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Original article

Design, synthesis and pharmacological evaluation of new 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy-propyl]-3,3-diphenylpyrrolidin-2-one derivatives with antiarrhythmic, antihypertensive, and α-adrenolytic activity

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ABSTRACT

A series of novel arylpiperazines bearing a 3,3-diphenylpyrrolidin-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-ethoxy-phenyl)-piperazin-1-yl]-2-hydroxy-propyl}-3,3-diphenylpyrrolidin-2-one (**7**), which binds with a $pK_i = 7.28$. The highest affinity for the α_2-AR was shown by 1-{3-[4-(2-methoxy-phenyl)-piperazin-1-yl]-2-hydroxy-propyl}-3,3-diphenylpyrrolidin-2-one (**5**), which binds with a $pK_i = 6.68$. Compound **7** was additionally evaluated in *in vitro* functional tests for its affinity for α_{1B}- and α_{1D}-AR, which gave pA_2 $\alpha_{1B} = 6.55$ and pA_2 $\alpha_{1D} = 7.26$. Among the compounds tested, compound **7** also had the highest prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia in anaesthetized rats. Its ED_{50} value was 1.1 mg/kg (i.v.). The compounds significantly decreased systolic and diastolic pressure in normotensive anaesthetized rats at doses of 2.5–5.0 mg/kg (i.v.) and their hypotensive effects lasted for longer than 1 h. It was found that the introduction of two phenyl ring substituents into the 3rd position of the pyrrolidin-2-one fragment gave compounds with affinity for both α_1- and α_2-AR. The substitution of the 2nd position in the phenyl piperazinyl fragment of the molecule was crucial for activity. To determine detailed information concerning the structure–activity relationship, a preliminary molecular modeling study was undertaken.

1. Introduction

Despite the significant progress made in prevention and treatment, cardiovascular diseases are still the main cause of death worldwide, and the number of deaths due to these diseases is increasing [1]. Hypertension and atherosclerosis are central to the pathogenesis of coronary artery disease (ischemia, angina, myocardial infarction), heart failure, cerebral (stroke) and peripheral vascular disease. Since these two risk factors have been widely investigated in the last few years, many therapeutic approaches are now available.

Hypertension affects approximately 1 billion people worldwide and is responsible for approximately 7.1 million deaths per year. Additionally, an estimated 45% of treated patients in the United States remained uncontrolled in 2004 [2,3].

Several factors account for this failure and include low antihypertensive efficacy of single-drug therapies, the use of inappropriate (or a combination of inappropriate) antihypertensive agents to treat patients and poor adherence to antihypertensive therapy [4,5]. Therefore, with the above findings in mind, the continued search for safer and more effective antihypertensive drugs is essential.

The α-adrenergic receptors (α-ARs) play a pivotal role in the regulation of a variety of physiological processes, particularly within the cardiovascular system and are divided into two main subtypes α_1- and α_2-ARs [6]. The α_1- and α_2-ARs are located in the vascular smooth muscle cell membrane, and upon stimulation by an appropriate agonist mediate vasoconstriction. The simultaneous occurrence of both receptor subtypes on vascular smooth muscles makes it conceivable that α_1- and α_2-ARs can contribute to the maintenance of peripheral arterial tone and may play an important role in resistance seen in hypertension.

α_1-ARs modulate intercellular biochemical processes in response to changes in extracellular concentrations of the neurotransmitter norepinephrine and the circulating hormone epinephrine [7–9]. Compounds acting as antagonists at various post-junctional α_1-ARs are frequently used in the therapy of high blood pressure, prazosin

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being the most common drug [6]. α_1-AR antagonists are also used in the treatment of benign prostatic hyperplasia, lower urinary tract symptoms and cardiac arrhythmia [9,10].

To date, a vast array of structurally unrelated α_1-AR antagonists have been identified, which makes it difficult to determine the structural requirements which lead to receptor selectivity [11–17]. However, some general rules have been postulated by Barbaro et al. [18] and Bremner et al. [19], which include an aromatic region, a basic nitrogen atom with at least one available protonation site and a semipolar region. Being phenylpiperazine derivatives, these compounds possess a positive ionizable nitrogen atom and a phenyl ring, corresponding to the positive ionizable atom (A) and the hydrophobic aromatic region (B). The second part of the molecule pyrrolidin-2-one, which is connected to the phenylpiperazine by a linker, is a hydrogen bond acceptor region (C) (Fig. 1).

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 modulators in the release of catecholamine and other neurotransmitters such as acetylcholine, γ-aminobutyric acid and nitric oxide [20,21]. In contrast to the α_1-AR, the post-junctional vascular α_2-ARs have not yet been established as a target for antihypertension therapy. The therapeutic uses of α_2-AR antagonists mainly concern the treatment of depression and diabetes. Other potential clinical applications include cardiovascular disease, obesity, Raynaud's disease, male sexual dysfunction, Alzheimer's disease and Parkinson's disease [22].

Non-selective α-AR antagonists such as phenoxybenzamine are effective in reducing radial artery spasm during coronary artery surgery [23].

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 showed marked hypotensive and antiarrhythmic activities. Among the compounds tested, the most active was 1-[2-hydroxy-3(4-phenylpiperazin-1-yl)-propyl]-pyrrolidin-2-one (**I**) and those which contained the hydroxyl- or chloro-substituent in the 2nd position of the phenyl ring [24–26]. In this context, the goal of our research was the development of novel α-AR antagonists, derivatives of arylpiperazine propyl-pyrrolidin-2-one. In this work, we report on the synthesis and *in vitro* and *in vivo* pharmacological studies of a series of new analogues of compound **I**; for these compounds, the influence of the introduction of two phenyl rings into the 3rd position of the pyrrolidin-2-one and modifications in the arylpiperazinyl moiety on their α_1- and α_2-AR affinity and their antiarrhythmic and hypotensive properties were studied. Taking into consideration the pharmacophore model presented above for α_1-AR antagonists, which suggested [18,19] that a hydrophobic group larger than a methoxy substituent may be accommodated by a hydrophobic pocket, 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 the introduction of 4-fluoro-; 2-methyl-; 2-trifluoromethyl-, and 2-, or 4-methoxy- into the phenyl ring (Fig. 2). The newly synthesized compounds (as water-soluble hydrochlorides) were tested for α_1- and α_2-AR affinity in addition to their antiarrhythmic and hypotensive activities.

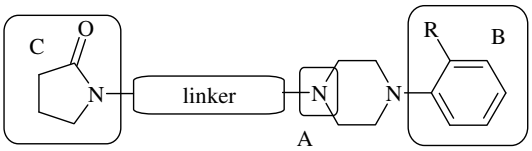


Fig. 1. General structure of compounds tested.

2. Chemistry

As a starting material for the synthesis of the title compounds (**4**–**10**), 3,3-diphenyl-dihydro-furan-2-one (**1**) was used. At the first stage, compound (**1**) was heated with allylamine giving 1-allyl-3,3-diphenylpyrrolidin-2-one (**2**) in 69% yield. Then, oxidation of compound (**2**) with meta-chloroperoxybenzoic acid (mCPBA) led to synthesized 1-oxiranylmethyl-3,3-diphenylpyrrolidin-2-one (**3**) in 66% yield. Finally, aminolysis of compound (**3**) with *N*-substituted arylpiperazines gave the relevant 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy]-propylpirolidin-2-ones (**4**–**10**). The yields of these reactions ranged from 50 to 80%. The structures of the new compounds were confirmed by elemental analysis and ^{1}H NMR spectral data. For the pharmacological assays, compounds (**4**–**10**) were converted to their water-soluble hydrochlorides, by dissolving them in anhydrous ethanol and saturating with gaseous HCl. Synthetic routes leading to the new compounds are presented in Fig. 3.

3. Pharmacology

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 (**4**–**10**).

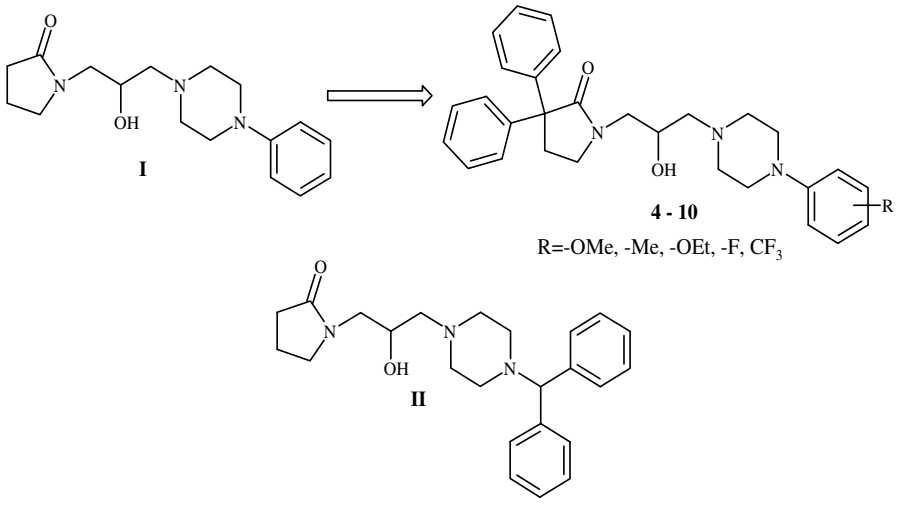


Fig. 2. Structures of compounds (**I**), (**II**) and obtained compounds (**4**–**10**).

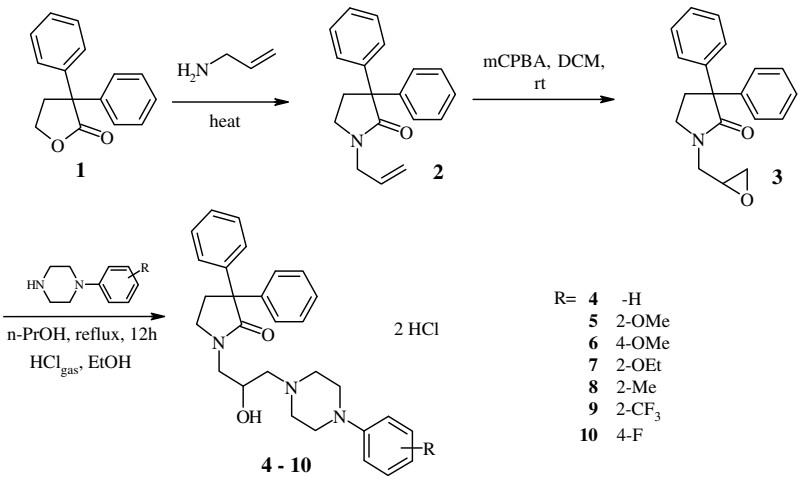


Fig. 3. General route of synthesis 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy-propyl]-3,3-diphenylpyrrolidin-2-one derivatives (**4**–**10**).

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- and α_2-ARs, respectively) on rat cerebral cortex [27,28]. All tested compounds displaced [^{3}H] prazosin from cortical binding sites ($pK_i = 5.36–7.28$) and [^{3}H] clonidine ($pK_i = 5.29–6.68$). The results obtained are presented in Table 1.

The most potent α_1-AR compound (**7**) was tested in *in vitro* functional studies to determine its affinity to the α_{1B}-AR and the α_{1D}-AR. The antagonist activity of compound (**7**) toward α_{1D}-AR present in rat aorta and toward α_{1B}-AR present in guinea-pig spleen was assessed by inhibition of noradrenaline-induced contractions. The investigated compound, concentration-dependently, shifted the noradrenaline response to the right. The Schild slopes did not differ significantly from unity, indicating a competitive interaction with the α_{1D}- and α_{1B}-adrenoceptors, and thus allowed determination of the pA_2 value. The results obtained are shown in Table 2 and Figs. 4 and 5.

Compounds (**4**–**10**) were tested for their prophylactic antiarrhythmic activity in an adrenaline-induced arrhythmia rat model [29]. Intravenous (i.v.) injections of adrenaline at a dose of 20 μg/kg caused reflex bradycardia (100%), supraventricular and ventricular extrasystoles (90%) bigeminy (75%) and blocks (100%), which led to death in approximately 80% of rats within 10 ± 5 min. Compounds (**4**–**5** and **7**–**9**) injected 15 min before adrenaline administration, decreased the occurrence of extrasystoles, bigeminy and reduced mortality. The most active compounds were (**7**) and (**8**) and when given at a dose of 5 mg/kg totally protected the animals from the above disturbances. The ED_{50} values obtained are presented in Table 3. The highest activity in this model of arrhythmia was displayed by compound (**7**), which had an ED_{50} value of 1.1 mg/kg.

The hypotensive activity of compounds (**4**–**10**) was determined after i.v. administration to normotensive anaesthetized rats at doses of 2.5–5.0 mg/kg. The results are presented in Table 4. Compounds (**4**–**9**) significantly decreased systolic and diastolic pressure. The observed effect lasted for more than 60 min. Compound (**10**) was found to be inactive.

The influence of compounds (**4**–**10**) on pressor responses to epinephrine, norepinephrine and methoxamine was tested. Intravenous epinephrine, norepinephrine and methoxamine were given to rats at a dose of 2 μg/kg and 150 μg/kg, respectively, to induce pressor response. Compounds (**4**, **5**, **7** and **8**) given at a dose of 5 mg/kg, significantly antagonized the pressor responses elicited by epinephrine, norepinephrine and methoxamine (Figs. 6–9). However, compounds (**6**, **9** and **10**) did not have any significant influence on systolic pressor response generated by epinephrine, norepinephrine and methoxamine (data not shown).

4. Results and discussion

All the newly synthesized compounds (**4**–**10**) 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 [24–26]. The highest affinity for the α_1-AR (pK_i 7.28) was displayed by compound **7**, which had an ethoxy-substituent in the 2-position of the phenyl ring. The highest affinity for the α_2-AR (pK_i 6.68) was shown by compound **5**, which had a methoxy group in the 2nd position of the phenyl ring. The introduction of two phenyl groups into the third position of pyrrolidin-2-one gave compounds which in comparison to the parent compound (**I**) had higher or comparable affinity for both α_1- and α_2-ARs. As expected, the introduction at the 2nd position of the phenyl ring of an alkoxy group (methoxy- (**5**) or ethoxy- (**7**)) resulted in enhancement of affinity to the α_1-AR. This effect which was proved in earlier observations showed that a hydrophobic group larger than methoxy group could be better

Table 1
Affinity of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy-propyl]-3,3-diphenylpyrrolidin-2-one derivatives (**4**–**10**) and model compounds (**I** and **II**) toward different α-AR subtypes in rat cerebral cortex.

Compound	$pK_i\alpha_1$ [^{3}H] prazosin (α_1 rec.)	$pK_i\alpha_2$ [^{3}H] clonidine (α_2 rec.)
I [24]	5.72	4.54
II [24]	6.57	5.27
4	6.74	6.64
5	6.80	6.68
6	5.36	6.00
7	7.28	6.38
8	6.69	6.51
9	5.89	5.29
10	5.68	5.32

The means pK_i values were obtained from three experiments. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff [28].

Table 2
Antagonistic activity for α_{1D}- and α_{1B}-receptors of compound (**7**) in functional *in vitro* studies.

Tissues/receptor	pA_2	Slope
Rat aorta α_{1D}	7.260 ± 0.051	0.882 ± 0.01
Guinea pig spleen α_{1B}	6.552 ± 0.279	0.996 ± 0.148

pA_2 value were obtained from the linear regression of Schild plot.
Each value was the mean ± SEM of 5–8 experimental results.

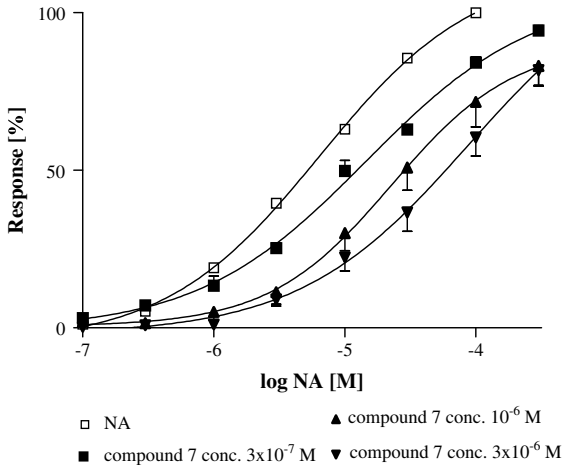


Fig. 4. Concentration–response curves to noradrenaline in the guinea pig spleen in the absence (□) or presence of **7** (■ 0.3, ▲1, ▼3 μM). Results are expressed as percentage of the maximal response to noradrenaline in the first concentration–response curve. Each point represents the mean ± SEM ($n = 5$).

accommodated by a hydrophobic pocket at the receptor binding site [18,19]. It was observed that the 2nd substitution at the phenyl ring was preferred. Compounds (**5**, **7**–**9**) had higher or comparable affinity for α_1-AR to that of the unsubstituted compound (**4**). The replacement of the methoxy group from the 2nd (**5**) into the 4th (**6**) position of the phenyl ring resulted in decreased affinity for both α_1- and α_2-ARs. The compounds tested displayed rather low selectivity for α_1-AR vs. α_2-AR subtypes. The highest selectivity was observed for compound **7**, which was about 10 times more potent against α_1-AR than α_2-AR (pK_i α_1 7.28 and pK_i α_2 6.38, respectively).

It is known that the α_{1B}-AR subtype of α_1-AR plays an important role in the regulation of blood pressure due to antagonistic activity and could be used in hypertension therapy. Antagonism of α_{1D}-AR may play a part in blood pressure control, because of the involvement of α_{1D}-ARs in the contraction of vessels [30]. Therefore, with these findings in mind, compound (**7**) which displayed the highest affinity for α_1-AR was tested for its ability to block α_{1B}- and α_{1D}-subtypes of the adrenoceptor in functional *in vitro* studies. It was found that compound (**7**) had slightly higher affinity for α_{1D}-AR ($pA_2 = 7.26$) than for α_{1B}-AR ($pA_2 = 6.55$). In both cases, compound (**7**) showed a competitive, surmountable type of antagonism where

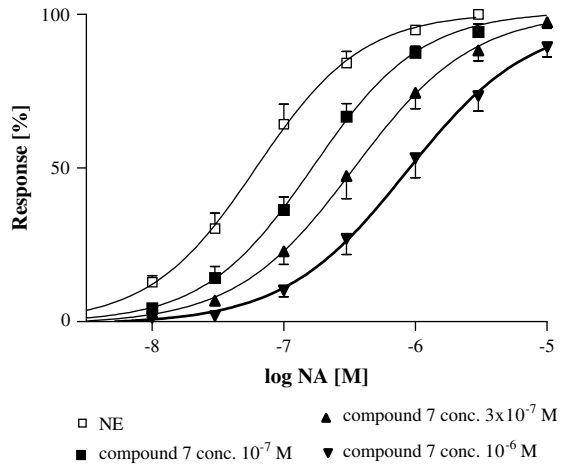


Fig. 5. Concentration–response curves to noradrenaline in the rat aorta in the absence (□) or presence of **7** (■ 0.1, ▲0.3, ▼1 μM). Results are expressed as percentage of the maximal response to noradrenaline in the first concentration–response curve. Each point represents the mean ± SEM ($n = 5$–8).

Table 3
The prophylactic anarrhytmic activity of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxypropyl]-3,3-diphenylpyrrolidin-2-one derivatives (**4**, **5**, **7**–**9**) and model compounds (**I** and **II**) in adrenaline-induced arrhythmia in anaesthetized rats after i.v. administration.

Compound	ED_{50} (mg/kg)
I [24]	7.6
II	n/a
4	1.9 (1.6–2.4)
5	2.05 (1.7–2.1)
7	1.1 (0.9–1.3)
8	1.4 (1.2–1.7)
9	3.9 (2.8–5.5)
Tolazoline	3.4 (2.6–4.4)
Propranolol	1.05 (0.64–1.73)

n/a – Not active in this test.
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 [42].

the pA_2 value was equal to the pK_B value [31]. The ratio of guinea-pig spleen K_B/rat aorta K_B was 5.2, thus compound (**7**) displayed modest tissue selectivity for α_{1D}- over α_{1B}-AR.

In order to better understand the mechanism of action displayed by the tested compounds, their influence on the pressor response to epinephrine, norepinephrine and methoxamine were tested. It is generally accepted that α_1-AR antagonists invert the pressor response to epinephrine, only partially invert the response to norepinephrine, diminish the pressor response to methoxamine, and potentiate the hypertensive effect of norepinephrine. Whereas the α_2-AR antagonists antagonize the pressor effect of norepinephrine, and reverse the pressor response to epinephrine. The results of these studies were in good agreement with the radioligand binding investigations and confirmed the α-AR antagonist activity of these compounds. Compound (**4**) and compounds (**5**, **7**, **8**), with the alkoxy- or methyl-substituent at the 2-position of the phenyl group, significantly decreased the pressor response elicited by epinephrine, norepinephrine and methoxamine. This effect can be explained by their affinity for the α_1- and α_2-AR profile. All these compounds possessed affinity for both α_1- and α_2-ARs which ranged from a pK_i value of 6.69–7.28 for α_1-AR and a pK_i value of 6.00–6.68 for α_2-AR. Compound (**7**) was the most active and possessed the highest affinity for α_1-AR (pK_i 7.28) of all the compounds tested. Compounds (**6**, **9** and **10**) which displayed low affinity for both α_1-AR and α_2-AR did not have a significant influence on the pressor response elicited by epinephrine, norepinephrine and methoxamine.

Compounds (**4**, **5**, **7**, **8** and **9**) diminished or prevented the appearance of epinephrine-induced arrhythmia symptoms. The most active in this test, compound (**7**) diminished the occurrence of extrasystoles in the anaesthetized rat in 37.5–100% of animals, and significantly prevented mortality in 100%. The ED_{50} value for compound (**7**) was 1.1 mg/kg and was three times lower than that displayed by tolazoline (a non-selective α-AR antagonist), comparable to that displayed by propranolol (the commonly used reference compound in adrenaline-induced models of arrhythmia) and 7 times lower than that displayed by compound **I**. These results are in good agreement with previously published data on non-selective and selective α-antagonists such as phentolamine, prazosin, and abanoquil which prevented arrhythmia induced by adrenaline or cocaine infusion [32,33]. In addition, the effectiveness of phentolamine and prazosin against ischemia-induced arrhythmia in a variety of animal models of arrhythmia has also been observed [34,35].

The obtained pyrrolidin-2-one derivatives (**4**–**10**) were also tested for their hypotensive activity in normotensive anaesthetized rats. Compounds possessing a substituent at the 2-position of the phenyl ring or 4-methoxy group (**4**–**9**) significantly decreased

Table 4
The hypotensive activity of 1-[3-(4-arylpiperazin-1-yl)-2-hydroxy-propyl]-3,3-diphenylpyrrolidin-2-one derivatives (**4**–**9**) in anaesthetized normotensive rats after i.v. administration.

Compound	Dose mg/kg	Blood pressure (mm Hg ± S.E.M.)	Time of observation (min)					
			0	5	10	20	30	60
4	5	Systolic	140.5 ± 1.1	135.5 ± 0.8*	134.5 ± 1.8**	135.5 ± 0.9**	129.0 ± 1.7***	126.5 ± 2.8***
		Diastolic	125.5 ± 0.6	119.0 ± 2.0	118.6 ± 1.5	116.5 ± 3.6	113.5 ± 4.4*	109.5 ± 4.6***
5	5	Systolic	139.0 ± 0.6	125.0 ± 6.0*	122.0 ± 6.0**	118.0 ± 3.0***	116.0 ± 3.5***	112.0 ± 3.0***
		Diastolic	120.0 ± 1.7	109.0 ± 1.5***	107.5 ± 1.9***	104.0 ± 1.9***	102.5 ± 0.6***	98.0 ± 0.9***
6	2.5	Systolic	125.6 ± 7.6	126.6 ± 7.2	115.2 ± 6.5	107.6 ± 7.9	105.6 ± 7.5	105.8 ± 9.8
		Diastolic	107.2 ± 7.3	107.0 ± 6.0	96.4 ± 5.5	89.8 ± 6.1	88.2 ± 5.9*	88.2 ± 9.5*
7	5	Systolic	142.5 ± 3.6	130.0 ± 1.2	118.0 ± 6.1***	111.0 ± 7.0****	113.5 ± 3.6****	114.0 ± 2.0****
		Diastolic	127.0 ± 8.1	117.0 ± 11.0	105.0 ± 6.0	98.0 ± 5.5*	96.0 ± 2.0*	95.5 ± 4.5*
8	5	Systolic	141.3 ± 6.4	132.3 ± 4.8	133.7 ± 2.2	136.3 ± 2.9	133.0 ± 3.6	116.0 ± 10.8***
		Diastolic	126.0 ± 4.6	116.7 ± 2.8	118.0 ± 2.1	119.7 ± 1.8	117.7 ± 0.9*	99.0 ± 11.7***
9	5	Systolic	138.0 ± 0.9	126.0 ± 5.0*	122.0 ± 7.1*	119.0 ± 3.1***	116.0 ± 3.9***	111.0 ± 3.1****
		Diastolic	119.0 ± 1.2	103.0 ± 1.5***	106.5 ± 2.9***	104.0 ± 1.8**	103.0 ± 3.0***	100.0 ± 0.7***

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

systolic and diastolic pressure. The observed effect persisted for more than 60 min.

In order to better define the structure–activity relationship of the investigated compounds, a molecular modeling study was undertaken. The three-dimensional structures of the pyrrolidin-2-one derivatives at their neutral state were obtained through full optimization based on the AM1 quantum chemical procedure. 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 our earlier research describing a pharmacophore model for α_1-AR, which included three features: an aromatic region, a positively ionizable group, and a hydrogen bond acceptor. The structures were compared to the earlier proposed pharmacophoretic features [24] using measurements of the appropriate distances and angles. All intramolecular distances and angles in the proposed pharmacophore series of investigated compounds were measured and are listed in Table 5.

In the preliminary analysis of the estimated distances and angles between the pharmacophoric features of these compounds, it was observed that the distances *a*, *b* and *c* were comparable, while the angle values were appreciably different. This indicated that, replacement of 3-(4-phenylpiperazin-1-yl) by the 3-(4-diphenylmethylpiperazin-1-yl) moiety or attachment of the diphenyl moiety at the 3,3-position of pyrrolidin-2-one had only limited influence on the distance between the center of the phenyl ring, the positively ionizable nitrogen atom and the hydrogen bond acceptor (compounds **I**, **II** and **4**). An extensive deflection in the angles measured was also found in compounds (**II**) and (**9**). For all other derivatives, the angle differences varied within 7°, and were about 4-fold less than that of the whole set (31.784°). This showed that the presence of the diphenyl moiety at the 3,3-position of pyrrolidin-2-one or modification of compound **4** by attachment of the trifluoromethyl group at the ortho position of the phenylpiperazine ring significantly influenced the ABC angle (compounds **I**, **II**, and **9**). As can be seen from Table 5, the set can be divided into two subsets, one consisting of compounds which had similar pharmacophoric features (**4**, **5**, **6**, **7**, **8**, **10**) and the second consisting of compounds which had a significant spread in the distances and angles between the pharmacophoric points (**I**, **II**, **9**). The values obtained for the first subset corresponded to the characteristic values of the most active compounds obtained in previously reported studies [26]. This suggested that this subset may contain the compounds with better antiarrhythmic or hypotensive activities together with α-adrenolytic activity. It was found that compounds (**4**, **5**, **7**, **8**) did possess the most active antiarrhythmic, hypotensive, and α-adrenolytic activities in this series. Compounds (**7**, **8**) had the most active profiles of antiarrhythmic activity. Compounds (**4**, **5**, **7**, **8**) significantly antagonized the pressor response. Furthermore, only compounds (**7**, **8**) were active in whole kinds of activity (hypotensive activity, see Table 3 and Figs. 7 and 8). Surprisingly, compounds (**6**) and (**10**) did not show either antiarrhythmic or hypotensive activity. Despite the successive prediction of the activity of compounds (**4**, **5**, **7**, **8**) this pharmacophore model did not explain the structure–activity relationship (SAR) of compounds (**6**, **10**).

In order to check the applied pharmacophore model more precisely, we extended our molecular modeling study to a two-part calculation experiment. In the first part, single point energy (SPE) quantum chemical calculations were carried out to predict the

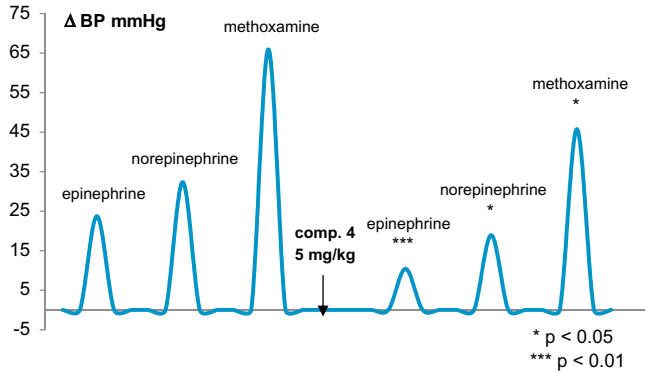


Fig. 6. The effect of compound (**4**) on blood pressor response to epinephrine, norepinephrine and methoxamine.

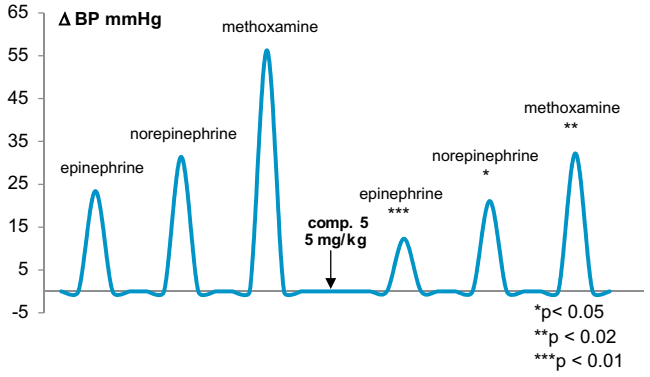


Fig. 7. The effect of compound (**5**) on blood pressor response to epinephrine, norepinephrine and methoxamine.

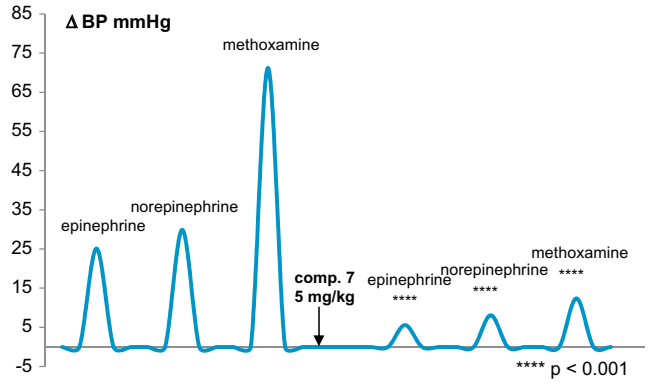


Fig. 8. The effect of compound (**7**) on blood pressor response to epinephrine, norepinephrine and methoxamine.

dipole moment values. In the second part, the electrostatic potential map (MEP) was generated by using quantum chemical calculations. The dipole moment values (μ) and calculated MEP of the studied compounds are listed in Table 6, for explicit understanding the corresponding molecular structures were plotted on the MEPs. For this investigation, the model derivatives, compounds **I** and **II**, were chosen. Compound **I** was a leading structure, while compound **II** displayed activity in the binding assays but was inactive in the *in vivo* tests.

The dipole moment values of the tested compounds varied within the range 2.76–6.44 D. It was observed that modification of compound **I** by replacing 3-(4-phenylpiperazin-1-yl) with the 3-(4-diphenylmethylpiperazin-1-yl) moiety or the introduction of two phenyl rings into the 3-position of pyrrolidin-2-one resulted in decreased μ values of less than 1 D (**II**, **4**). The modification of the compounds by replacing the methoxy substituent from the 4- to the 2-position of the phenyl ring resulted in decreased μ values of about 1.6 D (**5**, **6**). Introduction of a methyl group into the second position of the phenyl group or methoxy- into the 4- position did not change the μ value significantly (**6**, **8**). While introduction of strongly electronegative substituents such as CF_3 or F atom in the 2- or 4-positions of the phenyl moiety resulted in the maximum value of μ in the set (**9**, **10**). Low μ values were estimated for compounds with methoxy (**5**) or ethoxy (**7**) groups in the 2-position of the phenyl ring. This detailed variability in the dipole moment values demonstrated a multidirectional tendency.

For this reason, it was decided to include the electrostatic charge distribution in the molecule into the structure–activity considerations of the compounds in the current study. The MEP presented in Table 6, shows the view obtained by integrating two data sets together *e.g.* pharmacophoric features and the distribution of the

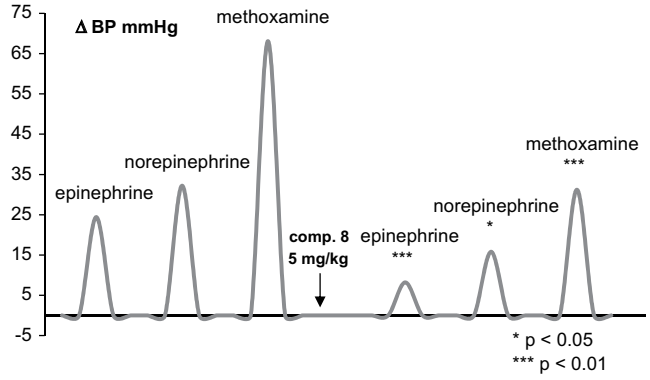
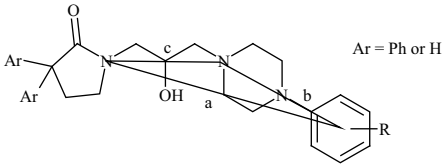


Fig. 9. The effect of compound (**8**) on blood pressor response to epinephrine, norepinephrine and methoxamine.

Table 5
The distances (Å) and angles (°) between pharmacophore features for compounds **I**, **II**, **4**–**10**.



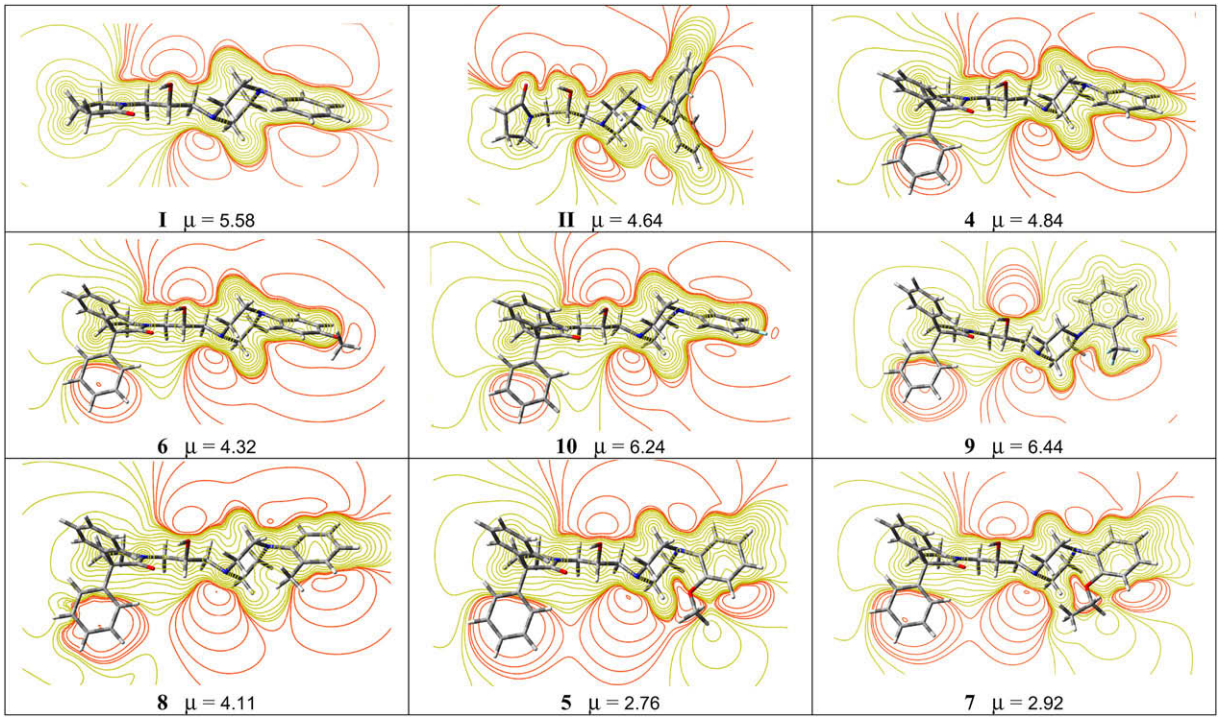
Compound	*a* (Å)	*b* (Å)	*c* (Å)	ABC (°)
I	8.719	3.946	4.957	156.501
II	8.600	3.825	4.857	164.086
4	8.725	3.946	4.967	156.278
5	8.748	4.046	4.969	151.891
6	8.725	3.945	4.967	156.327
7	8.748	4.053	4.970	151.472
8	8.814	4.164	4.970	149.486
9	8.477	4.298	4.966	132.302
10	8.721	3.946	4.967	156.057

charge in the molecule by plotting the electrostatic charge distribution contours in the plane of the pharmacophore triangle. In Table 6, for explicit understanding, the corresponding molecular structures are plotted on the MEP.

For each presented MEP, the green contours indicate less electronegative areas, which were generally found in the central region concentrated along the primary core of the compound's structure. Generally these green contours were due to atoms like C and H. The most important differences in the charge distribution concerned the variation in the localization of the negative charges (red coloured contours) due to the presence of strongly electronegative moieties (*e.g.* N, O, F, or phenyl ring) at a specific point in the molecular structure. For compound (**9**) there was a negative charge contour cluster enclosed by green contours localized in the upper central part of the MEP. This molecule was highly non-planar and had the highest number of strongly electronegative moieties (i.e. 3–N, 2–O, 3–F, and phenyl ring) in the set. For this reason, it differed significantly from the other compounds in the set which had similar negative charge distribution in the upper part of the MEP. In the MEP for compounds (**I**, **II**, **6**, **10**), the arylpiperazine terminal moiety was surrounded by red regions due to the presence of the electronegative substituent piperazine. This situation was correlated with low or absent antiarrhythmic activity. In the MEP (**4**, **5**, **7**, **8**, **9**) red areas interrupted by green contours in the plane of the molecular skeleton which were divided into separate upper and lower parts were observed. This type of charge distribution was correlated with the antiarrhythmic activity of the compound. The analysis of this activity based on the distribution of electrostatic charge in this subset, leads us to conclude that the extension of the negative charge in the upper part would be large, and the lower part of the MEP would be concentrated in clusters. In the case of the most active compound (**7**), there was a wide extension of red upper contours separated by broad green areas in which there were small clusters of negative charge in the lower part. When this cluster became larger and started to overlap, as in the case of compounds (**8**, **4**, **5**), the activity of the corresponding compounds decreased. It is also significant that any substitution leading to positioning of the terminal phenyl ring in a plane perpendicular to the pharmacophoric feature plan, decreased the antiarrhythmic activity or this activity was absent.

According to the results discussed in this work we suggest that the distribution resembled clusters as presented in the MEP (**7**).

Table 6
Calculated contour electrostatic potential maps and calculated dipole moment values for compounds (**4**–**10**) and parent molecules (**I**, **II**).



5. Conclusion

In summary, the synthesis of several new 1-[3-(4-arylpiperazin-1-yl)-propyl]-3,3-diphenylpyrrolidin-2-one derivatives is described. The new compounds were tested for their affinity for α-ARs, as well as their antiarrhythmic and hypotensive activities. As a result, each compound was found to possess affinity for α_1-AR. The most potent and selective for α_1-AR was compound (**7**) (1-{3-[4-(2-ethoxy-phenyl)-piperazin-1-yl]-2-hydroxy-propyl}-3,3-diphenylpyrrolidin-2-one; $pK_i\ \alpha_1 = 7.28$; $pK_i\ \alpha_2 = 6.38$) which was additionally tested in *in vitro* studies for its affinity to α_{1B}- and α_{1D}-ARs ($pA_2\ \alpha_{1B} = 6.55$ and $pA_2\ \alpha_{1D} = 7.26$). It was found that the introduction of two phenyl rings into the third position of pyrrolidin-2-one resulted in the compounds having higher affinity than the unsubstituted pyrrolidin-2-one derivatives for α_1-AR. It was also shown that the 2nd position could play a crucial role in improving the α_1-AR antagonist properties in terms of affinity and selectivity. The pharmacological results and binding studies suggested that the antiarrhythmic and hypotensive properties of these compounds were related to their adrenolytic properties. Theoretical investigations showed that the charge distribution in the molecules significantly influenced their antiarrhythmic activities. The pattern of the charge distribution in the plane of the pharmacophoric features was analyzed and a favorable charge distribution was found. More extensive structure–activity studies are in progress and will be reported in due course.

6. Experimental

6.1. Chemistry

Melting points were determined in open glass capillaries on Büchi 353 apparatus (Flawil, Switzerland) and were uncorrected. Elemental analyses (C, H, N) were carried out on an Elementar Vario EL III (Elementar Analysensystem, Hanau, Germany), which gave values within 0.4% of the theoretical values. ^{1}H NMR and ^{13}C NMR data were recorded on a Varian Mercury VX 300 MHz PFG instrument (Hansen Way, USA) in $CDCl_3$ at ambient temperature. Thin layer chromatography was carried out on Merck silica gel precoated F_{254} plates (0.2 mm) (Darmstadt, Germany). The plates were visualised with UV light or iodine solution (0.05 M in a 10% HCl).

6.1.1. 1-Allyl-3,3-diphenylpyrrolidin-2-one (**2**)

20.0 g (84 mmol) 3,3-diphenyl-dihydro-furan-2-one (**1**) and 19.2 g (336 mmol) allylamine were heated in an autoclave at 200 °C for 96 h. The obtained product was crystallized from acetone. Yield: 69.4%, Anal. calc. for $C_{19}H_{19}NO$ (277.37) C% 82.28, H% 6.90, N% 5.05, found C% 82.32, H% 6.97, N% 5.04; m.p. 91–92 °C; TLC $R_f = 0.67$ (toluene:ethyl acetate (7:3)); ^{1}H NMR ($CDCl_3$):$\delta = 2.75$ (t, NCH_2CH_2, $J = 6.5$ Hz, 2H), 3.33 (t, CH_2CH_2C, $J = 6.5$ Hz, 2H), 4.0 (dt, NCH_2CH, $J = 1.7$ Hz and $J = 6.0$ Hz, 2H), 5.08–5.20 (m, $CHCH_2$, 2H), 5.65–5.81 (m, CH_2CHCH_2, 1H), 7.18–7.40 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 37.8$ ($CH_2CH_2CPh_2$), 43.8 (NCH_2), 48.1 (CH_2CH), 63.4 (CPh_2), 117.4 (CH_2CH), 126.2, 128.2, 129.8, 129.2 (arom), 131.8 ($CHCH_2$), 176.1 (carbonyl).

6.1.2. 1-Oxiranylmethyl-3,3-diphenylpyrrolidin-2-one (**3**)

13.8 g (50 mmol) of compound (**2**) was dissolved in 50 mL of methylene chloride and 11.4 g (50 mmol) of mCPBA. The reaction mixture was stirred at room temperature for 2 h, and then 20.0 g of sodium thiosulfate and 50 mL water were added. The layer was separated and the organic layer was washed with saturated sodium carbonate solution (15 mL) and brine (15 mL), dried over sodium sulfate and evaporated in vacuo. The obtained residue was purified by column chromatography using a mixture of toluene and ethyl acetate (7:3). Yield: 65.7%; Anal. calc. $C_{19}H_{19}NO_2$ (293.36) calc. C% 77.79, H% 6.53, N% 4.77, found C% 77.83, H% 6.57, N% 4.79; m.p. 104–105 °C; TLC $R_f = 0.55$ (toluene:ethyl acetate (7:3)); ^{1}H NMR

($CDCl_3$):$\delta = 2.48$ (dd, $CHCH_2$, 1H, $J = 2.6$ Hz and $J = 4.7$ Hz), 2.74–2.82 (m, $CHCH_2$, NCH_2CH_2, 3H), 3.04–3.10 (m, CH_2CHCH_2, 1H), 3.20 (dd, NCH_2CH, 1H, $J = 6.2$ Hz, $J = 14.5$ Hz), 3.38–3.58 (m, CH_2CH_2C, 2H), 3.94 (dd, NCH_2CH, 1H, $J = 2.7$ Hz, $J = 14.5$ Hz), 7.20–7.38 (m, arom, 10 H); ^{13}C NMR ($CDCl_3$): $\delta = 37.7$ ($CH_2CH_2CPh_2$), 43.4 (NCH_2), 45.3 (OCH_2), 51.0 (NCH_2CH), 52.9 (NCH_2CH), 63.3 (CPh_2), 126.2, 128.2, 129.8, 129.2 (arom), 175.8 (carbonyl).

6.1.3. General procedure for the synthesis of the dihydrochloride of 1-(2-hydroxy-3-substituted arylpiperazin-1-yl-propyl)-3, 3-diphenylpyrrolidin-2-one (**4**–**10**)

A solution of 2.9 g (10 mmol) of compound (**3**) and 10 mmol of the corresponding arylpiperazine in *n*-propanol (20 mL) was heated under reflux for 12 h. After evaporating the solvent, the oily residue was purified by column chromatography using a mixture of chloroform and acetone (1:1). The obtained oil was then dissolved in EtOH and then EtOH saturated with HCl_{gas} was added until the mixture becomes acidic. The obtained precipitate was crystallized from EtOH.

*6.1.3.1. 1-[2-Hydroxy-3-(4-phenylpiperazin-1-yl)-propyl]-3,3-diphenylpyrrolidin-2-one dihydrochloride (**4**).* Yield: 86.5%; Anal. calc. $C_{29}H_{33}N_3O_2 \times 2$ HCl (528.51) calc. C% 65.90, H% 6.67, N% 7.95, found C% 66.03, H% 6.79, N% 8.09; m.p. 189–190 °C; TLC: $R_f = 0.48$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$): $\delta = 2.28$ (t, NCH_2CH_2, 2H), 2.39–2.50 (m, CH_2CH_2C, 2H), 2.65–2.78 (m, piper, 4H), 3.10–3.18 (m, piper, 4H), 3.34 (dd, $CH_2CH(OH)CH_2$, 1H), 3.46–3.62 (m, $CH(OH)CH_2N$, $NCH_2CH(OH)$, 3H), 3.82–3.96 (m, $NCH_2CH(OH)$, 1H), proton of OH group is not visible, 6.80–6.92, 7.17–7.37 (m, arom, 15H); ^{13}C NMR ($CDCl_3$): $\delta = 37.7$ ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 66.5 (*C*H), 114.3, 121.9, 126.2, 128.2, 129.2, 129.6, 129.8, 149.6 (arom), 175.8 (carbonyl).

*6.1.3.2. 1-{2-Hydroxy-3-[4-(2-methoxy-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**5**).* Yield: 52.6%; Anal. calc. $C_{30}H_{35}N_3O_3 \times 2$ HCl (558.54) calc. C% 64.51, H% 6.68%, N% 7.52, found C% 64.67, H% 6.78, N% 7.69; m.p. 202–203 °C; TLC: $R_f = 0.50$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$):$\delta = 2.30$ (t, NCH_2CH_2, 2H), 2.51–2.59 (m, CH_2CH_2C, 2H), 2.66–2.81 (m, piper, 4H), 3.03–3.16 (m, piper, 4H), 3.37 (dd, $CH_2CH(OH)CH_2$, 1H), 3.52–3.67 (m, $CH(OH)CH_2N$, 2H), 3.85 (s, OCH_3, 3H), 3.90–4.00 (m, $NCH_2CH(OH)$, 2H), proton of OH group is not visible, 6.82–7.02 (m, arom, 4H), 7.20–7.40 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 37.7$ ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 55.8 (OCH_3), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 66.5 (*C*H), 113.5, 121.9, 122.1, 123.0, 126.2, 128.2, 129.2, 129.6, 141.1, 162.2 (arom), 175.8 (carbonyl).

*6.1.3.3. 1-{2-Hydroxy-3-[4-(4-methoxy-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**6**).* Yield: 57.2%; Anal. calc. $C_{30}H_{35}N_3O_3 \times 2$ HCl (558.54) calc. C% 64.51, H% 6.68%, N% 7.52, found C% 64.73, H% 6.73, N% 7.65; m.p. 196–197 °C; TLC: $R_f = 0.52$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$):$\delta = 2.30$ (t, NCH_2CH_2, 2H), 2.44–2.50 (m, CH_2CH_2C, 2H), 2.66–2.81 (m, piper, 4H), 3.03–3.16 (m, piper, 4H), 3.37 (dd, $CH_2CH(OH)CH_2$, 1H), 3.52–3.67 (m, $CH(OH)CH_2N$, 2H), 3.78 (s, OCH_3, 3H), 3.90–4.00 (m, $NCH_2CH(OH)$, 2H), proton of OH group is not visible, 6.82–7.02 (m, arom, 4H), 7.20–7.40 (m, arom, 10H); ^{13}C NMR ($CDCl_3$):$\delta = 37.7$ ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 55.8 (OCH_3), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 66.5 (*C*H), 115.2, 115.3, 126.2, 128.2, 129.2, 129.8, 146.3, 152.8 (arom), 175.8 (carbonyl).

*6.1.3.4. 1-{2-Hydroxy-3-[4-(2-ethoxy-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**7**).* Yield: 58.8%; Anal. calc. $C_{31}H_{37}N_3O_3 \times 2$ HCl (572.56) calc. C% 65.03%, H% 6.87, N% 7.34, found C% 65.14, H% 6.96, N% 7.38; m.p. 149–151 °C; TLC: $R_f = 0.47$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$): $\delta = 1.45$ (t, CH_3, 3H), 2.33 (t, NCH_2CH_2, 2H), 2.50 (t, CH_2CH_2C, 2H), 2.65–2.78 (m, piper, 4H), 3.01–3.16 (m, piper, 4H), 3.35 (dd, $CH_2CH(OH)CH_2$, 1H), 3.45–3.69 (m, $CH(OH)CH_2N$, $CH_2CH(OH)CH_2N_{piper}$, 4H), 4.15 (qw, OCH_2, 2H), proton of OH group is not visible, 6.82–7.00 (m, arom, 4H), 7.18–7.33 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 14.8$ (CH_3), 37.7 ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 64.6 (OCH_2), 66.5 (*C*H), 113.6, 121.2, 121.7, 122.6, 126.2, 128.2, 129.2, 129.8, 142.2, 162.3 (arom), 175.8 (carbonyl).

*6.1.3.5. 1-{2-Hydroxy-3-[4-(2-methyl-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**8**).* Yield: 54.6%; Anal. calc. $C_{30}H_{35}N_3O_2 \times 2$ HCl (542.54) calc C% 66.41, H% 6.87, N% 7.75, found C% 66.57, H% 6.99, N% 7.92; m.p. 169–170 °C; TLC: $R_f = 0.59$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$): $\delta = 2.30$ (s, CH_3, 3H), 2.38 (t, NCH_2CH_2, 2H), 2.45 (t, CH_2CH_2C, 2H), 2.65–2.76 (m, piper, 4H), 2.80–2.95 (m, piper, 4H), 3.40 (dd, $CH_2CH(OH)CH_2$, 1H), 3.52–3.63 (m, $CH(OH)CH_2N$, $CH_2CH(OH)CH_2N_{piper}$, 4H), proton of OH group is not visible, 6.85–7.00 (m, arom, 4H), 7.12–7.49 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 17.9$ (CH_3), 37.7 ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 64.6 (OCH_2), 66.5 (*C*H), 118.9, 123.2, 126.2, 126.6, 128.2, 129.2, 129.8, 131.4, 132.8, 151.0 (arom), 175.8 (carbonyl).

*6.1.3.6. 1-{2-Hydroxy-3-[4-(2-triflouromethyl-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**9**).* Yield: 55.5%; Anal. calc. $C_{30}H_{32}O_2N_3F_3 \times 2$ HCl (596.51) calc. C% 60.40, H% 5.75, N% 7.04, found C% 60.58, H% 5.89, N% 7.19; m.p. 220–222 °C; TLC: $R_f = 0.52$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$):$\delta = 2.26$ (t, NCH_2CH_2, 2H), 2.45 (t, CH_2CH_2C, 2H), 2.65–2.79 (m, piper, 4H), 2.84–2.93 (m, piper, 4H), 3.45 (dd, $CH_2CH(OH)CH_2$, 1H), 3.57–3.67 (m, $CH(OH)CH_2N$, $CH_2CH(OH)CH_2N_{piper}$, 4H), proton of OH group is not visible, 7.15–7.40 (m, arom, 4H), 7.42–7.68 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 37.7$ ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 64.6 (OCH_2), 66.5 (*C*H), 110.4, 113.1, 118.5, 126.2, 127.4, 128.2, 129.2, 129.8, 132.9, 143.4 (arom), 125.8 (CF_3), 175.8 (carbonyl).

*6.1.3.7. 1-{2-Hydroxy-3-[4-(4-flouro-phenyl)-piperazin-1-yl]-propyl}-3,3-diphenylpyrrolidin-2-one dihydrochloride (**10**).* Yield: 53.8%; Anal. calc. $C_{29}H_{32}N_3O_2F \times 2$ HCl (546.50) calc. C% 60.40, H% 5.75, N% 7.04, found C% 60.54, H% 5.92, N% 7.14; m.p. 175–177 °C; TLC: $R_f = 0.52$ chloroform:acetone (1:1); ^1H NMR ($CDCl_3$): $\delta = 2.26$ (t, NCH_2CH_2, 2H), 2.45 (t, CH_2CH_2C, 2H), 2.65–2.79 (m, piper, 4H), 2.84–2.93 (m, piper, 4H), 3.45 (dd, $CH_2CH(OH)CH_2$, 1H), 3.57–3.67 (m, $CH(OH)CH_2N$, $CH_2CH(OH)CH_2N_{piper}$, 4H), proton of OH group is not visible, 7.15–7.40 (m, arom, 4H), 7.42–7.68 (m, arom, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 37.7$ ($CH_2CH_2CPh_2$), 44.0 ($NCH_2CH_2CPh_2$), 55.0 (NCH_2CHOH), 56.0, 56.3 (CH_2 piper), 63.3 (CPh_2), 63.8 ($CHCH_2$), 64.6 (OCH_2), 66.5 (*C*H), 113.2, 126.0, 127.4, 128.2, 129.2, 129.8, 152,9 (arom), 124.1 (CF_3), 175.8 (carbonyl).

6.2. Pharmacology

6.2.1. Materials and methods

6.2.1.1. Compounds. [^{3}H] clonidine (Amersham), epinephrine (Adrenalinum hydrochloricum, Polfa), norepinephrine (Levonor, Polfa), methoxamine (Sigma-Aldrich Chemie GmbH), [^{3}H] prazosin (Amersham), tyramine (Sigma-Aldrich Chemie GmbH), sodium heparin (Polfa), thiopental sodium (Biochemie GmbH, Vienna).

6.2.2. Animals

The experiments were carried out on male Wistar 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 and tap water which was given ad libitum. Control and experimental groups consisted of 8–10 animals in each group. All procedures were carried out according to the Animal Care and Use Committee Guidelines and approved by the Ethical Committee of the Jagiellonian University, Kraków, Poland.

6.2.3. Reference compounds

Compound **I** was used as a reference.

6.2.4. Statistical analysis

The data were expressed as mean ± S.E.M. The statistical significance was calculated using one-way ANOVA. Differences were considered significant when $p < 0.05$.

6.2.5. α-Adrenoceptor radioligand binding assay

These experiments were carried out on rat cerebral cortex. [^{3}H] prazosin (19.5 Ci/mmol, an α_1-adrenergic receptor antagonist) and [^{3}H] clonidine (70.5 Ci/mmol, an α_2-adrenergic receptor agonist) 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 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. To measure unspecific binding, phentolamine, 10 μM (in the case of [^{3}H] prazosin), or [^{3}H] clonidine, 10 μM (in the case of [^{3}H] clonidine), was applied [27]. 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 (PerkinElmer, USA). All assays were carried out in duplicate. Radioligand binding was analyzed using an iterative curve-fitting routine (GraphPAD/Prism, Version 4.0 – San Diego, CA, USA). K_i values were calculated by the method of Cheng and Prusoff [28].

6.2.6. In vitro functional studies

6.2.6.1. α_{1D}-Adrenoceptors: rat thoracic aorta. Male Wistar rats weighing 200–350 g were anaesthetized with thiopental sodium (75 mg/kg ip) and the aorta was dissected and placed in Krebs–Henseleit solution (NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.25 mM, $MgSO_4$ 1.64 mM, KH_2PO_4 1.18 mM, $NaHCO_3$ 24.88 mM, glucose 10 mM, $C_3H_3O_3Na$ 2.2 mM, and EDTA 0.05 mM) and surrounding fat tissues were cleaned. The thoracic aorta was denuded of endothelium and cut into approximately 4 mm long rings. The aorta rings were incubated in 30 mL chambers filled with Krebs–Henseleit solution at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1). Two stainless steel pins were inserted through the lumen of each arterial segment: one pin was attached to the bottom of the chamber and the other to an isometric FDT10-A force displacement transducer (BIOPAC Systems, Inc., COMMAT Ltd., Turkey). The aorta rings were stretched and maintained at an optimal tension of 2 g and allowed to equilibrate for 3 h. During the equilibration period, the preparations were stimulated three times with noradrenaline (NA) (0.3 μM). Two cumulative concentration–response curves to NA were determined for each arterial ring at an interval of 60 min in the absence and presence of antagonist. Antagonists were incubated for 30 min. Experiments were conducted in the presence of continuous yohimbine (0.1 μM) and propranolol (1 μM) to block α_2- and β-adrenoceptors [37].

6.2.6.2. α_{1B}-Adrenoceptors: guinea pig spleen. Male guinea pigs (350–400 g) were killed by cervical dislocation. The spleen was removed and cut longitudinally into strips of approximately 20 mm, which were set up in a 30 mL organ bath under a resting tension of 1 g to record isometric contractile responses in Krebs–Henseleit solution of the above composition at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1). During an equilibration period of 100 min, tissues were stimulated with NA (0.1–10 μM) followed by washout until the contractile response had become constant. Two cumulative concentration–response curves to NA were determined for each tissue at an interval of 60 min in the absence and presence of antagonist. Antagonists were incubated for 30 min. Experiments were conducted in the presence of continuous propranolol (1 μM) to block β-adrenoceptors [38].

Concentration–response curves were analyzed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). Contractile responses to vasoconstrictor (in the presence or absence of tested compounds) were expressed as a percentage of the maximal noradrenaline effect ($E_{max} = 100\%$), reached in the concentration–response curves obtained before incubation with the tested compounds. Data were expressed as the mean ± SEM of at least 5 separate experiments. Curves were fitted to all the data by non-linear regression to determine Hill slopes for the agonist concentration–response curves, and to calculate EC_{50} values. The EC_{50} value in the presence and absence of antagonists was used to determine the concentration ratio (CR). Schild analysis was performed, and where the slope was not significantly different from unity, the pA_2 value was determined by plotting the log (CR-1) against the −log of antagonist concentration [38].

6.2.7. Prophylactic antiarrhythmic activity in a model of adrenaline-induced arrhythmia according to Szekeres and Papp [29]

Arrhythmia was evoked in thiopental (60 mg/kg, ip) – anaesthetized rats by i.v. injection of adrenaline (20 μg/kg). The tested compounds were administered intravenously 15 min before adrenaline. The criterion for 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 [39] on ventricular premature beats (VBs), bigeminy, salvos (less than four successive VBs), ventricular tachycardia (VT, four or more successive VBs) and ventricular fibrillation (VF).

6.2.8. The influence on blood pressure

Normotensive male Wistar rats were anaesthetized with thiopental (50–75 mg/kg, ip). The right carotid artery 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 20 mg/kg or into the caudal vein after a 5 min stabilization period at a volume equivalent to 1 mL/kg.

6.3. Molecular modeling

To identify the effect of substituents on the antiarrhythmic and hypotensive activities of novel pyrrolidin-2-one derivatives a conformational analysis was preformed. An earlier report suggested that 1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-pyrrolidin-2-one (**I**)

[36] and 1-[2-hydroxy-3-(4-diphenylmethylpiperazin-1-yl)propyl]-pyrrolidin-2-one (**II**) [24] were model structures for the current investigations. The core for the synthesis of new species comprised a skeleton of 1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-3,3-diphenylpyrrolidin-2-one (**4**) containing the 4-(para-substituted phenyl)piperazin-1-yl moiety (**6**, **10**) or the 4-(ortho-substituted phenyl)piperazin-1-yl moiety (**5**, **7**, **8**, **9**).

For the molecular 3D structure calculations, the Gaussian 03 program [40] was used. Each compound was subjected to the following calculation procedure. Firstly, three-dimensional structures of the pyrrolidin-2-one derivatives at their neutral state were obtained through full optimization based on the AM1 quantum chemical procedure. Force field calculations were used to ascertain whether the resulting structures were energy minima. All the molecules were minimized until the root mean square (RMS) gradient value was smaller than 10^{-6} a.u. Then the single point energy (SPE) calculations were performed at the DFT/B3LYP level of theory using the 6-31G** basis set. Later, using the surface data generated from Gaussian checkpoint files, and the GaussView 4.1 program [41], the distribution of charge in a molecule was calculated. To obtain a 2D contour plot of the MEP, the electrostatic potential cube file was calculated from total SCF density. The contours were then drawn using the following parameters: the two grid direction fields specified as −15 to 15 Å, the distance between the grid point of 0.1 Å, and the isovalues were 0.001. The plane in the contour was determined by the same point as was used to define the pharmacophore model (i.e. A – the center of the phenyl ring, B – the less negative ionizable nitrogen atom in the piperazine ring and C – the hydrogen bond acceptor). All computations were performed on a HP-6200 wx workstation.

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