at a dose of 2.5, 5 or 10 mg/kg significantly antagonized the pressor response elicited by epinephrine, norepinephrine and methoxamine. Compounds **6**, **14**, **17**, **18** and **23**

Tab. 3. The hypotensive activity of tested compounds in anesthetized normotensive rats after *iv* administration

Comp.	Dose mg/kg	Blood pressure (mmHg ± SEM)	Time of observation (min)					
			0	5	10	20	30	60
6	2.5	systolic	153.5 ± 9.5	132.5 ± 5.5***	134 ± 2.0**	132.5 ± 0.5***	133.5 ± 0.5**	153.5 ± 9.5
		diastolic	144 ± 10	121.5 ± 5.5**	122 ± 3.0**	120.5 ± 1.5***	121 ± 1.0***	144 ± 10
7	10.0	systolic	141.5 ± 1.5	109.5 ± 8.5*	114.5 ± 5.5	111.5 ± 9.5*	109.5 ± 13.5*	110 ± 13*
		diastolic	126 ± 4	97.5 ± 8.5*	101 ± 5.0	98 ± 9*	96.5 ± 11.5*	96 ± 11*
11	5.0	systolic	135 ± 14	101 ± 18***	104 ± 11***	117 ± 14**	122 ± 6*	128 ± 14
		diastolic	109 ± 12	80 ± 11***	83 ± 12**	96 ± 15*	100 ± 12	105 ± 13
12	2.5	systolic	139 ± 6.4	114 ± 9***	120 ± 11*	121 ± 10	130 ± 10	131 ± 14
		diastolic	119 ± 10	98 ± 5**	105 ± 11*	114 ± 7	113 ± 9	117 ± 112
13	5.0	systolic	141.0 ± 0.6	127.0 ± 8.2*	128.3 ± 6.6	125.0 ± 3.6*	125.0 ± 4.0*	129.0 ± 2.5
		diastolic	126.7 ± 3.5	115.7 ± 8.2	115.3 ± 8.9	112.7 ± 5.9	111.0 ± 5.7	114.3 ± 3.3
15	2.5	systolic	133.7 ± 1.9	112.7 ± 5.4****	114.0 ± 3.6****	117.3 ± 3.4***	118.0 ± 3.1***	117.0 ± 4.0****
		diastolic	106.7 ± 2.8	81.0 ± 8.4**	86.3 ± 5.8*	89.7 ± 5.5	91.7 ± 4.9	89.3 ± 8.4
20	5.0	systolic	134.7 ± 1.3	125.0 ± 7.4	123.7 ± 1.7	118.3 ± 0.9	115.7 ± 2.6	120.3 ± 5.0
		diastolic	115.0 ± 2.5	103.3 ± 4.7	98.7 ± 2.0 *	94.7 ± 1.7**	91.3 ± 0.9***	96.0 ± 1.0*
21	2.5	systolic	135.0 ± 4.4	117.0 ± 1.2****	119.0 ± 1.0****	118.7 ± 0.9****	124.0 ± 2.6***	123.7 ± 3.2***
		diastolic	110.7 ± 2.2	96.3 ± 2.2***	97.0 ± 2.1***	96.7 ± 1.8***	98.3 ± 4.4***	98.0 ± 2.5
22	5.0	systolic	132.8 ± 4.4	115.5 ± 4.9***	114.8 ± 4.3***	113.3 ± 4.3***	113.0 ± 4.2***	117.8 ± 5.7**
		diastolic	119.3 ± 3.4	99.8 ± 1.4****	100.0 ± 1.1****	97.8 ± 1.5****	97.0 ± 0.9****	102.8 ± 3.1****
23	5.0	systolic	129.2 ± 0.7	119.2 ± 1.4	116.7 ± 2.1*	113.0 ± 2.9***	110.5 ± 3.9***	107.7 ± 6.0****
		diastolic	99.5 ± 4.1	88.0 ± 4.2	85.0 ± 5.3	82.2 ± 6.2*	81.0 ± 6.9*	77.2 ± 8.4**
Urapidil	0.625	systolic	135.5 ± 2.6	121 ± 2.6*	122 ± 2.5*	121 ± 2.6*	119.5 ± 3.1**	116.2 ± 4.6**
		diastolic	117.2 ± 1.7	102.7 ± 1.9**	104 ± 2**	101.5 ± 2.6***	101 ± 2.6***	99.7 ± 3.3***

The data are the means of six experiments ± SEM; statistical analyses were performed using a one-way ANOVA test. * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

given *iv* at a dose of 2.5 or 5 mg/kg significantly antagonized systolic pressor response elicited only by epinephrine. Compound **11** given *iv* at a dose of 5 mg/kg significantly antagonized pressor response elicited by epinephrine, norepinephrine, methoxamine and tyramine. Compounds **12**, **15**, **16** and **22** given *iv* at a dose 2.5 or 5 mg/kg significantly antagonized pressor response elicited by epinephrine and methoxamine. Compound **20** given *iv* at a dose 2.5 mg/kg significantly antagonized pressor response elicited by epinephrine, norepinephrine and methoxamine. (Figs. 3–20). However, compounds **9** and **19** did not have any significant influence on systolic pressor response generated by epinephrine, norepinephrine, methoxamine and tyramine (data not shown).

Discussion

All the newly synthesized compounds, **6**–**23,** were found to have an affinity toward α_1- and α_2-ARs, which was comparable to or higher than the affinity of the earlier reported compounds [15, 19, 20]. The highest affinity for α_1-AR (pK_i 7.13) was displayed by compound **7**, which has a chlorine atom in the 2-position of the phenyl ring. The highest affinity for α_2-AR (pK_i 7.29) was shown by compound **18**, which has a chlorine atom in the 4-position of the phenyl ring. Among the isomers with a methoxy- (**11**, **17** and **20**), hydroxy- (**11**, **16** and **19**) or chloro- (**7**, **14** and **18**) substituent in the phenyl ring, the af-

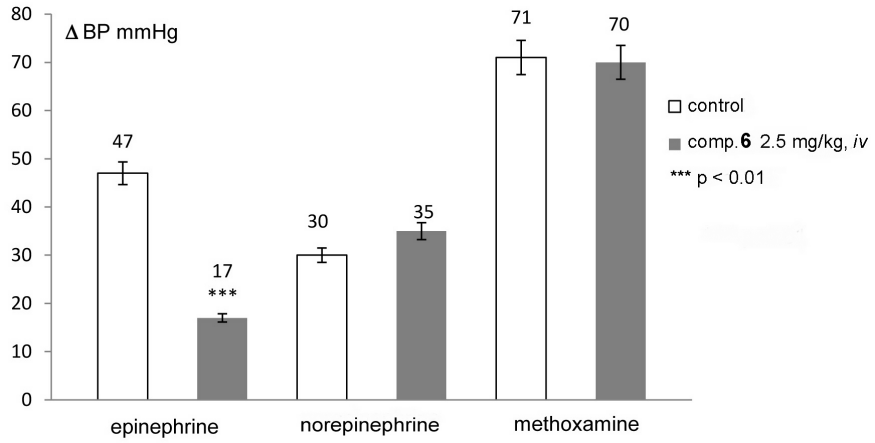

Fig. 3. The effect of comp. **6** on the blood pressure response to epinephrine, norepinephrine and methoxamine

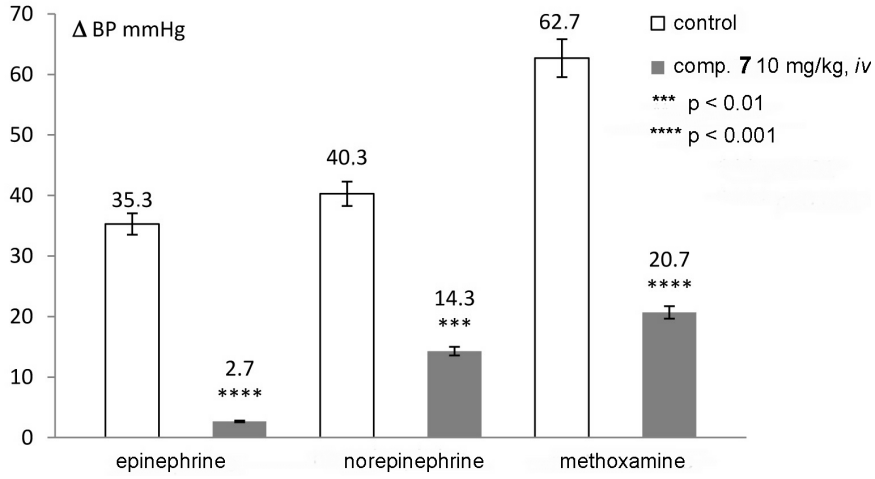

Fig. 4. The effect of comp. **7** on the blood pressure response to epinephrine, norepinephrine and methoxamine

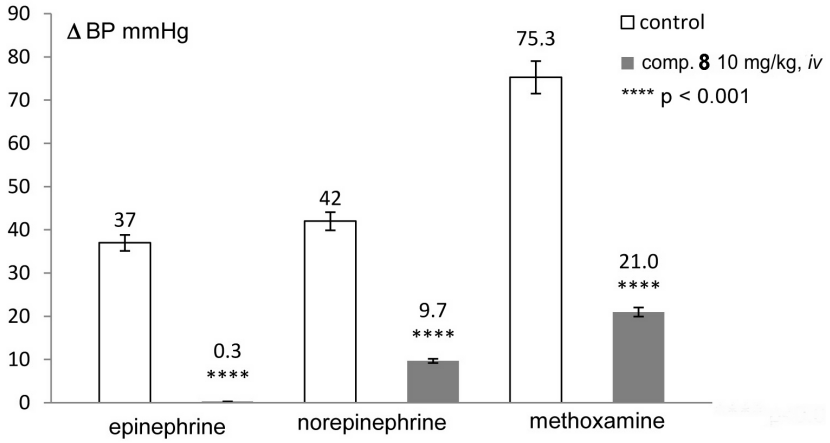

Fig. 5. The effect of comp. **8** on the blood pressure response to epinephrine, norepinephrine and methoxamine

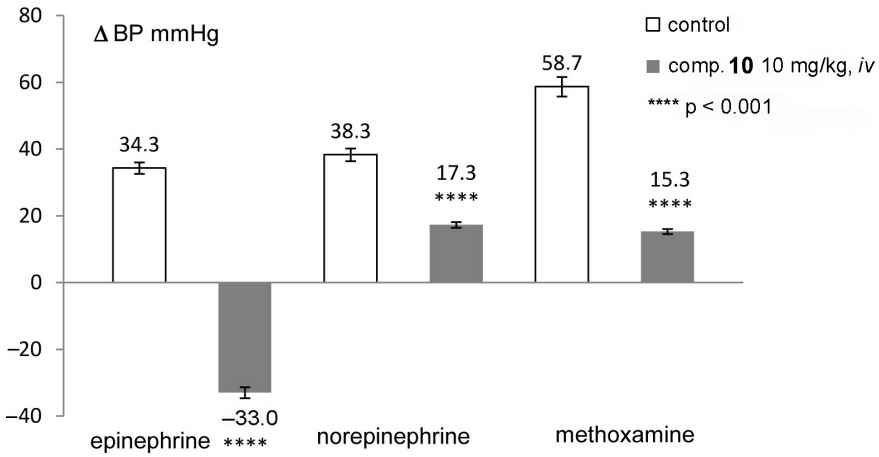

Fig. 6. The effect of comp. **10** on the blood pressure response to epinephrine, norepinephrine and methoxamine

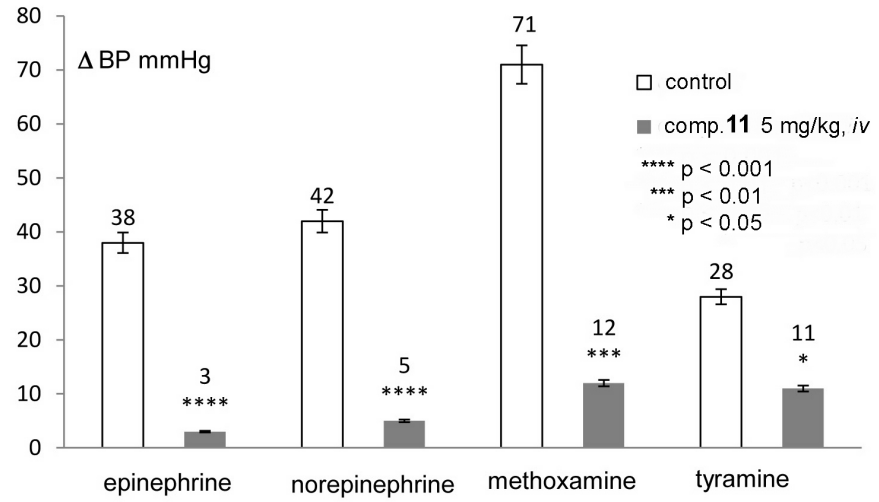

Fig. 7. The effect of comp. **11** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

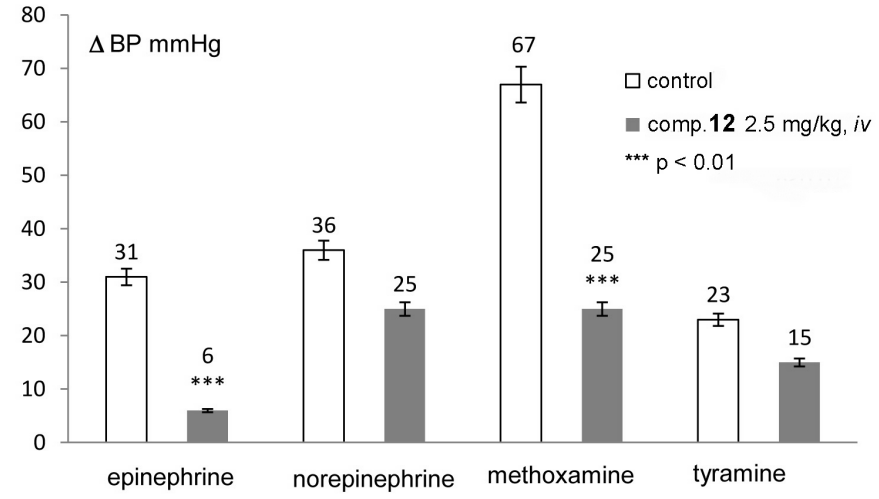

Fig. 8. The effect of comp. **12** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

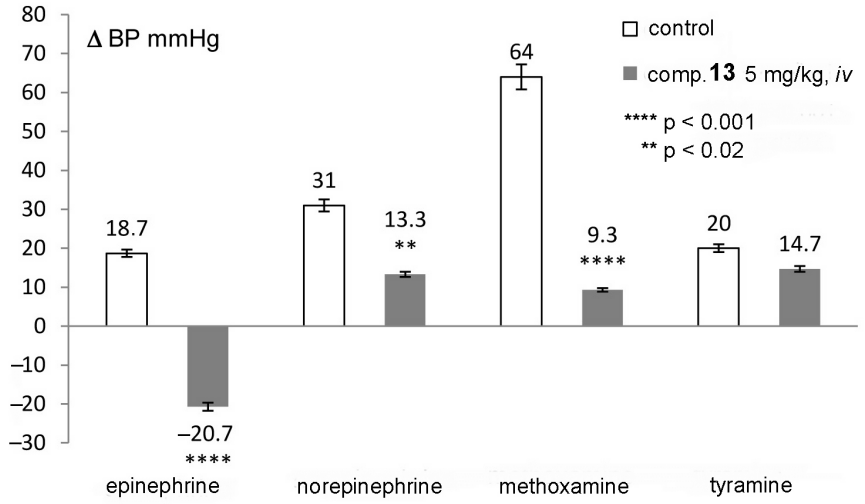

Fig. 9. The effect of comp. **13** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

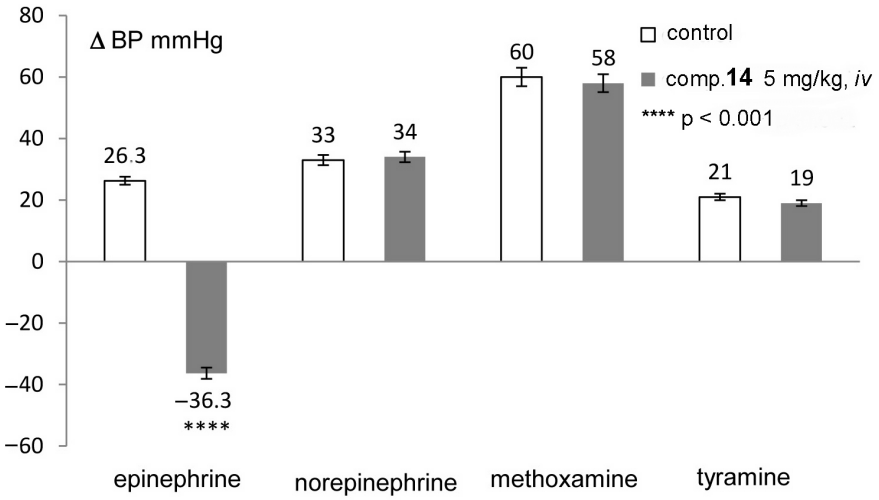

Fig. 10. The effect of comp. **14** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

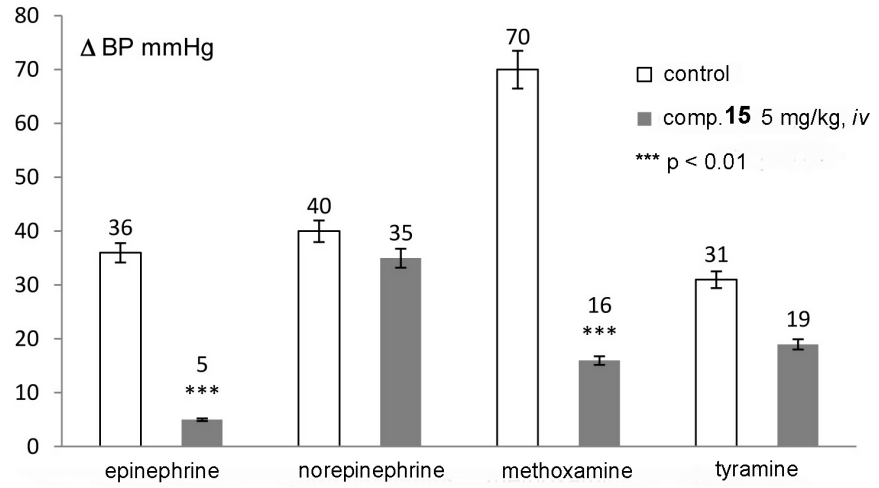

Fig. 11. The effect of comp. **15** on the blood pressure response to epinephrine, norepinephrine and methoxamine

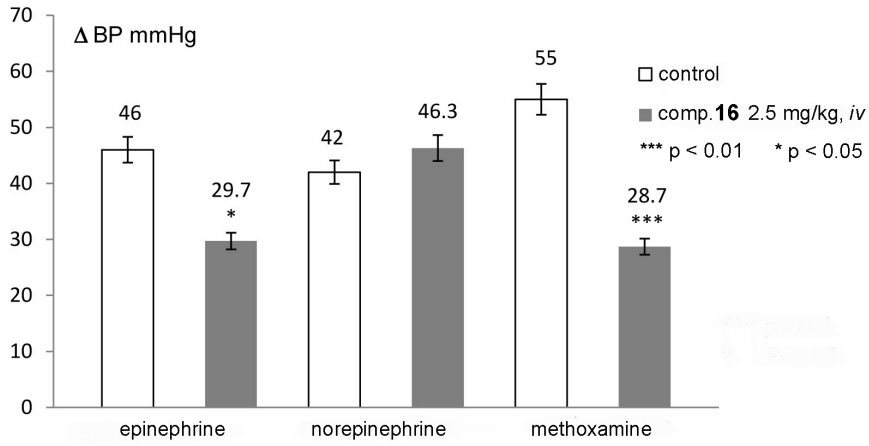

Fig. 12. The effect of comp. **16** on the blood pressure response to epinephrine, norepinephrine and methoxamine

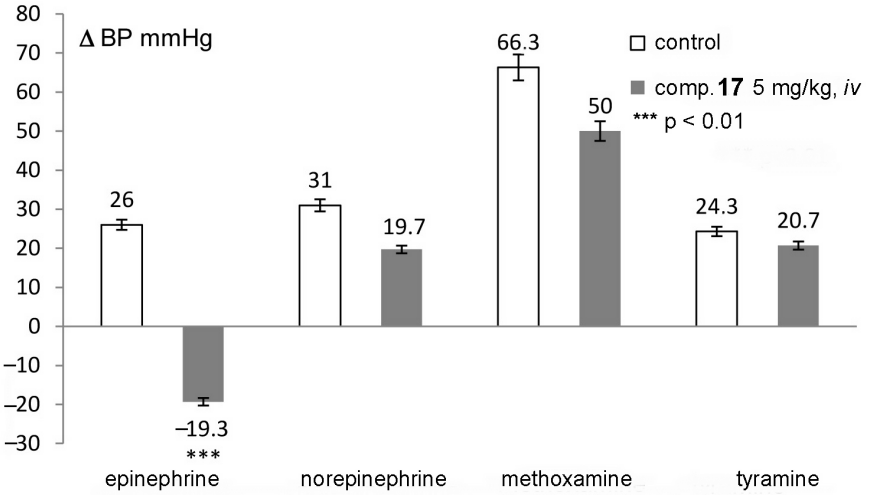

Fig. 13. The effect of comp. **17** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

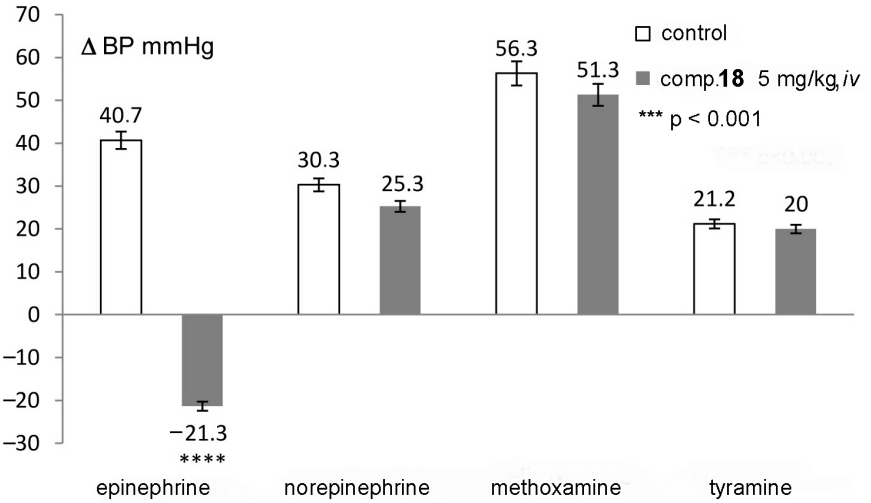

Fig. 14. The effect of comp. **18** on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

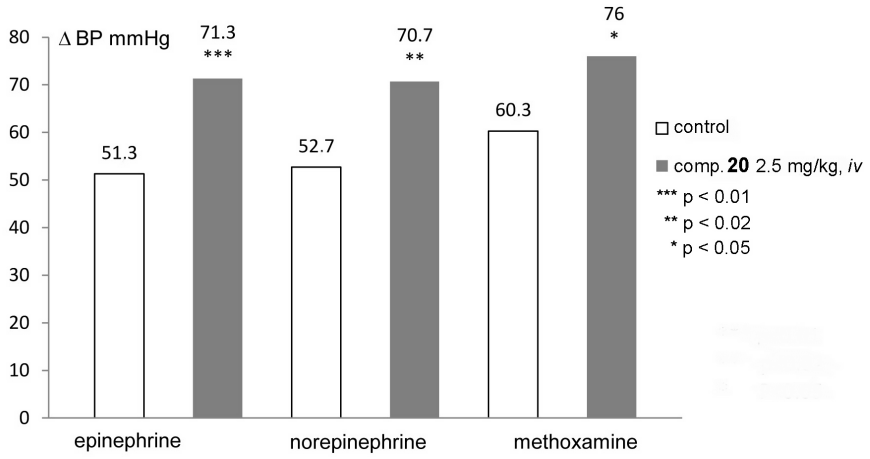

Fig. 15. The effect of comp. **20** on the blood pressure response to epinephrine, norepinephrine and methoxamine

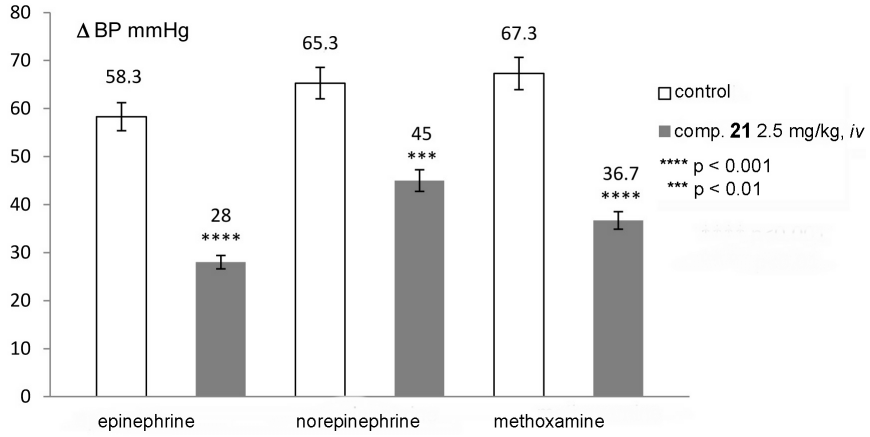

Fig. 16. The effect of comp. **21** on the blood pressure response to epinephrine, norepinephrine and methoxamine

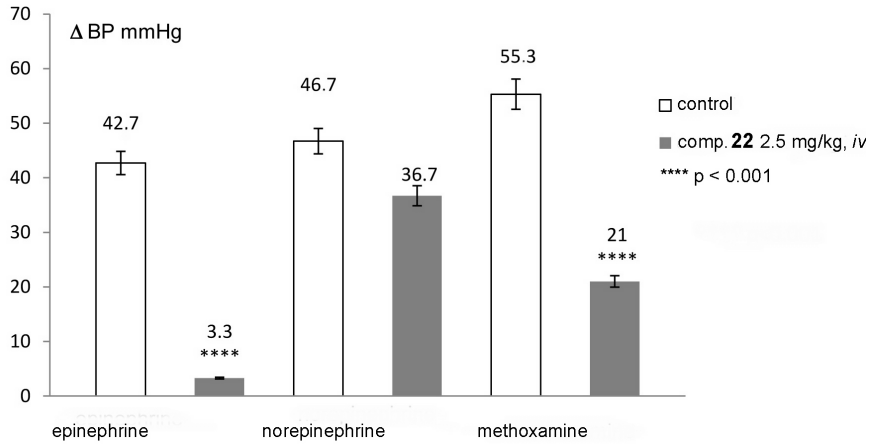

Fig. 17. The effect of comp. **22** on the blood pressure response to epinephrine, norepinephrine and methoxamine

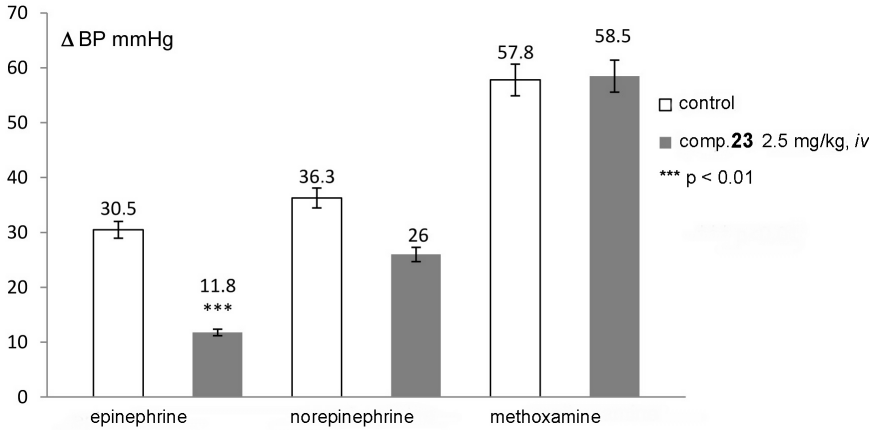

Fig. 18. The effect of comp. **23** on the blood pressure response to epinephrine, norepinephrine and methoxamine

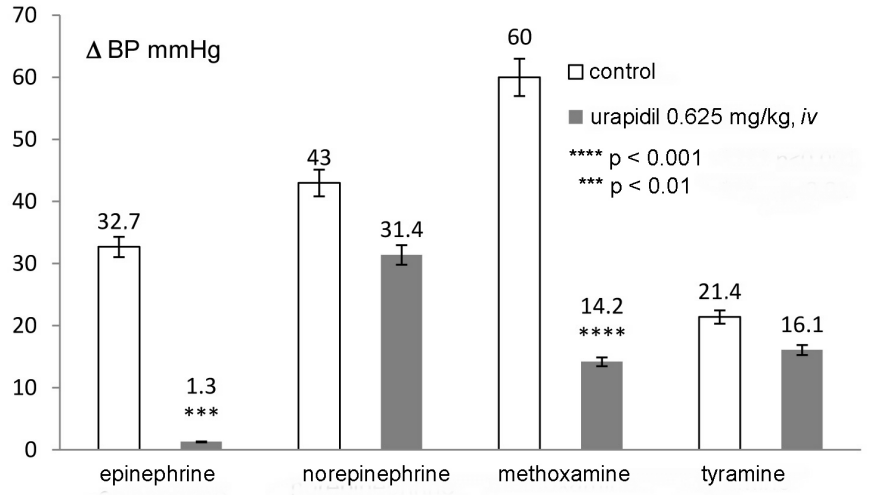

Fig. 19. The effect of urapidil on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

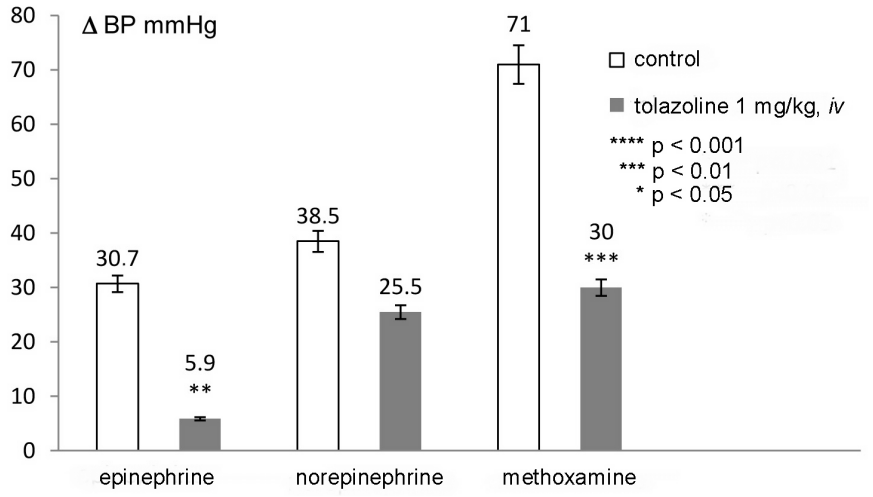

Fig. 20. The effect of tolazoline on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine

finity for α_1-AR decreased in the order of 2-, 3- and 4-. For isomers with methoxy- or hydroxy-groups, the affinity for α_2-AR decreased in the same order as for α_1-AR, while for the chlorine isomers the α_2-AR affinity decreased in order of 4-, 3- and 2-. The addition of the second substituent in the phenyl ring of **21** and **22** caused, in the case of compound **21,** a decrease in both the α_1-AR and α_2-AR binding affinity, while for compound **22** this modification resulted in an increase in the α_1-AR and α_2-AR binding affinity. The effect of protecting the hydroxy group in the 2-position of the phenyl group of compound **11** was observed as an increase in the affinity of compounds **12** and **13** for both α_1-AR and α_2-AR. The compounds tested displayed rather low selectivity for α_1-AR *vs.* α_2-AR subtypes. The highest selectivity was observed for compound **18**, which was 12 times more potent against α_2-AR then α_1-AR (pK_i α_1 6.21; pK_i α_2 7.29).

To better understand the mechanism of action displayed by the tested compounds, their influence on the pressor response to epinephrine, norepinephrine**,** methoxamine and tyramine was tested. It is generally accepted that α_1-AR antagonists invert pressor response to epinephrine and diminish the pressor response of methoxamine, norepinephrine and tyramine, whereas α_2-AR antagonists antagonize or cause no change in the pressor effect of norepinephrine or tyramine and reverse the pressor response to epinephrine [13, 14, 21]. The results of these studies are in good agreement with radioligand binding investigations and confirm the α-AR antagonist activity of the compounds obtained. The significant increase of the pressor response elicited by epinephrine, norepinephrine and methoxamine observed after the *iv* administration of compound **20** may be explained by taking into account its binding profile. Compound **20** possesses a rather weak affinity for both α_1- and α_2-AR (pK_i α_1 5.29; pK_i α_2-AR 6.08) and is slightly selective for α_2-AR. Blocking presynaptic α_2-AR and partiary blocking α_1-AR led to increasing norepinephrine release, which resulted in increasing blood pressure.

The compounds obtained diminished or prevented the appearance of epinephrine-induced arrhythmia symptoms. It was found that 3- or 4- substitution caused a diminished antiarrhythmic effect compared with 2-substitution. Compound **13** diminished the occurrence of extrasystoles in the anesthetized rat in 37.5–100% of animals, significantly preventing mortality in 100%. The ED_{50} value obtained for compound **13** was 1.0 mg/kg, comparable to that displayed by propranolol (the commonly used reference compound in the adrenaline-induced model of arrhythmia) and urapidil and was 7.5 times lower than that displayed by compound **1**. Compound **12** containing a 2-methyl-phenylpiperazine fragment prevented mortality in 100% of animals, but showed a weak protection with respect to the occurrence of extrasystoles at a dose of 5 mg/kg and increased this occurrence at higher doses. Hence, 2-methyl-substitution is considered to have a proarrhythmic effect.

The obtained pyrrolidin-2-one derivatives **6–23** were also tested for their hypotensive activity in normotensive anesthetized rats. Compounds possessing a substituent at the 2- or 3- and two substituents in the phenyl ring (**6**, **7**, **11–13**, **15** and **19–23**) significantly decreased the systolic and diastolic pressure. This effect persisted for more than 60 min. The highest hypotensive activity was displayed by compounds **19** and **21**.

In summary, the syntheis of several new 1-[3-(4-arylpiperazin-1-yl)propyl]-pyrrolidin-2-one derivatives was described. The new compounds were tested for their affinity for α_1- and α_2-AR and their antiarrhythmic and hypotensive activities. As a result, each compound was found to possess an affinity for α_1-AR. The highest affinity for the α_1-AR was displayed by 1-{3-[4-(2-chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one **7**, which binds with a pK_i = 7.13. The highest affinity for the α_2-AR was shown by 1-{3-[4-(4-chloro-phenyl)-piperazin-1-yl]-propyl}-pyrrolidin-2-one **18**, which binds with a pK_i = 7.29. The introduction of a propyl linker resulted in compounds having a higher affinity than 2-hydroxypropyl derivatives for both α_1-AR and α_2-AR. It was also shown that the substituent in the 2-position could play a crucial role in the antiarrhythmic and hypotensive activity of the compounds tested. The pharmacological results and binding studies suggested that the antiarrhythmic and/or hypotensive effects of these compounds were related to their adrenolytic properties. More extensive structure-activity relationship studies are in progress and will be reported in due course.

Acknowledgments:
This study was supported by the Polish Ministry of Science and Higher Education grant no. 2P05F02429. Ms. Cindy Spices and Ms. Christa Caspers were on leave from the Pharmaceutical Institute, University Bonn, within the European Erasmus students' exchange program. The authors would like to thank Prof. Gabriel Nowak and Dr. Dorota Maciąg for implementation of radioligand studies.

References:

1. Barbaro R, Botti L, Botta M, Corelli F, Giannaccini G, Maccari L, Manetti F et al.: Synthesis, biological evaluation, and pharmacophore generation of new pyridazinone derivatives with affinity toward α_1- and α_2-adrenoceptor. J Med Chem, 2001, 44, 2118–2132.
2. Bremner H, Coban B, Griffith R, Groenewoud KM, Yates BF: Ligand design for α_1 adrenoceptor subtype selective antagonists. Bioorg Med Chem, 2000, 8, 201–214.
3. Bujalska M, Araźna M, Makulska-Nowak H, Gumułka SW: Alpha$_1$- and alpha$_2$-adrenoceptor antagonist in streptozotocin- and vincristine-induced hyperalgesia. Pharmacol Rep, 2008, 60, 499–507
4. Chapple CR: Pharmacological therapy of benign prostatic hyperplasia/lower urinary tract symptoms: an overview for the practising clinician. BJU Int, 2004, 94, 738–744.
5. Cheng YC, Prusoff WH: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem Pharmacol, 1973, 22, 3099–3108.
6. Chiu G, Li S, Connolly PJ, Pulito V, Liu J, Middleton SA: (Phenylpiperazinyl)cyclohexyl-ureas: Discovery of $\alpha_{1a/1d}$-selective adrenergic receptor antagonists for the treatment of benign prostatic hyperplasia/lower urinary tract symptoms (BPH/LUTS). Bioorg Med Chem Lett, 2008, 18, 640–644.
7. Crassous P-A, Denis C, Paris H, Senard JM: Interest of α_2-adrenergic agonists and antagonists in clinical practice: background, facts and perspectives. Curr Top Med Chem, 2007, 7, 187–194.
8. Filipek B, Sapa J, Malawska B, Kulig K, Antkiewicz-Michaluk L: Synthesis, antiarrhythmic, antihypertensive and α-adrenoreceptor blocking activity of novel 1-(2-hydroxy-3-amino)-propylpyrrolidin-2-one derivatives. Arch Pharm Med Chem, 1997, 330, 225–231.
9. Graham RM, Perez DM, Hwa J, Piascik MT: α_1-Adrenergic receptor subtypes: molecular structures, function, and signaling. Circ Res, 1996, 78, 737–749.
10. Gentili F, Pigini M, Piergentili A, Giannella M: Agonists and antagonists targeting the different α_2-adrenoceptor subtypes. Curr Top Med Chem, 2007, 7, 163–186.
11. Hielbe JP: Subclassification and nomenclature of α- and β-adrenoceptors. Curr Topics Med Chem, 2007, 7, 129–134.
12. Jain KS, Bariwal JB, Kathiravan MK, Phoujdar MS, Sahne RS, Chauhan BS, Shah AK, Yadav MR: Recent advances in selective α_1-adrenoreceptor antagonists as antihypertensive agents. Bioorg Med Chem, 2008, 16, 4759–4800.
13. Kalkman HO, Timmermans PB, Van Zwieten PA: Characterization of the antihypertensive properties of ketanserin (R 41 468) in rats. J Pharmacol Exp Ther, 1982, 222, 227–231.
14. Koss MC: Prazosin resistant α-adrenoceptor vasoconstriction in the cynomolgus monkey. Pharmacol Comm, 1993, 4, 11–17.
15. Kulig K, Sapa J, Maciag D, Filipek B, Malawska B: Synthesis and pharmacological evaluation of new 1-[3-(4-Arylpiperazin-1-yl)-2-hyroxypropyl]-pyrrolidin-2-one derivatives with anti-arrhythmic, hypotensive, and α-adrenolytic activity. Arch Pharm Chem Life Sci, 2007, 340, 466–475.
16. Leonardi A, Barlocco D, Montesano F, Cignarella G, Motta G, Testa R, Poggesi E et al.: Synthesis, screening, and molecular modeling of new potent and selective antagonists at the α_{1d} adrenergic receptor. J Med Chem, 2004, 47, 1900–1918.
17. Litchfield JT, Wilcoxon FA: A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther, 1949, 96, 99–113.
18. Maj J, Klimek V, Nowak G: Antidepressant drugs given repeatedly increase binding to α_1-adrenoceptors in the rat cortex. J Pharmacol, 1985, 119, 113–116.
19. Malawska B, Kulig K, Filipek B, Sapa J, Maciąg D, Zygmunt M, Antkiewicz-Michaluk L: Synthesis, antiarrhytmic and antihypertensive effects of novel 1-substituted pyrrolidin-2-one and pyrrolidine derivatives with adrenolytic activity. Eur J Med Chem, 2002, 37, 183–195.
20. Malawska B, Kulig K, Gippert A, Filipek B, Sapa J, Maciąg D: Synthesis and development of new 2-substituted 1-[3-(4-arylpiperazin-1-yl)propyl]-pyrrolidin-2-one derivatives with antiarrhythmic, hypotensive, and α-adrenolytic activity. Il Farmaco, 2005, 60, 793–803.
21. Mouille P, Dabire H, Joly G, Schmitt H: Interactions between α, α and β-adrenoceptors on tensionnal efects of adrenaline and noradrenaline in the dog (French). J Pharmacol, 1983, 14, 325–332.
22. Marien MR, Colpaert FC, Rosenquist AC: Noradrenergic mechanism in neurodegenerative diseases: a theory. Brain Res Brain Res Rev, 2004, 45, 38–78.
23. Piascik MT, Soltis EE, Piascik MM, Macmilian LB: α-Adrenoceptors and vascular regulation: Molecular, pharmacologic and clinical correlates. Pharmacol Ther, 1996, 72, 215–241.
24. Saina A, Lindsay J, Ahmed E-G: Clinical and potential use of pharmacological agents to reduce radial artery spasm in coronary artery surgery. Ann Thorac Surg, 2008, 85, 1483–1489.
25. Szekeres L, Papp G, Handbook of Experimental Pharmacology. Eds. Schmier J, Eichler O, Springer-Verlag, Berlin, 1975.
26. Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse M.J, Yellon DM, Cobbe SM et al.: The Lambeth Conventions: Guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. Cardiovasc Res, 1988, 22, 447–455.

Received:
March 6, 2009; in revised form: January 21, 2010.