Full Paper

Synthesis and Pharmacological Evaluation of New 1-[3-(4-Arylpiperazin-1-yl)-2-hydroxypropyl]-pyrrolidin-2-one Derivatives with Anti-arrhythmic, Hypotensive, and α-Adrenolytic Activity

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A series of novel arylpiperazines bearing a pyrrolidin-2-one fragment was synthesized and evaluated for the binding affinity of the α_1- and α_2-adrenoceptors (AR) and for the antiarrhythmic and hypotensive activities of the compounds. The most potent and selective compound 1-[2-hydroxy-3-[4-[(2-hydroxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one **8** binds with pK_i = 6.71 for α_1-AR. Derivative **8** was also the most active in the prophylactic antiarrhythmic test in adrenaline-induced arrhythmia in anaesthetized rats. Its ED_{50} value equals 1.9 mg/kg (i.v.). Compounds with substituents such as a fluorine atom **4**, a methyl **5**, or a hydroxyl **8** group, or two substituents such as fluorine/chlorine atoms and methoxy groups in the phenyl ring, significantly decreased the systolic and diastolic pressure in normotensive anesthetized rats at a dosages of 5 – 10 mg/kg (i.v.). It was found that the presence of the piperazine ring and a hydroxy group in the second position of the propyl chain are critical structural features in determining the affinity of the compounds tested.

Keywords: α-Adrenoceptor blocking activity / Antiarrhythmic / 1-[3-(4-Arylpiperazin-1-yl)-2-hydroxypropyl]-pyrrolidin-2-one derivatives / Hypotensive activity / Molecular modeling

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Introduction

In the recent years, the search for novel and selective α_1-adrenoceptors (α_1-ARs) antagonists has intensified, mainly due to their therapeutic potential in the treatment of hypertension and benign prostatic hyperplasia (BPH) [1 – 3]. Recently, a number of α_1-AR selective antagonists representing different structural classes of compounds were disclosed. These include quinazolines, phenylalkylamines, piperidines, arylpiperazines, and related compounds [4 – 7]. In spite of the differences between these classes of compounds, some common structural elements for α_1-AR have been found. According to the DeMarinis' model [8], a typical α_1-AR antagonist contains three major features corresponding to a basic (positively ionizable) nitrogen atom, an aromatic molecular portion, and a moderately polar moiety. Two additional sterically sensitive features have been identified within the model defining the shape and size properties of the ligands. Of the numerous structures that have been synthesized in this field, the arylpiperazine fragment constitutes one of the most versatile templates for obtaining new molecules that show affinity for α_1-AR. Recently, the structural properties of many arylpiperazine derivatives

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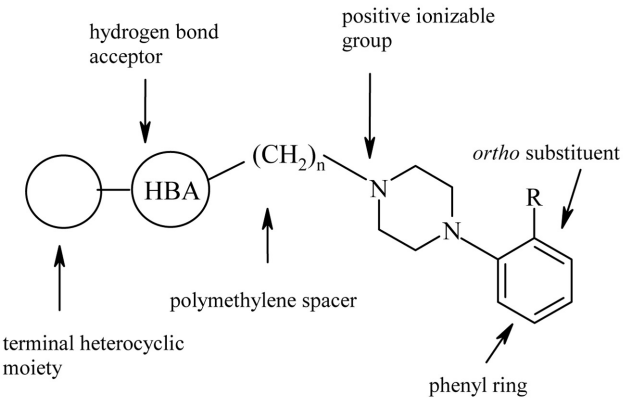


Figure 1. The pharmacophore model of α_1-AR antagonists [12].

that possess affinity towards α_1-ARs have been summarized [9 – 11].

Based on these findings, a three-dimensional pharmacophore model for α_1-AR antagonist among arylpiperazines was proposed [12] (Fig. 1).

In this model, the following structural properties of an ideal α_1-AR were suggested. They are a positively ionizable group, corresponding to the more basic nitrogen atom of the piperazine ring, and an *ortho*- or *meta*-substituted phenyl ring constituting the arylpiperazine system. Moreover, a polar group able to provide a hydrogen-bond acceptor feature incorporated or not incorporated into the second heterocyclic terminal ring, is required at the edge of the molecule, opposite to the arylpiperazine moiety. Finally, a three- or four-carbon atom spacer representing the optimal polymethylene chain is used to connect these two elements [12].

We have previously reported that a series of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]- or 2-acetoxy]propyl-pyrrolidin-2-one derivatives possess affinity for α_1- and α_2-ARs and 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 which contain the methoxy **2** or chloro **3** substituent in the *ortho*-position of the phenyl ring [13 – 15]. In this context, the goal of our research was the development of novel α-ARs antagonists, derivatives of arylpiperazine-propyl-pyrrolidin-2-one. In order to better understand the structure-activity relationship within the synthesized compounds, a molecular study was undertaken.

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** – **2**; for them, the influence of the modifications in the arylpiperazinyl moiety on their α_1- and α_2-ARs affinity and their antiarrhythmic and hypotensive properties were studied. Moreover, a preliminary molecular modeling study consisting in the comparison of structures of the obtained compounds and the selected α_1-AR antagonist was carried out.

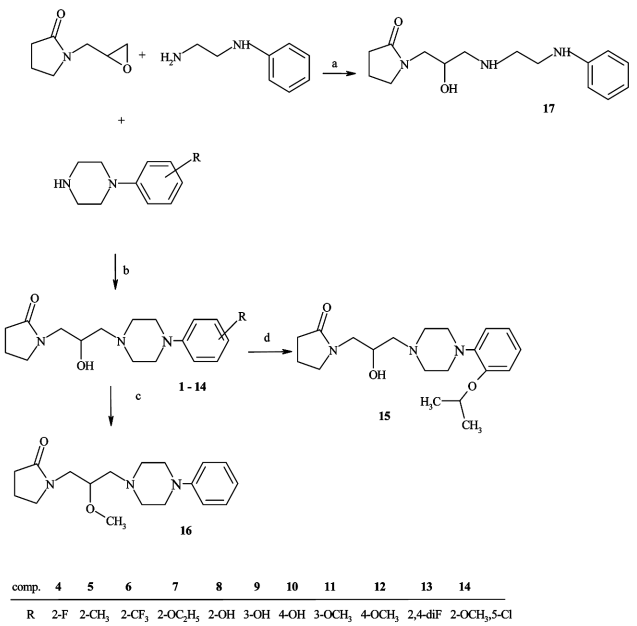
Taking into consideration the above presented pharmacophore model for α_1-AR antagonists, suggesting [12] that a hydrophobic group larger than a methoxy substituent can be accommodated by a hydrophobic pocket where the substituted phenyl ring binds to the piperazine compounds with alkoxy moieties larger than a methoxy group at the *ortho*-position of the phenyl ring were obtained. The modifications in the arylpiperazinyl moiety also included an introduction of one (2-fluoro-; 2-, 3-, or 4-hydroxy-; 2-methyl; 2-trifluoromethyl-) or two different substituents (2,4-difluoro-; and 2-methoxy-5-chlorophenyl-) into the phenyl ring. In order to verify the role of the hydroxy group in the propyl chain, compound **16** with a hydroxy group blocked by a methyl group was obtained. Finally, a more flexible analog of **1** was investigated by synthesizing compound **17**. The newly synthesized compounds (as water-soluble hydrochlorides) were tested for α_1- and α_2-ARs as well as for their antiarrhythmic and hypotensive activity.

Chemistry

As staring material for the synthesis of the new compounds **4** – **17** the earlier described 1-(2,3-epoxypropyl)-pyrrolidin-2-one was used [16]. Its aminolysis with *N*-substituted arylpiperazines and 1-phenylethylenediamine yielded the relevant 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]-propylpirolidin-2-ones **4** – **14** and compound **17**. The required 1-(2-hydroxyphenyl)piperazine was synthesized according to the method described in the literature [17] by heating 1-(2-methoxyphenyl)-piperazine in 47% HBr (57% yield). The 1-[2-hydroxy-4-(2-isopropyloxyphenylpiperazin-1-yl)propyl]pyrrolidin-2-one **15** was obtained through the reaction of compound **8** with isopropyl bromide in acetone, in the presence of potassium carbonate and potassium iodide. The 1-[2-methoxy-4-(phenylpiperazin-1-yl)propyl]pyrrolidin-2-one **16** was obtained through the reaction between compound **1** and methyl iodide in the presence of 1,4,7,10,13-pentaoxacyclopentadecane (15-crown-5) as a catalyst [18]. Yields for these reactions were in the range from 52 to 79%. The structures of the new compounds were confirmed by elemental analysis and spectral data. For the pharmacological assays, compounds **4** – **17** were converted into their water-soluble hydrochlorides. Synthetic routes leading to these new compounds **4** – **17** are presented in Fig. 2.

Pharmacology

In the presented study, several pharmacological tests were carried out to assess α_1- and α_2-AR affinity as well as



Reagents and conditions: a; 1-propanol, reflux, 12 h; b; 1-propanol, reflux, 8-12 h; c; CH_3I, NaH, 15-crown-5, toluene, 70°C, 6 h; d; isopropyl bromide, KI, K_2CO_3, acetone, reflux, 24 h. All compounds were isolated as hydrochloride salts using HCl_{gas} in anhydrous EtOH.

Figure 2. Synthesis route of compounds **1**–**17**.

antiarrhythmic and hypotensive activity of novel pyrrolidin-2-ones.

The pharmacological profile of the new compounds was evaluated by radioligand-binding assays (ability to displace [^{3}H]prazosin or [^{3}H]clonidine from α_1- and α_2-ARs, respectively) on rat cerebral cortex [19, 20]. All tested compounds displaced [^{3}H]prazosin from cortical binding sites (pK_i = 4.07–6.71) and [^{3}H]clonidine (pK_i = 4.31–6.68). The obtained results are presented in Table 1.

It is known that α_1-AR play many roles in the myocardium ranging from positive inotropic and chronotropic effects, through ischemic preconditioning to arrhythmogenesis and cardiac hypertrophy [21, 22]. For example, the nonselective and selective α-adrenoceptor antagonist (phentolamine and prazosin) abanoquil, a highly selective α_{1A}-receptor blocker, prevented arrhythmias induced by adrenaline or cocaine infusions [24, 24]. Many studies also implicate adrenoceptors in the formation of arrhythmia during myocardial ischemia and reperfusion in the isolated heart [25]. It was also shown that α_1-blocking drugs such as prazosin and phentolamine are also effective against ischemia-induced arrhythmias in a variety of animal models [26, 27]. Taking the above into consideration, the prophylactic antiarrhythmic activity of compounds **4**–**17** was determined using a model of adrenaline-induced arrhythmia in rats [28]. Intravenous injections of adrenaline at a dose of 20 μg/kg caused reflex bradycardia (100%), supraventricular and ventricular extrasystoles (100%), bigeminy, and ventricular tachycardia (50%) in rats, which led to the death of approx. 50% of animals within 10 ± 5 min. Compounds **4**–**8**, **11**, and **13**–**15** injected intravenously 15 min before the adrenaline administration diminished the occurrence of extrasystoles and reduced mortality. The ED_{50} value is presented in Table 2. These data show that compound **8** was the most active one; its ED_{50} value is equal to 1.9 mg/kg.

The hypotensive activity of compounds **4**–**17** was determined after i.v. administration to normotensive anaesthetized rats in doses of 5–10 mg/kg. The results

Table 1. Affinity towards 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 [13]	5.72	4.54
2 [14]	5.89	4.39
3 [14]	6.57	4.79
4	5.80	5.89
5	6.00	6.15
6	5.70	6.68
7	6.15	5.96
8	6.71	5.64
9	4.63	4.31
10	4.95	4.81
11	5.36	5.21
12	4.07	5.64
13	6.14	6.11
14	6.45	6.00
15	6.25	5.85
16	4.17	5.27
17	5.41	5.80

K_i value was obtained from three experiments.

Table 2. The prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia in anaesthetized rats. (Route of administration: intravenously.)

Compound	ED_{50} (mg/kg)
1 [13]	7.6 (6.9–8.4)
2 [14]	12 (9.7–14.8)
3 [14]	2.7 (1.95–3.7)
4	5.2 (3.8–6.4)
5	8.9 (7.1–10.2)
6	18.2 (13.2–22.4)
7	3.7 (2.4–4.8)
8	1.9 (1.1–2.6)
11	7.6 (5.5–8.4)
13	9.8 (5.9–14.2)
14	12.3 (8.8–16.1)
15	6.2 (4.9–7.4)
Tolazoline	3.4 (2.6–4.4)
Propranolol	1.05 (0.64–1.73)

Table 3. The hypotensive activity of tested compounds in anaesthetized normotensive rats following i.v. administration.

Compound	Dose (mg/kg)	Blood pressure (mmHg ± S.E.M)	Time of observation (min)					
			0	5	10	20	30	60
4	10	systolic	148 ± 9.9	118 ± 19**	128 ± 19*	127 ± 15*	139 ± 16	139 ± 18
		diastolic	123 ± 11	100 ± 19**	115 ± 17	115 ± 17	120 ± 10	122 ± 17
5	10	systolic	140 ± 10	110 ± 8***	111 ± 13**	129 ± 10*	129 ± 7*	134 ± 11
		diastolic	120 ± 11	101 ± 10***	110 ± 9*	115 ± 8	112 ± 11	114 ± 8
8	5	systolic	142 ± 9.5	113 ± 15.5**	121 ± 13*	124 ± 18*	129 ± 17	129 ± 16
		diastolic	129 ± 11.5	104 ± 13**	111 ± 11.5*	111 ± 14*	118 ± 13	116 ± 14
13	10	systolic	132 ± 3.5	123 ± 2*	122.5 ± 3.5*	129 ± 8.5	128.5 ± 4	128 ± 1
		diastolic	118 ± 4.5	103 ± 5*	111 ± 1.5*	117 ± 2.5	116 ± 4	116.5 ± 1
14	10	systolic	145 ± 6	121 ± 5.5**	120 ± 8.9**	129 ± 9*	136 ± 4	135 ± 6
		diastolic	116.5 ± 3	101 ± 6.9*	102.4 ± 8.2*	105 ± 4*	115 ± 6.2	114.7 ± 5

* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$.

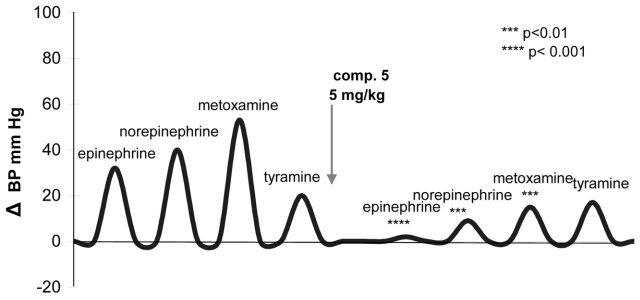


Figure 3. The effect of compound **5** on the blood pressure response to epinephrine, norepinephrine, methoxamine, and tyramine.

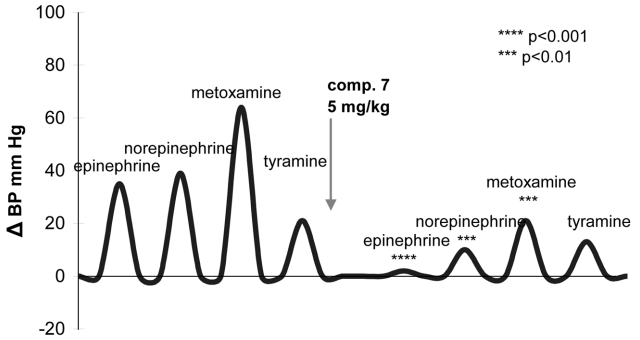


Figure 4. The effect of compound (**7**) on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine.

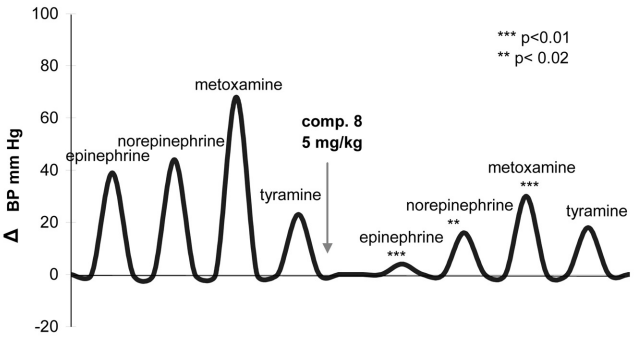


Figure 5. The effect of compound **8** on the blood pressure response to epinephrine, norepinephrine, methoxamine, and tyramine.

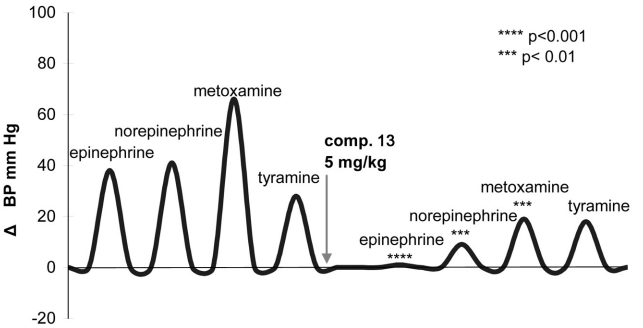


Figure 6. The effect of compound (**13**) on the blood pressure response to epinephrine, norepinephrine, methoxamine and tyramine.

are presented in Table 3. It was found that only compounds **4**, **5**, **8**, **13**, and **14** significantly decreased systolic and diastolic pressure. The observed effect persisted for about 20 min.

In order to examine the mechanism of the hypotensive effects of these compounds, their influence on the pressor responses to epinephrine, norepinephrine, methoxamine, and tyramine were studied. These compounds administered intravenously to rats in the following doses: epinephrine 2 μg/kg, norepinephrine 2 μg/kg, methoxamine 150 μg/kg, tyramine 200 μg/kg caused a pressor response. Compounds **5**, **7**, **8**, **13**, and **14** administered intravenously in doses of 5 mg/kg antagonized the

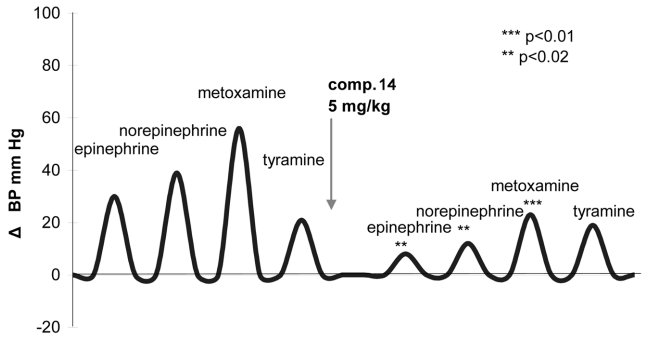


Figure 7. The effect of compound **14** on the blood pressure response to epinephrine, norepinephrine, methoxamine, and tyramine.

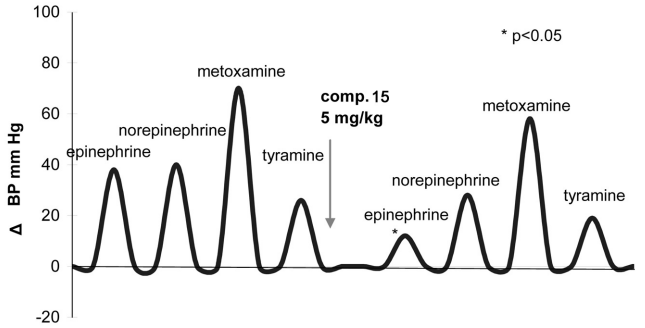


Figure 8. The effect of compound **15** on the blood pressure response to epinephrine, norepinephrine, methoxamine, and tyramine.

pressor response elicited by epinephrine, norepinephrine, and methoxamine (Figs. 3–7) statistically significantly. Only compound **15** administered intravenously in a dose of 5 mg/kg decreased the systolic pressor response evoked by epinephrine statistically significant (Fig. 8). Compounds **4**, **6**, **11** had no statistically significant influence on the systolic pressor response generated by epinephrine, norepinephrine, methoxamine, and tyramine.

Results and discussion

All the newly synthesized compounds **4**–**17** were found to possess an affinity toward α_1- and α_2-ARs that was comparable to or higher than the affinity of the earlier reported compounds **1**–**3**. As expected, replacing the *ortho*-methoxy substituent in the phenylpiperazine moiety of compound **2** with larger alkoxy groups enhanced the affinity. In fact, while compound **7** (1-[2-hydroxy-3-[4[(2-ethoxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one) exhibited an approximately threefold improvement in affinity, compound **15** (1-[2-hydroxy-3-[4[(2-*iso*-propyloxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one) had affinity four times higher than that of the reference compound **1**.

Among the compounds tested, higher affinity for α_1-AR was displayed by compound **8**, pK_i = 6.71, bearing the hydroxy substituent in the *ortho*-position in the phenylpiperazine moiety. Higher affinity for α_2-adrenoceptors was exhibited by compound **6** (1-[2-hydroxy-3-[4[(3-triflouromethylphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one) pK_i = 6.68. It is interesting to note that compound **8** was the sole compound characterized by selectivity for α_1-AR with respect to α_2-AR, (α_2/α_1 ratio 12). Among the isomers with a methoxy substituent in the phenyl ring (**11**, **12**, and **2**), affinity for α_1-AR increased in the order: *para*-, *meta*-, *ortho*-. The addition of a second substituent in the phenyl ring **13**, **14** caused (in comparison with the parent compounds **4** and **2**) further increase in the α_1-AR binding affinity. The effect of protecting the hydroxy group in the second position of the propyl chain of compound **1** was observed as a decrease in the affinity of compound **16** for α_1-AR and an increase for α_2-AR, respectively. A similar effect was observed when the piperazine ring of compound **1** was substituted by ethyldiamine **17**.

In order to better define the structure-activity relationship within the investigated compounds, a molecular modeling study was undertaken. The aim of this approach was the identification of a pharmacophore, which is a template derived from the structure of these compounds, and represents the geometry of the receptor site as a collection of functional groups in 3D space. This work was based on the above presented pharmacophore model for α_1-AR, which includes three features: an aromatic region, a positively ionizable group, and a hydrogen-bond acceptor (Fig. 9). The compounds obtained were modeled and minimized using the PM5 (MOPAC) method of the CAChe program [29]. Conformational analysis has been performed by randomly changing five torsion angles common to all molecules. Although this approach explores only a part of all possible conformations, the results gave an estimation of the range of energy in the geometry of isolated molecules. Finally, the geometry of calculated conformers was optimized. The obtained values are presented in Table 4.

The distances and angle between pharmacophoric features measured for the tested compounds (Fig. 10) fall in the range: a: 5.958–9.176 Å, b: 3.728–4.903 Å, c: 2.940–5.728 Å, and ABC: 126.20–170.75°. It was found that protecting the hydroxy group with a methyl moiety **16** in the second position of the propyl chain of compound **1** had in particular influence on the contraction of the distance between the protonable nitrogen atom and hydro-

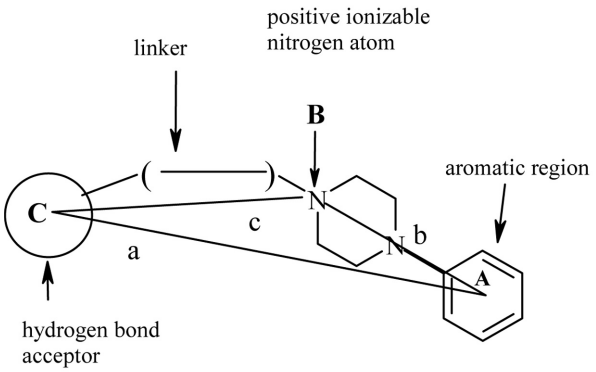


Figure 9. The pharmacophore model of α_1-AR antagonists proposed for arylpiperazynepropylpyrrolidin-2-one derivatives.

Table 4. The angle (°) and distances (Å) between pharmacophore features for compounds **1**–**17**.

Compound	a (Å)	b (Å)	c (Å)	ABC (°)
1	9.117	4.273	5.183	148.13
2	9.176	4.043	5.196	166.50
3	8.912	3.880	5.728	135.23
4	7.755	4.035	5.035	117.10
5	8.932	4.244	4.875	156.59
6	8.967	4.135	5.046	149.73
7	8.883	4.107	5.035	149.37
8	8.884	4.111	5.044	132.48
9	8.704	3.885	4.973	158.45
10	8.702	3.882	4.975	158.38
11	8.383	4.127	5.103	130.21
12	8.657	3.876	4.970	156.06
13	8.841	3.989	4.980	160.49
14	8.697	4.034	4.979	161.50
15	8.755	4.065	5.032	140.83
16	5.958	3.728	2.940	126.20
17	9.877	4.903	4.986	170.75

gen-bond acceptor (c = 2.940 Å). The introduction of an alkoxy substituent in the second position of the phenyl ring caused a decrease of the angle ABC in the following order: methyl **2**, ethyl **7**, and *iso*-propyl **15**. It is also worth noting that among the three isomers with the hydroxyl group in the phenyl ring, the angle between the pharmacophoric features for *meta* **9** and *para* **10** isomers which displayed lower affinity for α-AR was higher (ABC = 158.45° **9**, ABC = 158.38° **10**) than that of the *ortho*-one **8** (ABC = 132.45°). The importance of the presence the phenylpiperazine fragment in the tested series was also confirmed in molecular modeling studies. In the case of the 1-phenylethyldiamine derivative **17**, a significant increase of the angle between the pharmacophoric features was observed (ABC = 170.75°). The obtained results have shown that the character of the substituent in the second position of the phenyl ring could be essential for their α_1-AR affinity.

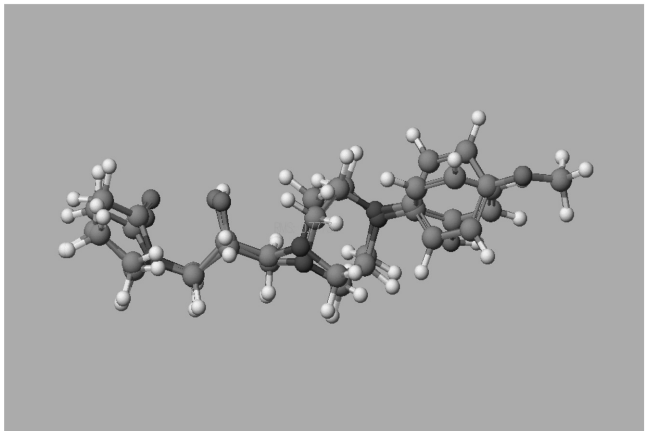


Figure 10. Superimposition of compounds **8** and **12**, which displayed the highest and lowest α_1-AR affinity.

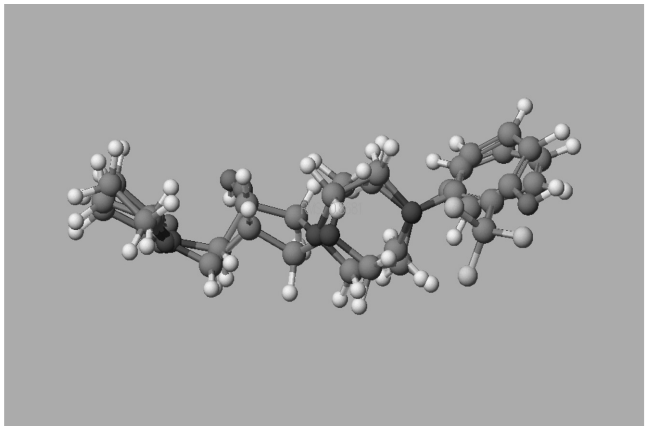


Figure 11. Superimposition of compounds **6** and **9**, which displayed the highest and lowest α_2-AR affinity.

To obtain additional information concerning the shape of the investigated molecules, the compounds which displayed the highest and lowest affinity for α_1 and α_2-ARs were chosen for superimposition. The atoms (except hydrogen atoms) common to these molecules were selected for the fitting procedure. Their similarity was calculated as a RMS fit. The RMS routine provided estimates of how closely molecules fit to each other. The lower the RMS value, the better the similarity. The RMS deviations for each group are as follows: 0.7773 Å (compounds **8** and **12**, α_1-AR; Fig. 10) and 0.8681 Å (compounds **6** and **9**, α_2-AR; Fig. 11). Comparison of the above compounds showed similarity in the orientation of the pyrrolidin-2-one ring and the propyl chain, while the phenylpiperazine fragment possessed different orientation. These results confirm that the phenylpiperazine group is an important structural unit for their activity.

Conclusion

In summary, the synthesis of several new 1-[3-(4-arylpiperazin-1-yl)propyl]pyrrolidin-2-one derivatives is described. As a result, each compound was found to possess affinity for α_1-AR comparable or higher than the affinity of the reference compound **1**. Some structural features of these derivatives have been demonstrated as important for the affinity for α_1-ARs. The hydroxyl group at the *ortho*-position of the phenylpiperazine moiety **8** led to the best α_1-AR affinity and selectivity profile. It was also shown that the *ortho*-position could play a crucial role in the improvement of the α_1-AR antagonist properties in terms of affinity and selectivity. The presence of a piperazine ring and a hydroxy group in the second position of the propyl chain is a critical structural feature in determining the affinity of compounds **4**–**17**. In fact, as demonstrated by binding and molecular modeling studies, blocking the hydroxy group in the second position of the propyl chain by a methyl group or replacing the piperazine ring with ethylenediamine led to compounds with lower affinity for α_1-AR than their parent compounds. Our results indicate that compounds **4**-**8** and **11**–**15** prevented the cardiac arrhythmia caused by administration of adrenaline and that this effect was more potent than its hypotensive action. 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.

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Experimental

Chemistry

Melting points were determined in open glass capillaries on the Büchi (353 m.p.) apparatus and are uncorrected (Büchi Labortechnik, Flawil, Switzerland). Elemental analyses (C, H, N) were carried out within 0.4% of the theoretical values. ^{1}H-NMR spectra were recorded on a Varian Mercury VX 300 MHz PFG instrument (Varian Inc., Palo Alto, CA, USA) in [d_6]-DMSO at ambient temperature using the solvent signal as an internal standard. Mass spectra were measured at 70 eV with a 95 MAT S Sigimann spectrometer (Sigimann, USA). Thin layer chromatography was carried out on Merck silica gel pre-coated F_{254} plates (0.2 mm; Merck, Darmstadt, Germany) using: S_1: chloroform/acetone (1 : 1), S_2: chloroform/methanol/acetic acid (60 : 10 : 5), and S_3: methanol/25% ammonia (98 : 2) as a developing system. The plates were visualized with UV light or iodine solution (0.05 M in 10% HCl).

Compounds: [^{3}H]Clonidine (Amersham), epinephrine (adrenalinum hydrochloricum, Polfa, Warschaw, Poland), norepinephrine (Levonor, Polfa), methoxamine (Sigma, Aldrich Chemie GmbH, München, Germany), [^{3}H]prazosin (Amersham), tyramine (Sigma/Aldrich), sodium heparin (Polfa), thiopental sodium (Biochemie GmbH, Vienna, Austria). Reference compounds: compound **1** was used as a reference.

*General procedure for the synthesis of 1-(2-hydroxy-3-substituted aminopropyl)pyrrolidin-2-one **4**–**14** and **17***

A solution of 1.4 g (10 mmol) of 1-(2,3-epoxypropyl)pyrrolidin-2-one and 10 mmol of the corresponding amine in *n*-propanol (20 mL) was heated under reflux for 12 h. The progress of the reaction was monitored by TLC. After evaporating the solvent, the oily residue was dissolved in anhydrous EtOH and then EtOH saturated with HCl gas was added until the mixture became acidic. The obtained precipitate was crystallized from EtOH.

*1-{3-[4-(2-Fluorophenyl)piperazin-1-yl]-2-hydroxypropyl}-pyrrolidin-2-one dihydrochloride **4***

Yield: 65.4%. Anal. Calc. for $C_{17}H_{24}FN_3O_2 \times 2HCl$; M_r 394.40; mp. 185.0–186.6°C; TLC R_f = S_1(0.43), S_2(0.32); MS (70 eV), *m*/*z* (%) 321 (5.23) [M$^+$], 303 (12.4), 194 (100), 179 (5.6), 98 (8.2), 70 (32.8); ^{1}H-NMR ([d_6]-DMSO): δ = 1.94 (qw, $CH_2CH_2CH_2$, 2H), 2.28–2.37 (m, CH_2CO, NCH_2CH_2, 4H), 2.45–2.59 (m, CH_2 piper., 4H), 2.78–2.88 (m, CH_2 piper., 4H), 3.20 (dd, CH_2CH_2N, 2H), 3.4 (s, O*H*, 1H), 3.51–3.63 (m, CH_2CH_2N, CH, 3H), 6.84–7.00 (m, arom, 4H).

*1-[2-Hydroxy-3-(4-o-tolylpiperazin-1-yl)-propyl]pyrrolidin-2-one dihydrochloride **5***

Yield: 68.9%. Anal. Calc. for $C_{18}H_{27}N_3O_2 \times 2HCl$; M_r 390.40; mp. 189.3–189.7°C ; TLC R_f = S_1(0.12), S_2(0.65); MS (70 eV), *m*/*z* (%) 317 (2.67) [M$^+$], 299 (8.5), 190 (100), 179 (8.1), 98 (12.2), 70 (43.7); ^{1}H-NMR ([d_6]-DMSO): δ = 1.98 (qw, $CH_2CH_2CH_2$, 2H), 2.19–2.27 (m, CH_2CO, NCH_2CH_2, 4H), 2.35 (s, CH_3, 3H), 2.55–2.63 (m, CH_2 piper., 4H), 2.85-2.98 (m, CH_2 piper., 4H), 3.15 (dd, CH_2CH_2N, 2H), 3.37 (s, O*H*, 1H), 3.53–3.70 (m, CH_2CH_2N, C*H*, 3H), 6.47–6.88 (m, arom, 4H).

*1-{2-Hydroxy-3-[4-(2-trifluoromethylphenyl)piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride **6***

Yield: 56.3%. Anal. Calc. for $C_{18}H_{24}F_3N_3O_2 \times 2$ HCl; M_r 444.41; mp. 167.3–168.7°C; TLC R_f = S_1(0.71), S_2(0.62); MS (70 eV), m/z (%) 371 (1.43) [M$^+$], 353 (9.2), 243 (100), 179 (5.9), 98 (23.9), 70 (41.9); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.21–2.32 (m, CH_2CO, NCH_2CH_2, 4H), 2.51–2.68 (m, CH_2 piper, 4H), 2.75–2.88 (m, CH_2 piper, 4H), 3.21 (dd, CH_2CH_2N, 2H), 3.41 (s, O*H*, 1H), 3.59–3.67 (m, CH_2CH_2N, C*H*, 3H), 6.34–6.71 (m, arom, 4H).

*1-{3-[4-(2-Ethoxyphenyl)piperazin-1-yl]-2-hydroxypropyl}-pyrrolidin-2-one dihydrochloride **7***

Yield: 85.3%. Anal. Calc. for $C_{19}H_{29}N_3O_3 \times 2$ HCl; M_r 420.38; mp. 187.5–188.7°C; TLC R_f = S_1(0.63), S_2(0.45); MS (70 eV), *m*/*z* (%) 347 (2.13) [M$^+$], 332 (5.9), 329 (10.5), 318 (6.78), 315 (3.8), 300 (14.2), 205 (100), 179 (4.2), 98 (31.4), 70 (52.9); ^{1}H-NMR ([d_6]-DMSO): δ = 1.33 (t, CH_3, J = 3.5 Hz, 3H), 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.18–2.25 (m, CH_2CO, NCH_2CH_2, 4H), 2.61–2.72 (m, CH_2 piper, 4H), 2.80–2.93 (m, CH_2 piper, 4H), 3.19 (dd, CH_2CH_2N, 2H), 3.32 (s, O*H*, 1H),

3.53 – 3.63 (m, CH_2CH_2N, C*H*, 3H), 3.98 (qw, CH_2, 2H), 6.34 – 6.71 (m, arom, 4H).

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

Yield: 57.5%. Anal. Calc. for $C_{17}H_{25}N_3O_3 \times 2$ HCl; M_r 392.33; mp. 209.8 – 11.3°C; TLC R_f = S_2(0.76), S_3(0.52); MS (70 eV), *m/z* (%) 319 (3.48) [M^+], 301 (7.9), 191 (100), 179 (6.8), 98 (38.4), 70 (49.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.21 – 2.35 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.26 (s, O*H*, 1H), 3.43 – 3.58 (m, CH_2CH_2N, C*H*, 3H), 6.42 – 6.64 (m, arom, 4H).

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

Yield: 78.3%. Anal. Calc. for $C_{17}H_{25}N_3O_3 \times 2$ HCl; M_r 392.33; mp. 132,3 – 133,8°C; TLC R_f = S_1(0.45), S_2(0.63); MS (70 eV), *m/z* (%) 319 (2.32) [M^+], 301 (6.3), 191 (100), 179 (5.4), 98 (41.2), 70 (49.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.26 – 2.38 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.46 (s, O*H*, 1H), 3.58 – 3.72 (m, CH_2CH_2N, C*H*, 3H), 6.06 – 6.15 (m, arom, 4H).

1-{2-Hydroxy-3-[4-(4-hydroxyphenyl)piperazin-1-yl]-propyl}-pyrrolidin-2-one dihydrochloride ***10***

Yield: 45.3%. Anal. Calc. for $C_{17}H_{25}N_3O_3 \times 2$ HCl; M_r 392.33; mp. 189.2 – 190,2°C; TLC R_f = S_1(0.54), S_2(0.62); MS (70 eV), *m/z* (%) 319 (1.28) [M^+], 301 (5.9), 191 (100), 179 (8.2), 98 (48.9), 70 (39.5); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.26 – 2.38 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.46 (s, O*H*, 1H), 3.58 – 3.72 (m, CH_2CH_2N, C*H*, 3H), 6.42 – 6.55 (m, arom, 4H).

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

Yield: 76.2%. Anal. Calc. for $C_{18}H_{27}N_3O_3 \times 2$ HCl; M_r 405.92; mp. 190.2-191.6°C; TLC R_f = S_1(0.21), S_2(0.63); MS (70 eV), *m/z* (%) 333 (0.98) [M^+], 318 (3.7), 315 (5.9), 205 (100), 190 (37.1) 179 (9.4), 98 (32.1), 70 (31.5); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.36 – 2.48 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.46 (s, O*H*, 1H), 3.52 – 3.68 (m, CH_2CH_2N, C*H*, 3H), 3.73 (s, CH_3, 3H), 5.99 – 6.43 (m, arom, 4H).

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

Yield: 75.2%. Anal. Calc. for $C_{18}H_{27}N_3O_3 \times 2$ HCl; M_r 405.92; mp. 165.2 – 166.9°C; TLC R_f = S_1(0.56), S_2(0.48); MS (70 eV), *m/z* (%) 333 (1.05) [M^+], 318 (2.1), 315 (4.1), 205 (100), 190 (42.1) 179 (11.7), 98 (39.5), 70 (21.8); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.36 – 2.48 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.46 (s, O*H*, 1H), 3.52 – 3.68 (m, CH_2CH_2N, C*H*, 3H), 3.83 (s, CH_3, 3H), 6.32 – 6.55 (m, arom, 4H).

1-{3-[4-(2,4-Difluorophenyl)piperazin-1-yl]-2-hydroxypropyl}-pyrrolidin-2-one dihydrochloride ***13***

Yield: 65.2%. Anal. Calc. for $C_{17}H_{23}N_3O_2F_2 \times 2$ HCl; M_r 412.31; mp. 154.3 – 155.8°C; TLC R_f = S_1(0.42), S_2(0.65); MS (70 eV), *m/z* (%) 339 (1.05) [M^+], 321 (8.2), 211 (100), 190 (45.4) 179 (12.5), 98 (41.7), 70 (25.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.46 – 2.68 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.36 (s, O*H*, 1H), 3.45 – 3.51 (m, CH_2CH_2N, CH, 3H), 6.39 – 6.52 (m, arom, 3H).

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

Yield: 78.3%. Anal. Calc. for $C_{18}H_{26}N_3O_3Cl \times 2$ HCl; M_r 440.80; mp. 145.2 – 146.8°C; TLC R_f = S_1(0.24), S_2(0.65); MS (70 eV), *m/z* (%) 369 (0.3) [M^++2] 367 (1.05) [M^+], 352 (3.1), 349 (5.6), 239 (100), 190 (45.4) 179 (12.5), 98 (41.7), 70 (25.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.32 – 2.48 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.43 (s, O*H*, 1H), 3.57 – 3.68 (m, CH_2CH_2N, C*H*, 3H), 3.79 (s, CH_3, 3H), 6.29 – 6.58 (m, arom, 3H).

1-[2-Hydroxy-3-(2-phenylaminoethyl)amino-propyl]pyrrolidin-2-one dihydrochloride ***1***

Yield: 20.5%. Anal. Calc. for $C_{15}H_{23}N_3O_2 \times 2$ HCl; M_r 350.29; mp. 145.3 – 146.2°C; TLC R_f = S_2(0.17), S_3(0.35); MS (70 eV), *m/z* (%) 277 (3.05) [M^+], 259 (1.7), 135 (100), 98 (32.6), 70 (36.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.91 (qw, $CH_2CH_2CH_2$, 2H), 2.22 – 2.36 (m, CH_2CO, NCH_2CH_2, 4H), 2.88 (t, CH_2, 2H), 3.07 (dd, CH_2CH_2N, 2H), 3.32 (t, CH_2, J = 4.8 Hz, 2H), 3.43 (s, O*H*, 1H), 3.57 – 3.68 (m, CH_2CH_2N, C*H*, 3H), 3.79 (s, CH_3, 3H), 6.43 – 7.04 (m, arom, 4H).

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

To a solution of 5.2 mmol (1.66 g) of **8** in 60 mL acetone 5.2 mmol (0.64 g) isopropylbromide and 10 mmol (1.38 g) anhydrous K_2CO_3 and 0.03 mmol (0.005 g) of dry potassium iodide were added. The reaction mixture was stirred at room temperature for 24 h. Then, the inorganic salt was filtered, the solvent was evaporated, and the oily residue was purified by column chromatography using a mixture of methanol and ammonia (98 : 2). After evaporating the solvent, the oily residue was dissolved in EtOH and then EtOH saturated with HCl gas was added until the mixture became acidic. The obtained precipitate was crystallized from EtOH.

Yield: 61.3%. Anal. Calc. for $C_{20}H_{31}N_3O_3 \times 2$ HCl; M_r 434.41; mp. 194.4 – 195.6°C; TLC R_f = S_2(0.53), S_3(0.57); MS (70 eV), *m/z* (%) 361 (2.5) [M^+], 343 (7.6), 233 (100), 190 (43.7) 179 (11.8), 98 (47.1), 70 (25.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.38 (d, CH_3, 6H), 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.32 – 2.48 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.43 (s, O*H*, 1H), 3.57 – 3.68 (m, CH_2CH_2N, C*H*, 3H), 4.04 (t, C*H*, J = 3.8 Hz, 1H), 6.78 – 7.12 (m, arom, 4H).

1-[2-Methoxy-3-(4-phenylpiperazin-1-yl)propyl]pyrrolidin-2-one dihydrochloride ***16***

NaH (6 mmol; 60% suspension in mineral oil) and 0.5 mmol (0.1 mL) of 1,4,7,10,13-pentaoxacyclopentadecane (15-crown-5) were added to a solution of 5 mmol (1.51 g) 1-[2-hydroxy-3-(4-phenyl-piperazin-1-yl)-propyl]-pyrrolidin-2-one **1** in 10 mL dry toluene. The reaction was stirred at room temperature for 8 h. Then, 10 mmol (1.9 g) of iodomethane was added and the mixture was heated on an oil bath at 70°C for 20 h. After completion of hydrolysis, the solution was cooled and toluene was evaporated. The remaining oily residue was dissolved in 20 mL of ace-

tone; 30 mL 2M HCl was added and the mixture was heated on an oil bath at 70°C for 2 h. After completion of hydrolysis, the solution was cooled and acetone was evaporated *in vacuo*. Subsequently, 50 mL of ethyl ether was added to the residue. The water layer was washed twice with 50 mL ethyl ether, alkalized with Na_2CO_3, and twice extracted with 25 mL dichloromethane. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated. The obtained oil was purified by column chromatography using a mixture of methanol and 25% ammonia (98 : 2) as a solvent. After evaporating the solvent, the oily residue was dissolved in EtOH and then EtOH saturated with HCl gas was added until the mixture became acidic. The obtained precipitate was crystallized from EtOH.

Yield: 21.3%. Anal. Calc. for $C_{18}H_{27}N_3O_2 \times 2$ HCl; M_r 390.45; mp. 135.4–136.7°C; TLC R_f = S_2(0.32), S_3(0.72); MS (70 eV), *m/z* (%) 361 (2.5) [M^+], 343 (7.6), 233 (100), 190 (43.7) 179 (11.8), 98 (47.1), 70 (25.1); ^{1}H-NMR ([d_6]-DMSO): δ = 1.96 (qw, $CH_2CH_2CH_2$, 2H), 2.32–2.48 (m, CH_2CO, NCH_2CH_2, 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, 2H), 3.57–3.68 (m, CH_2CH_2N, CH, 3H), 3.79 (s, CH_3, 3H), 6.78–7.12 (m, arom, 5H).

Pharmacology

Animals

The experiments were carried out on male Wistal rats (180–250 g). Animals were housed in constant temperature facilities exposed to a 12/12 h light-dark cycle, and maintained on a standard pellet diet, with tap water 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.

Antiarrhythmic activity

Prophylactic antiarrhythmic activity in a model of adrenaline-induced arrhythmia according to [28].

Arrhythmia was evoked in thiopental (60 mg/kg, i.p.)-anaesthetized rats by intravenous injection of adrenaline (20 μg/kg). The tested compounds were administered intravenously 15 min before adrenaline. The criterion of anti-arrhythmic activity was the lack of premature beats and the inhibition of rhythm disturbances in comparison with the control group (ventricular bradycardia, atrioventicular 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 [30] on ventricular premature beats (VBs), bigeminy, salvos (less than four succecive VBs), ventricular tachycardia (VT, four or more successive VBs), and ventricular fibrillation (VF).

The influence on blood pressure

Male Wistar normotensive rats were anaesthetized with thiopental (50–75 mg/kg, i.p.) The right carotid was cannulated with a polyethylene tube filled with heparin in saline to facilitate pressure measurement using the Datamax apparatus (Columbus Instruments Internationa, Columbus, OH, USA). The studied compounds were injected in a single dose of 2.5 or 20 mg/kg or into the cadual vein after a 5 min stabilization period at a volume equivalent to 1 mL/kg.

α-Adrenoceptor radioligand binding assay

The experiment was carried out on 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. The brains were homogenized in 20 vol 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 the Tris-HCl buffer and centrifuged again. Radioligand binding assays were performed in plates (MultiScreen/Millipore, Billerica, MA, 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 concentration (10^{-11}–10^{-4}M) of the tested compounds. To measure the unspecific binding, 10 μM of phentolamine (in the case of [^{3}H]prazosin), or 10 μM of clonidine (in the case of [^{3}H]clonidine), was applied. The incubation was terminated by rapid filtration over glass fibre and placed in scintillation vials with a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter (Perkin Elmer, Norwalk, CT, USA). All assays were made in duplicate. The radioligand binding were analyzed using an iterative curve-fitting routine (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff [20].

Statistical analysis

The data are expressed as mean ± S.E.M. Statistical significance was calculated using one-way ANOVA. Differences were considered significant for $P < 0.05$.

Molecular modelling

The conformational analysis and the measurement of minimizing energy of compounds were performed by the MM/PM5 method of the CAChe programme [29]. Conformational analysis was carried out by changing the five torsion angles marked in Fig. 12 as ϕ_1–ϕ_5, common to all molecules. The adopted convergence criterion was an energy gradient of 0.1 kcal/mol Å.

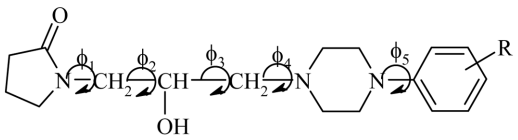

Figure 12. Schematic structure of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]-propylpyrrolidin-2-ones derivatives.

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