Arch. Pharm. Chem. Life Sci. 2016, *349*, 211–223

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Full Paper

Synthesis and Pharmacological Activity of a New Series of 1-(1*H*-Indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)-propan-2-ol Analogs

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β-Adrenergic receptor antagonists are important therapeutics for the treatment of cardiovascular disorders. In the group of β-blockers, much attention is being paid to the third-generation drugs that possess important ancillary properties besides inhibiting β-adrenoceptors. Vasodilating activity of these drugs is produced through different mechanisms, such as nitric oxide (NO) release, β_2-agonistic action, α_1-blockade, antioxidant action, and Ca^{2+} entry blockade. Here, a study on evaluation of the cardiovascular activity of five new compounds is presented. Compound **3a** is a methyl and four of the tested compounds (**3b**–**e**) are dimethoxy derivatives of 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)-ethylamino)propan-2-ol. The obtained results confirmed that the methyl and dimethoxy derivatives of 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and their enantiomers possess α_1- and β_1-adrenolytic activities and that the antiarrhythmic and hypotensive effects of the tested compounds are related to their adrenolytic properties.

Keywords: α_1-, α_2-, β_1-Adrenoreceptor antagonists / Cardiovascular activity / Enantiomers / Synthesis

Received: July 8, 2015; Revised: January 19, 2016; Accepted: January 20, 2016

DOI 10.1002/ardp.201500234

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Introduction

In the group of β-blockers, much attention is being paid to the third-generation drugs which possess important ancillary properties besides inhibiting β-adrenoceptors [1]. Vasodilating activity of the third generation β-adrenolytics is produced through different mechanisms, such as nitric oxide (NO) release, β_2-agonistic action, α_1-blockade, antioxidant action, and Ca^{2+} entry blockade [2]. Vasodilating β-blockers were developed to use initially for hypertension. Currently, these anti-hypertensive drugs are used for congestive heart failure (CHF) [3]. Vasodilating ability may ameliorate some of the therapeutic problems associated with traditional β-blockade, such as the adverse effects on metabolic and lipid parameters as well as peripheral circulatory and respiratory disturbances that impaired quality of life [4].

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In the last decade, a new generation of β-blockers with additional α-adrenoceptor blocking activity was introduced to the therapy. The α/β-blockers (bucindolol, carvedilol, labetalol) have vasodilating properties via relaxation of arterial smooth muscle, with no reflex tachycardia as a result of β-adrenoceptor blockade [5].

In the search for structures with potential circulatory activity, our attention was focused on the carvedilol analogs. These compounds have the characteristic structural fragment of every β_1-adrenergic blocking agent, namely the aminopropan-2-ol moiety. In addition, they contain substituted indole moiety instead of carbazole moiety characteristic for carvedilol.

A methodology was designed for synthesis of new aminopropan-2-ol derivatives, analogs of carvedilol, which show similar or weaker pharmacological effects of carvedilol [6, 7]. Seeking an answer for structure–activity relationship, this paper reports on the preparation of five analogs of 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol as a racemic mixture and their enantiomeric forms, compounds **3a–e**. In contrary to already described compounds, new compounds were modified in the aromatic ring.

Results and discussion

Chemistry

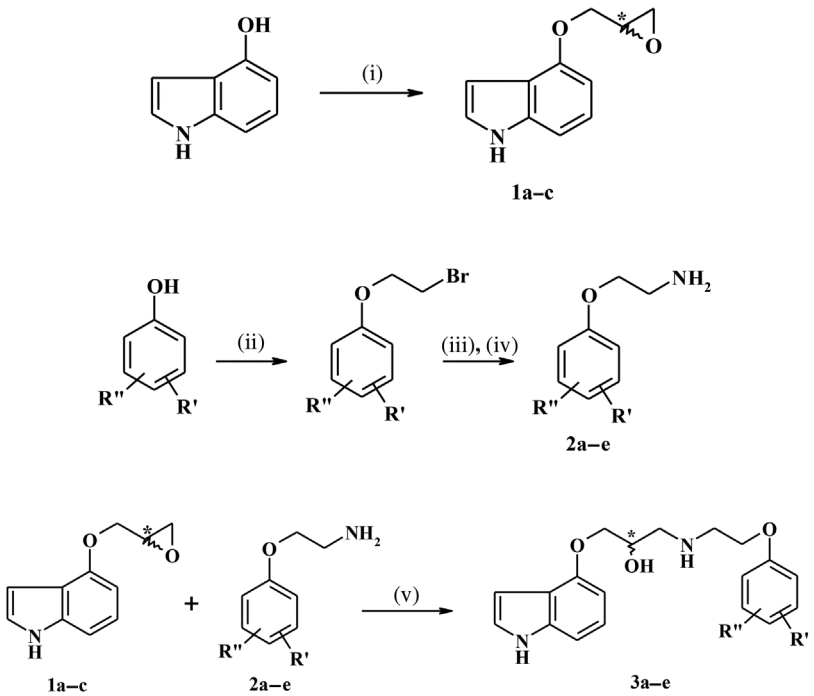
The synthesis of objective compounds is outlined in Scheme 1.

In contrary to the discussion in our previous paper, for synthesis of epoxide derivatives **1a–c**, we used commercially available 4-hydroxyindole and epichlorohydrin as a racemic mixture along with its optical pure forms. Condensation reaction goes smoothly with yield from 64 to 73% (after off crystallization). All three transformations were accompanied with the production of chlorohydrins derivatives (5–10%, estimated from thin layer chromatography (TLC)), which after isolation decomposed during storage. Primary amine derivatives **2a–e** were obtained through known Gabriel synthesis from an appropriate alkyl halide using Manske modification to release the primary amine [8]. Yield of these three steps was moderately low, from 19 to 47%, because in the condensation of phenol derivatives with 1,2-dibromoethane, phenol substrate was recovered, and in synthesis of *N*-alkylphthalimide intermediate, by-products were formed. It is strongly recommended to use analytically pure *N*-alkylphthalimide for hydrazinolysis. For phthalimide of **c** row, the pure crystals of 2-(2-(2,6-dimethoxyphenoxy)ethyl)isoindole-1,3-dione were obtained and X-ray analysis was done (Fig. 1a) [9]. Moreover, the addition of amine **2a–e** to the epoxy function of **1a–c** always gives by-product **4** (Fig. 1b). It was the consequence of the reaction of two molecules of the final product, which occurred during isolation over silica–gel chromatography. From the reaction mixture of **3c**, by-product **4** was isolated in pure crystalline form, which was suitable for X-ray analysis (Fig. 1b) [10].

Pharmacology

Radioligand receptor binding assays for adrenergic receptors

The affinity data for different adrenaline receptor subtypes are summarized in Table 1 (see also the Supporting Information). All tested compounds showed high affinity to



Scheme 1. Synthesis of compounds **3a–e**. Reagents and conditions: (i) (±) or (+)- or (−)-epichlorohydrin, 1 N NaOH, 1,4-dioxane, rt; (ii) 1,2-dibromoethane, NaOH or KOH, 80°C; (iii) potassium phthalimide, DMF, 50–60°C; (iv) NH_2-$NH_2 \cdot H_2O$, EtOH, 78°C or H_2O, rt; (v) CH_3CN, 80°C, argon, followed by salicylic aldehyde. Where: **3a** R′ = 2-CH_3, R″ = H; **3b** R′ = 2-OCH_3, R″ = 3-OCH_3; **3c** R′ = 2-OCH_3, R″ = 6-OCH_3; **3d** R′ = 3-OCH_3, R″ = 4-OCH_3; and **3e** R′ = 3-OCH_3, R″ = 5-OCH_3.

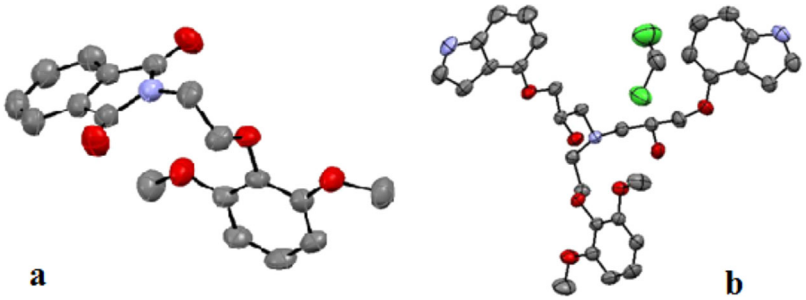


Figure 1. ORTEP diagram of 2-(2-(2.6-dimethoxyphenoxy)ethyl)isoindole-1.3-dione. Small crystal twinning was observed and twin data were ommited during automatic structure solving process carried out by AutoChem. Summary of data CCDC 969942 [9] (a), ORTEP diagram of (*R*)-1-((2-(2.6-dimethoxyphenoxy)ethyl)-((*S*)-2-hydroxy-3-(1*H*-indol-4-yloxy)propyl))amino)-3-(1*H*-indol-4-yloxy)propan-2-ol · CH_2Cl_2 with the hydrogens omitted for clarity. Summary of data CCDC 969943 [10] (b).

the α_1-adrenoceptors. The most potent in binding to these receptors was compound **3a**, for which the K_i value was in the range of 24.6 (racemic mixture) to 43.3 nM (*S*-enantiomer). There were no essential differences between enantiomers of the tested compounds in affinity for adrenergic α_1-adrenoceptors, depending on the spatial form. Other compounds (**3b–e**) showed about 6–23 times less affinity for these receptors. This is consistent with the literature in which a fragment responsible for binding to the α_1-adrenergic receptor assumed phenoxyethylamine moiety which has no asymmetric carbon [11]. In addition, it can be observed that the affinity to the receptor decreases, when added to the phenyl ring with two methoxy groups, practically regardless of the point of substitution.

Of all the tested compounds, only **3a** weakly binds to the α_2-adrenergic receptors (K_i ranged from 3 to 37 μM). The dimethoxy derivatives were not displaced at a concentration of 10^{-5}M [^{3}H]clonidine from the binding site in the rat cerebral cortex. There was no essential difference between enantiomers of the compound **3a** in affinity for adrenergic α_2-adrenoceptors, depending on the spatial form, but substitution of the phenyl ring of two methoxy groups, regardless of their location, decreased affinity for α_2-adrenergic receptors. Described in a previous paper [7], compounds **Ib** and **Ic** and their enantiomers, having one methoxy group on the indole ring, displaced [^{3}H]clonidine from cortical binding sites in the low concentration range (K_i = 43–277 nM). However, substitution of the phenyl ring with one methoxy group, compound **Ia** [6], inhibited [^{3}H]clonidine binding with K_i ranging from 365.5 to 1400 nM. If the phenol ring possesses two methoxy groups, compounds **3b–e**, deprived the affinity for α_2-adrenergic receptor.

The greatest differences between enantiomers were shown in affinity to β_1-adrenoceptor. All tested compounds in the form of enantiomer *S* possess 60 to 355-fold greater affinity to β_1-adrenoceptors than *R*-enantiomers. These results give evidence of the relationship of spatial configuration and affinity with β-adrenoceptors and lack of this relationship for α-adrenoceptors. The same results are observed for enantiomers of carvedilol and other β-adrenergic antagonists as well as

Table 1. Affinity for different adrenoceptor types in the rat cerebral cortex.

Compound	[^{3}H]Prazosin K_i [nM] ± SEM	[^{3}H]Clonidine K_i [μM] ± SEM	[^{3}H]CGP12177 K_i [nM] ± SEM
(*RS*)-**3a**	24.6 ± 1.3	3.0 ± 0.4	2.5 ± 0.2
(*R*)-**3a**	29.1 ± 0.9	37.4 ± 5.2	126.9 ± 1.6
(*S*)-**3a**	43.3 ± 1.1	5.0 ± 0.7	1.6 ± 0.2
(*RS*)-**3b**	390.5 ± 12.4	–	1.5 ± 0.3
(*R*)-**3b**	407.6 ± 16.1	–	131.5 ± 20.4
(*S*)-**3b**	262.9 ± 5.1	–	2.2 ± 0.3
(*RS*)-**3c**	291.1 ± 9.3	–	12.8 ± 0.9
(*R*)-**3c**	215.3 ± 14.1	–	1600 ± 132.4
(*S*)-**3c**	363.0 ± 21.6	–	4.5 ± 0.5
(*RS*)-**3d**	926.0 ± 68.2	–	6.3 ± 0.4
(*R*)-**3d**	415.7 ± 43.1	–	217.4 ± 16.5
(*S*)-**3d**	880.7 ± 61.8	–	2.0 ± 0.1
(*RS*)-**3e**	626.0 ± 51.2	–	12.8 ± 1.4
(*R*)-**3e**	146.7 ± 23.1	–	377.3 ± 26.5
(S)-**3e**	792.5 ± 61.8	–	12.0 ± 1.1
Carvedilol	2.2 ± 0.2[a)]	3.4 ± 0.9	0.81 ± 0.06[a)]

[a)] Ref. [40].

enantiomers of compounds 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxy phenoxy)ethylamino)propan-2-ol (**Ia**), 1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol (**Ib**), and 1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol (**Ic**), tested before [6, 7, 11–13]. Comparable affinity for β_1-adrenoceptor can be explained by the fact that the tested compounds were modified only in part of the molecule responsible for binding to the α-adrenergic receptors without asymmetric carbon.

The effect on adrenaline-induced arrhythmia in rats

In anesthetized rats, intravenous (*iv*) injections of adrenaline ($20\,\mu g \cdot kg^{-1}$) caused sinus bradycardia (100%), atrioventricular disturbances, and ventricular and supraventricular extrasystoles (100%), which led to the death of approximately 50% of animals. The tested compounds administered 15 min prior to adrenaline injection decreased the number of premature ventricular and supraventricular beats and reduced mortality.

The ED_{50} values, defined as a dose producing a 50% inhibition of premature ventricular beats, in the adrenaline-induced arrhythmia, are presented in Table 2. These compounds administered 15 min before adrenaline prevented and/or reduced in a statistically significant manner the number of premature ventricular beats. Compound **3c** and their enantiomers exhibited important antiarrhythmic effects with ED_{50} values ranging between 0.96 and 1.49 $mg \cdot kg^{-1}$. The tested compounds **3a** and **3b** and their enantiomers diminished the occurrence of extrasystoles and reduced mortality with ED_{50} values ranging between 0.48 and 1.67 $mg \cdot kg^{-1}$, and 0.43 and 1.38 $mg \cdot kg^{-1}$, respectively. Compound **3d**, only as the enantiomer *S* reduced the number of premature ventricular beats with ED_{50} value of 1.49 $mg \cdot kg^{-1}$. Compound **3e** exhibited antiarrhythmic effect with ED_{50} values ranging between 2.48 (for the enantiomer *S*) and 3.97 $mg \cdot kg^{-1}$ for the racemic mixture.

Table 2. Prophylactic antiarrhythmic activity in anesthetized rats.

Compound	ED_{50} *iv* ($mg \cdot kg^{-1}$)
(*RS*)-**3a**	0.96 (0.84–1.09)
(*R*)-**3a**	1.67 (1.48–1.87)
(*S*)-**3a**	0.48 (0.42–0.55)
(*RS*)-**3b**	1.29 (0.94–1.78)
(*R*)-**3b**	1.38 (1.12–1.69)
(*S*)-**3b**	0.43 (0.30–0.58)
(*RS*)-**3c**	1.49 (1.07–2.07)
(*R*)-**3c**	0.96 (0.84–1.09)
(*S*)-**3c**	1.22 (1.09–1.36)
(*RS*)-**3d**	>2
(*R*)-**3d**	>2
(*S*)-**3d**	1.49 (1.07–2.07)
(*RS*)-**3e**	3.97 (2.59–6.08)
(*R*)-**3e**	3.44 (2.44–4.88)
(*S*)-**3e**	2.48 (1.87–3.3)
Carvedilol	0.25 (0.12–0.53)[a]
Propranolol	1.05 (0.64–1.73)[b]

[a] Ref. [6].
[b] Ref. [41].

The antiarrhythmic effects of the novel compounds were examined on rats using the model of adrenaline-induced arrhythmia. The tested compounds **3a–e** and their enantiomers administered *iv* 15 min before an arrhythmogen, prevented or attenuated the symptoms of adrenaline-induced arrhythmia. The antiarrhythmic activity of the tested compounds was lower than carvedilol. All of the tested compounds demonstrated similar activity to propranolol in this test. The antiarrhythmic activity of pure β-adrenergic blocking agents has been known for years. The antiarrhythmic properties of β-blockers (Class II antiarrhythmic) are related to their ability to inhibit sympathetic influences on cardiac electrical activity. Sympathetic nerves increase the sinoatrial automatism, increase conduction velocity, and stimulate aberrant pacemaker activity. These effects are mediated through β_1-adrenergic receptors. Therefore, β-blockers may alleviate the adverse effects and thereby reduce sinus rhythm and conduction velocity (which can block reentry mechanisms) and inhibit the activity of pacemaker incorrect.

Cardiac α_1-adrenoceptors can alter myocardial hypertrophy, electrophysiological properties, and myocardial inotropy and chronotropy [14, 15]. α_1-Adrenoceptors in the heart can be of importance in the genesis of ischemia- and reperfusion-related ventricular arrhythmias [16]. Because the density of α_1-adrenoceptors in rat heart seems to be exceptionally high, five times greater than in the human heart [17], these rodent models could not be good predictors of new agents' with α_1-adrenolytic activity effectiveness in arrhythmias in humans. The recent studies indicate an important role of cardiac α_1-adrenoceptors in sustaining cardiac contractility in the failing human heart. In physiological state, the density of human cardiac α_1-adrenoceptors: α_{1A}, α_{1B}, and α_{1D} is only about 10–15% of that of β-adrenoceptors, but pathological settings are involved with decrease of β_1-adrenoceptors proportion, whereas the proportion of α_1-adrenoceptors is maintained. As a result, the function of α1-adrenoceptors under pathological conditions seems essential in sustaining cardiac increase [15, 18].

The antiarrhythmic effect of carvedilol may be due to blockade of both types of adrenergic receptors. The electrophysiological properties of carvedilol and its application in antiarrhythmic therapy were described previously [6, 19–22]. Based on our results, we hypothesize that the antiarrhythmic effects of the tested compounds are probably related to the blockade of β_1-adrenoceptor and to a lesser extent α_1-adrenoreceptors in heart tissue.

The influence on blood pressure in rats

Hypotensive activity of the tested compounds (as a racemic mixture and both enantiomers) was determined after *iv* administration to normotensive-anesthetized rats.

Compound **3a** in the form of racemic mixture statistically significantly decreased systolic (13.1–23%) and diastolic blood pressure (15.8–29.2%) in the range of doses 0.25–1.0 mg · kg^{-1}; in the dose of 0.25 mg · kg^{-1} hypotensive activity occurred 30 min after intravenous administration but in higher doses, this activity was observed from the beginning of measurement (Tables 3–5). The *R*-enantiomer of the compound **3a** significantly lowered systolic and diastolic blood pressures at a dose of 1.0 mg · kg^{-1} by 13.6–19.2 and 13.9–21.3%, respectively, and in a dose of 0.5 mg · kg^{-1} by 10.0–15.7 and 9.9–19.1%, respectively. The *S*-enantiomer of compound **3a** significantly decreased systolic and diastolic blood pressures in the range of doses 0.125–1.0 mg · kg^{-1} by 10.7–28.6 and 14.0–28.4%, respectively.

Compound **3b** in the form of enantiomer *S* as well as racemic mixture possessed similar hypotensive activity. They decreased systolic and diastolic blood pressures in the range of doses 0.125–1.00 mg · kg^{-1} (7–23%) from the 10th minute of measurement. The enantiomer *R* exhibited hypotensive activity only in a dose of 1 mg · kg^{-1} (it lowered systolic and diastolic blood pressures by 7.4–11.4 and 8.9–15.6%, respectively).

Compound **3c** as a racemic mixture, administered in a dose range 0.5–1.0 mg · kg^{-1}, significantly decreased the systolic and diastolic blood pressures by 12.5–15.7 and 12.6–13.9%, respectively. The racemic mixture of compound **3c** only at the highest dose of 1.0 mg · kg^{-1} decreased diastolic blood pressure from the 40th minute till the end of measurement. In the lower dose (0.25 mg · kg^{-1}), the hypotensive effect disappeared. The *R*-enantiomer of the compound **3c** in a dose of 1.0 mg · kg^{-1} only for a short period (15–30 min) significantly lowered the systolic blood pressure by 9.3–11%. The *S*-enantiomer of the compound **3c** only at a dose of

Table 3. Changes of systolic blood pressure after *iv* administration of tested compounds.

Compound	Dose	Time of observation (min)					
		0	5	10	20	40	60
Control	–	128.8 ± 2.7	128.3 ± 2.5	128.5 ± 4.9	127.8 ± 4.3	127.3 ± 2.8	127.5 ± 4.7
(*RS*)-**3a**	1	131.3 ± 4.0	102.5 ± 5.2*	105.8 ± 4.3**	106.5 ± 2.3**	107.3 ± 3.4**	108.5 ± 2.7**
	0.5	137.0 ± 2.7	113.5 ± 2.7*	112.2 ± 4.8*	112.0 ± 2.2*	114.3 ± 2.1*	113.5 ± 2.1*
	0.25	137.7 ± 3.3	123.7 ± 3.7	124.0 ± 3.2	119.7 ± 3.7***	116.7 ± 3.2**	114.3 ± 6.2**
	0.125	142.0 ± 3.0	135.7 ± 2.9	136.7 ± 4.4	132.3 ± 6.1	126.0 ± 5.9	126.0 ± 5.9
(*R*)-**3a**	1	136.5 ± 3.4	112.3 ± 3.6*	113.3 ± 3.4*	113.0 ± 1.5*	114.5 ± 2.9**	118.0 ± 3.6***
	0.5	132.0 ± 3.2	117.0 ± 1.7**	116.5 ± 1.2**	117.3 ± 1.4**	115.8 ± 2.8*	112.8 ± 2.7*
	0.25	135.7 ± 4.3	125.3 ± 3.7	127.0 ± 3.2	124.0 ± 3.5	122.3 ± 2.7	122.7 ± 1.9
(*S*)-**3a**	1	135.8 ± 2.6	99.5 ± 8.9**	105.0 ± 3.7***	105.8 ± 3.2***	106.0 ± 1.4***	106.3 ± 1.0***
	0.5	136.3 ± 3.8	103.5 ± 4.3*	109.3 ± 2.4*	105.0 ± 4.4*	99.3 ± 5.8*	104.0 ± 3.5*
	0.25	139.0 ± 2.1	121.3 ± 2.7***	121.3 ± 3.7***	116.7 ± 4.3**	108.0 ± 4.2*	109.0 ± 3.8*
	0.125	142.7 ± 2.3	130.0 ± 4.6	128.0 ± 5.0	119.7 ± 4.5***	113.0 ± 5.5**	112.3 ± 5.2*
	0.0631	139.0 ± 1.5	137.3 ± 1.5	133.3 ± 2.2	126.7 ± 2.6	120.0 ± 6.4**	120.0 ± 4.0**
(*RS*)-**3b**	1	135.6 ± 1.9	124.2 ± 1.3**	123.0 ± 1.7**	123.2 ± 3.2**	119.4 ± 1.2*	119.4 ± 1.4*
	0.5	134.5 ± 3.0	124.3 ± 0.5**	123.3 ± 0.8**	119.5 ± 1.2*	115.5 ± 2.5*	115.8 ± 3.1*
	0.25	133.5 ± 1.7	124.8 ± 1.6**	124.5 ± 2.7**	122.0 ± 1.1*	122.8 ± 2.1**	123.3 ± 1.9**
	0.125	130.8 ± 0.5	133.0 ± 0.9	130.0 ± 1.1**	126.3 ± 1.3*	126.3 ± 1.7*	124.8 ± 0.9*
	0.063	141.8 ± 1.3	138.3 ± 1.4	135.5 ± 2.1	131.5 ± 2.6**	125.5 ± 1.6*	125.0 ± 2.8*
	0.031	137.3 ± 2.6	144.3 ± 3.0	140.7 ± 3.4	134.0 ± 4.0	132.0 ± 1.7	133.0 ± 3.1
(*R*)-**3b**	1	134.8 ± 1.9	121.5 ± 1.9*	124.8 ± 1.9***	123.8 ± 0.8**	124.0 ± 0.4**	125.0 ± 0.4***
	0.5	136.7 ± 4.3	131.0 ± 3.0	131.3 ± 0.5	129.7 ± 3.2	127.0 ± 4.0	128.3 ± 3.2
(*S*)-**3b**	1	130.3 ± 3.1	113.5 ± 4.3**	115.3 ± 3.0***	115.3 ± 1.9***	110.5 ± 4.5*	111.3 ± 3.3**
	0.5	134.8 ± 2.1	126.0 ± 1.8	122.0 ± 1.6**	116.0 ± 1.2*	112.0 ± 4.6*	111.3 ± 3.0*
	0.25	138.5 ± 2.6	131.8 ± 2.5	128.5 ± 2.7	124.8 ± 2.7**	123.5 ± 1.9**	124.5 ± 2.3**
	0.125	135.2 ± 2.5	130.4 ± 2.7	127.2 ± 2.3	123.4 ± 1.7***	120.4 ± 1.5*	118.6 ± 2.9*
	0.063	119.0 ± 7.8	123.8 ± 6.7	120.0 ± 8.1	116.8 ± 6.9	115.5 ± 5.2	115.3 ± 5.6
(*RS*)-**3c**	1	134.7 ± 3.5	121.0 ± 1.0**	119.7 ± 3.4*	117.7 ± 6.2*	120.0 ± 5.3**	117.7 ± 3.8*
	0.5	134.0 ± 3.3	124.0 ± 3.5	123.3 ± 3.0	118.7 ± 2.3**	113.0 ± 2.1****	115.7 ± 6.4*
	0.25	129.0 ± 3.8	122.7 ± 4.4	120.0 ± 3.5	119.7 ± 2.6	116.3 ± 2.4**	112.7 ± 2.0*
	0.125	130.0 ± 10.3	127.0 ± 11.2	124.0 ± 8.9	125.3 ± 9.7	117.0 ± 6.6	119.0 ± 6.5
(*R*)-**3c**	1	132.3 ± 2.0	119.0 ± 1.0***	121.7 ± 2.7	120.3 ± 2.9***	124.0 ± 2.9	122.3 ± 2.9
	0.5	138.0 ± 1.7	131.3 ± 2.7	131.3 ± 2.7	129.7 ± 0.3	126.3 ± 1.5	125.3 ± 2.9
(*S*)-**3c**	1	131.7 ± 2.9	121.3 ± 1.5***	118.7 ± 1.9**	115.7 ± 2.6*	113.3 ± 1.5*	114.0 ± 1.5*
	0.5	119.8 ± 2.6	115.5 ± 2.0	114.8 ± 1.6	114.0 ± 2.3	112.3 ± 2.1	111.5 ± 2.7

Values are the mean ± SEM of six experiments. Statistical analyses were performed using a one-way ANOVA test.
$^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

Table 4. Changes of systolic blood pressure after *iv* administration of tested compounds.

Compound	Dose	Time of observation (min)					
		0	5	10	20	40	60
(*RS*)-**3d**	1	138.0 ± 1.9	129.8 ± 0.5*	129.3 ± 1.5***	124.5 ± 3.6****	120.8 ± 2.8****	118.8 ± 1.9****
	0.5	137.5 ± 4.6	129.3 ± 2.4**	127.0 ± 2.4*	124.0 ± 2.1***	120.3 ± 1.8****	119.8 ± 1.3****
	0.25	139.8 ± 5.7	124.5 ± 1.9	120.5 ± 1.7***	116.3 ± 2.6***	113.5 ± 3.1****	112.8 ± 3.8****
	0.125	139.0 ± 3.1	134.3 ± 0.9	131.7 ± 0.9*	128.3 ± 1.5***	124.7 ± 2.3****	123.0 ± 3.8****
	0.063	135.3 ± 3.4	135.3 ± 3.6	133.8 ± 4.3	130.3 ± 5.0	125.3 ± 5.3	122.8 ± 4.6**
(*R*)-**3d**	1	139.3 ± 3.2	135.0 ± 2.3	132.3 ± 2.7	129.0 ± 5.6	123.0 ± 6.7**	120.0 ± 6.1***
	0.5	135.7 ± 1.9	136.3 ± 2.2	136.7 ± 0.9	132.0 ± 1.0	130.3 ± 2.3	126.3 ± 3.4
(*S*)-**3d**	1	136.3 ± 2.0	134.0 ± 2.7	133.0 ± 2.1	127.0 ± 2.7*	124.7 ± 2.6***	122.3 ± 3.0****
	0.5	139.0 ± 3.5	125.7 ± 3.9***	125.0 ± 2.1***	119.0 ± 2.7****	109.7 ± 3.0****	107.3 ± 2.7****
	0.25	138.3 ± 0.9	131.3 ± 2.3	128.7 ± 3.4**	123.0 ± 3.1***	119.3 ± 4.2****	119.7 ± 4.8****
	0.125	137.3 ± 1.5	129.3 ± 4.1	126.7 ± 4.9	121.7 ± 5.8**	119.7 ± 6.2*	120.0 ± 5.9**
	0.063	137.7 ± 1.9	132.0 ± 3.0	131.3 ± 3.3	128.7 ± 2.8**	125.0 ± 2.9***	121.3 ± 3.0****
	0.031	134.8 ± 3.2	129.3 ± 2.9	128.8 ± 3.9	124.8 ± 4.2	119.8 ± 4.3***	117.8 ± 4.5***
	0.016	130.0 ± 3.9	132.0 ± 3.0	127.0 ± 2.5	124.3 ± 3.1	118.0 ± 2.5*	114.8 ± 2.0***
	0.008	130.0 ± 6.5	130.3 ± 5.6	126.8 ± 6.0	122.0 ± 4.9	117.0 ± 4.5	114.5 ± 4.1**
(*RS*)-**3e**	4	134 ± 7.3	114.8 ± 4.9**	113.3 ± 5.3*	125.3 ± 2.9**	117 ± 3.9**	116.3 ± 1.7**
	2	137 ± 8.3	124.3 ± 6.5	123 ± 5.7	124.3 ± 3.2	127.3 ± 6.9	112 ± 9.4*
(*R*)-**3e**	4	141.3 ± 4.1	125.3 ± 6.7	124.3 ± 4.2	125.3 ± 2.9	124.5 ± 4.4	118 ± 3.5
	2	132.8 ± 4.4	128.7 ± 3.5	127.2 ± 3.8	126.5 ± 4.2	127.0 ± 4.3	124.5 ± 5.5
(*S*)-**3e**	4	135.2 ± 4.8	117.9 ± 2.1**	116.9 ± 2.8*	116.5 ± 3.0**	118.5 ± 3.2**	121.1 ± 3.5
	2	140.6 ± 4.4	123.6 ± 2.7**	122.3 ± 3.4**	121.4 ± 2.4**	110.0 ± 3.5*	116.2 ± 2.9*
	1	133.4 ± 5.1	121.7 ± 5.8	119.5 ± 6.2	120.5 ± 4.8	122.1 ± 5.2	123.1 ± 4.5
Carvedilol	1	134.4 ± 4.5	111.4 ± 2.3****	106.6 ± 2.4****	104.6 ± 1.8****	104.4 ± 3.0****	107.8 ± 6.3****
	0.5	127.6 ± 9.0	102.2 ± 7.1**	103.6 ± 7.5**	102.4 ± 8.2**	101.6 ± 7.6**	102.4 ± 8.2**
	0.25	137.2 ± 2.7	107.3 ± 3.8****	106.3 ± 4.3****	104.5 ± 4.1****	105.7 ± 4.2****	106.3 ± 4.0****
	0.125	139.0 ± 5.8	112.0 ± 5.1****	111.7 ± 5.1****	109.7 ± 6.5****	113.5 ± 4.9***	113.5 ± 6.3***
	0.062	129.2 ± 5.9	110.0 ± 3.1***	109.5 ± 3.0***	107.5 ± 3.3****	103.5 ± 3.0****	102.0 ± 1.5****
	0.031	139.6 ± 2.6	126.4 ± 5.0**	125.4 ± 3.8**	116.2 ± 4.8****	104.4 ± 4.7****	107.6 ± 4.4****
	0.015	151.2 ± 1.6	139.0 ± 1.6	137.2 ± 1.4	132.2 ± 1.0*	125.5 ± 1.3***	120.5 ± 2.4****
	0.007	137.5 ± 4.5	133.7 ± 4.3	133.7 ± 4.8	132.7 ± 6.3	131.5 ± 6.7	131.2 ± 7.1

Values are the mean ± SEM of six experiments. Statistical analyses were performed using a one-way ANOVA test.
*$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, ****$p < 0.001$.

1.0 mg · kg^{-1} significantly decreased blood pressure, both systolic and diastolic.

Compound **3d** as a racemic mixture in the range of doses 0.125–1.00 mg · kg^{-1} decreased systolic blood pressure (6–23%) throughout the whole observation period and diastolic blood pressure (6–22%). In the lower dose (0.061 mg · kg^{-1}), this compound decreased only systolic blood pressure from the 50th minute after administration. The enantiomer *S* of the compound **3d** displayed a significant hypotensive activity to the dose 0.0625 mg · kg^{-1}. The enantiomer *R* of the compound **3d** decreased systolic and diastolic blood pressure in the dose of 1.0 mg · kg^{-1}.

Compound **3e** administered as the enantiomer *R* and racemic mixture decreased blood pressure only in a dose of 4.0 mg · kg^{-1}. The enantiomer *S* of the compounds **3e** produced hypotensive activity only in a dose of 2.0 mg · kg^{-1}.

Carvedilol as a reference drug, administered in a dose range 0.015–1.0 mg · kg^{-1}, significantly decreased the systolic and diastolic blood pressure. In the dose 0.007 mg · kg^{-1}, the hypotensive activity disappeared.

Hypotensive activity of the investigated compounds was determined after their *iv* administration to normotensive-anesthetized rats. Compounds **3a**, **3b**, **3d**, and **3e** show a high correlation of hypotensive activity of spatial configuration. The most active as hypertensive agent is enantiomer *S*, while the *R*-enantiomer is 2–32 times weaker. The hypotensive effect probably results from adrenoceptor blockade (α_1 in arteries and β_1 in heart). In radioligand binding studies, we found the difference only in affinity of enantiomers to β_1- not α_1-adrenoceptor. This relationship is consistent with literature reports. β-Blockers decrease arterial blood pressure by reducing cardiac output. Acute treatment with a β-blocker is not very effective in reducing arterial blood pressure because of a compensatory increase in systemic vascular resistance. This effect can be reversed by blocking the adrenergic receptors and relaxation of vascular smooth muscle. The compound **3c** diminished blood pressure at a range of doses 0.25–1.0 mg · kg^{-1} in a form of racemic mixture. Both enantiomers of compound **3c** showed a similar activity, reducing blood pressure only in a dose of

Table 5. Changes of diastolic blood pressure after *iv* administration of tested compounds in selected doses.

Compound	Dose	Time of observation (min)					
		0	5	10	20	40	60
Control	–	93.7 ± 2.7	93.5 ± 4.7	93.2 ± 3.3	94.0 ± 1.5	92.3 ± 5.3	93.7 ± 2.6
(*RS*)-**3a**	0.5	106.7 ± 2.0	89.8 ± 1.6*	86.3 ± 4.2***	87.0 ± 1.3***	86.2 ± 2.0***	85.2 ± 1.8***
	0.25	107.7 ± 2.9	96.0 ± 3.6	96.3 ± 3.8	92.3 ± 3.3	88.3 ± 3.2**	86.3 ± 6.2****
	0.125	112.7 ± 1.5	108.0 ± 2.3	107.0 ± 3.5	105.3 ± 4.7	98.0 ± 4.9	95.7 ± 5.0**
(*R*)-**3a**	0.5	106.3 ± 2.4	94.0 ± 1.2**	94.3 ± 1.4**	92.8 ± 1.7****	88.3 ± 2.7*	90.0 ± 1.7*
	0.25	112.3 ± 1.5	103.0 ± 2.3	106.7 ± 0.7	100.7 ± 2.4**	99.3 ± 4.1**	101.0 ± 3.1**
(*S*)-**3a**	0.5	106.5 ± 4.0	86.8 ± 2.1***	84.0 ± 3.4***	78.3 ± 3.1***	78.3 ± 1.5***	78.3 ± 1.5***
	0.25	109.7 ± 3.5	95.0 ± 2.7	94.3 ± 3.5**	90.7 ± 4.8*	82.7 ± 3.9***	81.3 ± 3.8***
	0.125	111.0 ± 1.5	104.3 ± 3.2	103.3 ± 3.7	95.0 ± 2.5**	89.3 ± 3.9*	85.7 ± 3.0***
	0.0631	115.3 ± 1.9	113.3 ± 1.8	111.0 ± 3.0	103.7 ± 3.5	96.33 ± 6.7**	94.7 ± 7.8**
(*RS*)-**3b**	0.125	105.3 ± 1.3	100.8 ± 1.7	96.7 ± 2.9	93.5 ± 2.5*	92.7 ± 3.5*	93.2 ± 1.0*
	0.063	109.8 ± 1.7	107.3 ± 2.0	105.0 ± 2.0	99.7 ± 3.9	93.0 ± 3.6*	93.0 ± 2.2***
	0.031	102.0 ± 1.2	107.7 ± 1.2	107.0 ± 1.5	101.7 ± 2.4	98.7 ± 0.3	100.3 ± 2.8
(*R*)-**3b**	1	104.0 ± 0.9	90.7 ± 2.8****	94.2 ± 2.4**	93.2 ± 1.3**	92.3 ± 2.2****	94.7 ± 1.0**
	0.5	104.3 ± 3.8	100.0 ± 2.6	101.3 ± 3.7	99.0 ± 2.5	98.7 ± 1.7	100.3 ± 2.2
(*S*)-**3b**	0.25	105.3 ± 0.9	95.5 ± 1.0****	92.8 ± 0.9*	89.0 ± 1.6*	86.5 ± 1.8***	89.5 ± 1.3***
	0.125	105.4 ± 2.0	100.2 ± 2.1	97.4 ± 1.9*	93.6 ± 1.2*	88.2 ± 1.8***	89.4 ± 2.4***
	0.063	89.5 ± 6.5	92.2 ± 5.4	89.7 ± 6.0	86.5 ± 4.4	82.4 ± 5.1	80.2 ± 4.5
(*RS*)-**3c**	0.5	106.7 ± 1.2	98.7 ± 4.5	99.3 ± 4.5	97.0 ± 1.4****	89.3 ± 1.5***	93 ± 2.1*
	0.25	108.3 ± 2.3	105.7 ± 3.7	104.3 ± 3.5	104.0 ± 2.9	101.0 ± 1.9****	99.1 ± 1.4*
	0.125	103.7 ± 2.8	103.7 ± 2.9	102.3 ± 5.4	101.3 ± 5.1	97.3 ± 3.2	95.1 ± 3.5
(*R*)-**3c**	1	100.1 ± 2.3	96.2 ± 5.2	95.1 ± 6.1	93.3 ± 4.2	88.7 ± 4.1	86.3 ± 4.1
	0.5	102.7 ± 1.9	97.3 ± 2.9	99 ± 3.4	97.7 ± 6.0	93 ± 2.9	91.7 ± 2.9
(*S*)-**3c**	1	96.7 ± 3.7	86.7 ± 1.8**	85.3 ± 2.1**	82.3* ± 5.3	81.7 ± 1.8*	82.7 ± 1.8*
	0.5	83.3 ± 3.5	81.2 ± 4.1	80.5 ± 3.3	79.8 ± 4.9	78.2 ± 3.5	77 ± 4.8
(*RS*)-**3d**	0.25	108.0 ± 4.6	100.0 ± 3.0	96.8 ± 3.1**	93.8 ± 4.0*	91.8 ± 4.3*	91.5 ± 4.3*
	0.125	112.0 ± 2.7	106.3 ± 1.5	104.3 ± 0.7	102.3 ± 1.8*	98.0 ± 2.9***	95.7 ± 3.7***
	0.063	103.8 ± 3.1	103.3 ± 4.1	102.5 ± 4.9	99.8 ± 5.0	95.5 ± 5.5	93.0 ± 4.8
(*R*)-**3d**	1	114.7 ± 2.3	111.0 ± 2.7	110.3 ± 1.8	108.3 ± 1.3	102.7 ± 1.2*	101.7 ± 0.9***
	0.5	116.3 ± 3.2	113.3 ± 1.8	113.7 ± 0.3	111.0 ± 1.5	108.3 ± 2.6	105.0 ± 4.0**
(*S*)-**3d**	0.125	110.0 ± 4.0	106.3 ± 3.2	105.7 ± 3.5	101.0 ± 3.5	98.3 ± 2.2**	98.0 ± 3.1**
	0.063	104.8 ± 6.3	104.0 ± 4.7	101.5 ± 5.1	98.8 ± 4.5	94.3 ± 4.5	90.8 ± 3.8**
	0.031	109.5 ± 4.6	106.5 ± 3.2	105.5 ± 4.0	101.5 ± 4.5	97.5 ± 5.1	96.3 ± 6.3
(*RS*)-**3e**	4	93.3 ± 3.1	84.5 ± 7.3	84 ± 3.4	89.8 ± 2.9	86.5 ± 2.8	88 ± 1.9
	2	115.8 ± 3.3	100.8 ± 6.4	102.5 ± 4.9	102.3 ± 6.1	105.5 ± 3.1	88.3 ± 11.8
(*R*)-**3e**	4	119.8 ± 5.6	104.8 ± 4.3	104.3 ± 3.1	105.5 ± 3.0	105.3 ± 4.3	115.3 ± 10.1
	2	112.3 ± 4.3	109.8 ± 2.5	107.8 ± 2.9	107.2 ± 2.4	108.8 ± 2.5	105.5 ± 4.3
(*S*)-**3e**	4	105.8 ± 1.8	91 ± 1.64*	92.3 ± 1.2*	92.5 ± 1.32*	92.5 ± 1.2*	95.3 ± 0.97****
	2	104.8 ± 8.2	91 ± 8.6	90.4 ± 8.2	85.8 ± 8.7	94.4 ± 9.4	93 ± 6.7
Carvedilol	0.125	130.0 ± 3.6	102.7 ± 4.8***	102.5 ± 4.7***	101.5 ± 6.6***	104.0 ± 4.1*	106.0 ± 6.4*
	0.062	115.2 ± 6.8	98.0 ± 4.6	97.5 ± 4.7*	95.0 ± 5.1**	90.5 ± 5.2*	88.7 ± 3.2*
	0.031	119.6 ± 2.0	106.6 ± 3.9****	105.6 ± 4.2**	96.0 ± 5.7****	88.6 ± 3.1***	90.4 ± 2.8***
	0.015	133.5 ± 2.4	122.5 ± 1.2	121.5 ± 1.2	117.0 ± 1.1**	110.2 ± 0.2*	104.5 ± 2.3***
	0.007	115.5 ± 7.9	112.5 ± 5.7	113.0 ± 5.1	113.7 ± 5.4	111.7 ± 6.0	111.2 ± 5.6

Values are the mean ± SEM of six experiments. Statistical analyses were performed using a one-way ANOVA test.
$^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

1.0 mg · kg^{-1}. In our research, we confirmed essential differences in hypotensive activity between enantiomers of the tested compounds **3a**, **3b**, **3d**, and **3e** after *iv* administration. Similarly to carvedilol and other compounds, which were tested before, there was a relationship between spatial configuration and hypotensive activity [6, 7].

Molecular modeling

The most potent compound **3a** has been regarded as a high affinity ligand of both α_{1A}- and β_1-adrenergic receptors. It has been synthesized and thoroughly evaluated as a racemate as well as both the single enantiomers. The affinity for α_{1A} is characterized by nanomolar K_i values for both *R*- and *S*-

enantiomers, whereas the affinity for β_1 receptors is noticeably biased. Such observations are in line with the established SAR data for the reference compound carvedilol (CYT). In the current study, molecular interactions displayed by the representative compound **3a** have been defined by using molecular modeling. The aim was to evaluate putative binding mode of the novel ligand and to point out the crucial interactions which would explain *in vitro* data.

A molecule of compound **3a** (*S*-enantiomer), was characterized by distinctive binding mode in α_{1A}- and β_1-receptors. In both sites, it adopted linear conformation locating its terminal fragments in each of the opposite cavities (one formed by transmembrane helices (TMHs) 3–6 and the second located between TMHs 1, 2, and 7). The main anchoring interaction in both sites was a charge-reinforced hydrogen bond between the protonated nitrogen atom of the ligand and carboxyl group of Asp3.32, supported by H-bond interaction between the hydroxyl group substituted in the alkyl linker of the ligand and the latter receptor residue. The second essential and common point for the interaction of both targets was CH–π contact between the aromatic moiety and Phe6.52. For β_1 receptor, the ligand's basic nitrogen was capable of forming additional interaction of H-bond nature with Asn7.39. Moreover, such specific contact arrangement determined the favorable geometry of the molecule, which was able to form aromatic CH–π interaction between substituted phenoxyethanamine and Trp3.28 (Fig. 2A). Formation of those interactions resulted in superior affinity for β_1-receptor, which is characteristic for *S*-enantiomers only – the eutomers. Such conclusion is consistent with *in vitro* binding data (see Table 1). The central fragment of the *R*-enantiomer (distomer in SAR studies) formed H-bond with Asp3.32 only, and no specific interaction of 2-methylphenoxyethanamine (Fig. 2C). Both the enantiomers bound to the deeper receptor cavity in the same manner, forming CH–π contact between Phe6.52 and 1*H*-indol-4-yloxy moiety, which served at the same time as H-bond donor to Ser5.42 (Fig. 2A and C).

In the α_{1A}-receptor-ligand complex, the amine and the hydroxy groups of compound **3a** formed H-bonds to Asp3.32, but the present Phe7.39 residue (instead of H-bond accepting Asn in the β_1-receptor) enforced the molecule to take the reversed pose in the binding site. The 1*H*-indol-4-yloxy moiety attached to the longer 1-aminopropan-2-ol linker caused less steric clashes with the bulky phenyl side residue of Phe7.39 than the 2-methylphenoxyethanamine fragment did. Therefore, the 1*H*-indol-4-yloxy moiety interacted with Phe2.64 (CH–π stacking), while the 2-methylphenoxy residue formed similar aromatic interaction with Phe6.52 (Fig. 2B).

The proposed binding mode of the evaluated compound **3a** is in line with the one of reference β_1-receptor ligand, carvedilol (*S*-enantiomer (Fig. 2A)), and satisfies the common interactions for monoaminergic receptor ligands [23, 24].

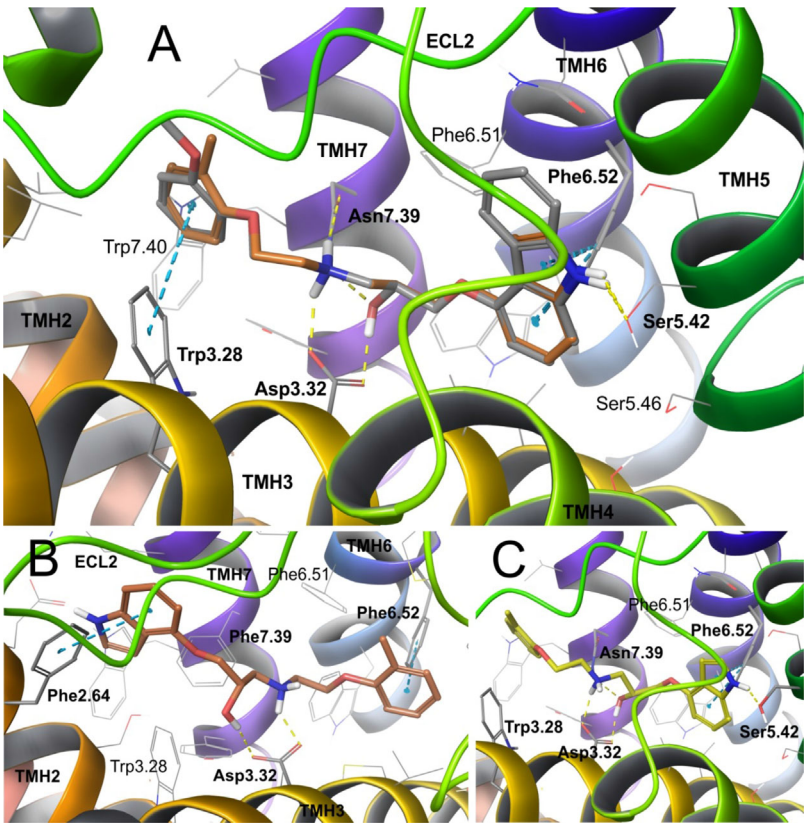


Figure 2. The predicted binding mode of the *S*-enantiomer (eutomer) of compound **3a** (orange), displayed together with the reference carvedilol molecule (gray) (A) and the *R*-enantiomer (distomer, yellow) (B) in the site of adrenergic β_1-receptor. The *S*-enantiomer of **3a** docked in the site of α_{1A}-receptor (C). Amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are displayed as sticks, whereas crucial residues, e.g., forming H-bonds (dotted yellow lines) or π–π/CH–π stacking interactions (dotted cyan lines) are represented as thick sticks. ECL2 residues were hidden for clarity of view. TMH, transmembrane helix; ECL, extracellular loop.

Moreover, it closely resembles the crystallographic experimental data. RMSD value between carvedilol molecule co-crystallized (4AMJ) and docked to the optimized receptor model derived from 4AMJ was 0.32 Å.

Conclusions

Our pharmacological tests show that new methyl and dimethoxy derivatives of **Ia** possess high affinity to the α_1- and β_1-adrenoceptors but not to α_2-adrenoceptors. Substitution of the phenyl ring of two methoxy groups, regardless of their location, decreased affinity for α_2-adrenergic receptors. The greatest differences between enantiomers were shown in affinity to β_1-adrenoceptor. The tested compounds **3a–e** and their enantiomers prevented or attenuated the symptoms of adrenaline-induced arrhythmia and decreased systolic and diastolic blood pressures but the antiarrhythmic and hypotensive effects of the tested compounds were lower than carvedilol. The results suggest that the antiarrhythmic and hypotensive effects of the tested compounds are related to their adrenolytic properties. Most of the pharmacological effects of the tested compounds and their enantiomers, especially enantiomer *S*, were qualitatively similar or qualitatively weaker than those of carvedilol.

The next step should be to examine the activity of tested compounds for each subtype of α_1-adrenoceptors as well as other mechanisms that may affect the pharmacological activity such as effects on the level of nitric oxide, antioxidant activity, or ability to bind to other GPCR receptors like angiotensin II receptor (AT_1) or endothelin receptor (ET_A, ET_B).

Experimental

Chemistry

Melting points were determined on a Boëtius or MEL-TEMP® apparatus and have gone uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker (500 MHz) instrument. Chemical shifts are expressed in ppm (δ) referred to TMS, coupling constants (*J*) are given in Hertz. IR and UV spectra were recorded on PerkinElmer and Hewlett Packard 8453 instruments, respectively. IR spectra were recorded using KBr pellets and wavenumbers are expressed in cm^{-1}. Elemental analysis was done on AE 1108 Carlo Erba apparatus. Mass spectra were obtained on AMD-604 spectrometer. Optical rotation was done on Jasco P-2000 polarimeter. TLC plates precoated with silica gel 60 F_{254} were used for monitoring, and silica gel 230–400 mesh was used for flash column chromatography (both from Merck). The X-ray structures were determined on SuperNova apparatus (Oxford Diffraction).

Materials

4-Hydroxyindole was supplied by ABCR, (±)-1-chloro-2,3-epoxypropane was purchased from Aldrich Chemicals, *S*-(+)- and *R*-(−)-1-chloro-2,3-epoxypropane were delivered by Chemos GmbH with optical purity of 99.9 and 99.8%, respectively. Phenol derivatives (2-methyl, 2,3-dimethoxy, 2,6-dimethoxy, 3,4-dimethoxy, and 3,5-dimethoxy) and other common materials were commercially available and used as obtained without further purification. Solvents were distilled and dried if required. [^{3}H]Prazosin, [^{3}H]clonidine, and [^{3}H]CGP12177 were supplied by PerkinElmer. The reference compound, carvedilol, was supplied by Pharmaceutical Research Institute, Warsaw, Poland.

*Preparation of 4-oxiranylomethoxy-1H-indols **1a–c***

4-Hydroxyindole was treated with racemic 1-chloro-2,3-epoxypropane, and their *R*,*S*-enantiomeric forms in the presence of stoichiometric quantity of base in 1,4-dioxane and water according to procedure described in literature [6], and gave the epoxide derivatives **1a–c**. Compounds were obtained as solids and yields were 73, 64, and 69%, respectively. NMR data, mps, for all three compounds, optical rotation for enantiomers confirmed the chemical structures and were agreeable with literature [6, 25].

*Preparation of 2-phenoxyethylamine derivatives **2a–e***

Appropriate phenol derivatives were converted to 2-phenoxyethylamine derivatives according to the procedure in literature [6]. All compounds were obtained as oil or meltable solids at room temperature. After standard work-up, amine derivatives were identified by ^{1}H-NMR data and intended to further transformation. They were sensitive to air exposure. Yield was calculated for three steps.

*2-o-Tolyloxyethylamine **2a***

Yield 47% [26]. ^{1}H NMR: δ: 7.13 (t, *J* 7.15, 2H), 6.86 (t, *J* 7.15, 1H), 6.80 (d, *J* 8.4, 1H), 3.96 (t, *J* 5.17, 2H), 3.07 (t, *J* 5.17, 2H), 2.24 (s, 3H), 1.44 (brs, 2H).

*2-(2,3-Dimethoxyphenoxy)ethylamine **2b***

Yield 17% [26]. ^{1}H NMR: δ: 6.97 (t, *J* 8.35, 1H), 6.57 (d, *J* 8.35, 2H), 4.04 (t, *J* 5.23, 2H), 3.86 (s, 6H), 3.09 (t, *J* 5.23, 2H), 1.49 (brs, 2H).

*2-(2,6-Dimethoxyphenoxy)ethylamine **2c***

Yield 27% [27]. ^{1}H NMR: δ: 6.99 (t, *J* 8.39, 1H), 6.58 (d, *J* 8.39, 2H), 4.04 (t, *J* 5.01, 2H), 3.85 (s, 6H), 2.93 (t, *J* 5.01, 2H), 1.79 (brs, 2H).

*2-(3,4-Dimethoxyphenoxy)ethylamine **2d***

Yield 19% [28]. ^{1}H NMR: δ: 6.77 (d, *J* 8.71, 1H), 6.55 (d, *J* 2.81, 1H), 6.40 (dd, *J* 8.71 and 2.81, 1H), 3.95 (t, *J* 5.18, 2H), 3.85 and 3.83 (2s, 6H), 3.07 (t, *J* 5.18, 2H), 1.85 (brs, 2H).

*2-(3,5-Dimethoxyphenoxy)ethylamine **2e***

Yield 19% [26, 28]. ^{1}H NMR: δ: 6.10 (s, 3H), 3.94 (t, *J* 5.18, 2H), 3.76 (s, 6H), 3.06 (t, *J* 5.18, 2H), 1.49 (brs, 2H).

*Preparation of the final compounds **3a–e***

A procedure described in the literature was used for addition of appropriate amine **2a–e** to epoxide function of **1a–c** [6].

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Spectral and physical data confirmed the structures of the obtained compounds.

*(2RS)-1-(1H-Indol-4-yloxy)-3-(2-(2-methylophenoxy)-ethylamino)propan-2-ol, (RS)-**3a***
Yield 56% as colorless crystals from acetone, mp 93–95°C. IR (KBr): 3430, 3393, 2945, 1586, 1501, 1363, 1245, 1124, 1056, 740. ^{1}H NMR ($CDCl_3$): 8.22 (brs, 1H), 7.15–7.06 (m, 4H), 7.02 (d, *J* 8.10, 1H), 6.86 (t, *J* 7.35, 1H), 6.82 (d, *J* 8.10, 1H), 6.63 (t, *J* 2.22, 1H), 6.53 (d, *J* 7.60, 1H), 4.18–4.14 (m, 3H), 4.08 (t, *J* 5.10, 2H), 3.09 (t, *J* 5.10, 2H), 3.02–3.0 (m, 1H), 2.95–2.92 (m, 1H), 2.60–2.40 (brs, 2H), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$): 156.8, 152.3, 137.3, 130.7, 126.84, 126.79, 122.74, 122.70, 120.6, 118.7, 111.1, 104.8, 100.9, 99.9, 70.6, 68.5, 67.4, 51.8, 48.9, 16.3. UV ($CHCl_3$), (nm), λ_{max}: 267 (lgε 3.95); 290 (lgε 3.82) (c 0.416 mg/10 mL). EA [%] for $C_{20}H_{24}N_2O_3$, calculated: C 70.57, H 7.11, N 8.23; found: C 70.63, H 7.30, N 8.40.

(*R*)-**3a**: Yield 68%, mp 101–103°C (methylene chloride/hexane), $[\alpha]_D^{20} = +2.4°$ (1.0, $CHCl_3$).

(*S*)-**3a**: Yield 46%, mp 101–103°C (methylene chloride/hexane), $[\alpha]_D^{20} = -2.5°$ (1.0, $CHCl_3$).

*(2RS)-1-(2-(2,3-Dimethoxyphenoxy)ethylamino)-3-(1H-indol-4-yloxy)propan-2-ol, (RS)-**3b***
Yield 43% as colorless crystals from methanol/methylene chloride, mp 109–111°C. IR (KBr): 3318, 2935, 1616, 1600, 1588, 1496, 1479, 1248, 1107, 997. ^{1}H NMR (CD_3OD): 7.12 (d, *J* 3.12, 1H), 7.04–7.01 (m, 3H), 6.70 (d, *J* 8.40, 2H), 6.58 (d, *J* 3.12, 1H), 6.54 (dd, *J* 6.15 and 2.25, 1H), 4.23–4.17 (m, 1H), 4.16–4.13 (m, 4H), 3.86 (s, 3H), 3.78 (s, 3H), 3.09 (t, *J* 5.30, 2H), 3.05 (dd, *J* 12.12 and 3.85, 1H), 2.91 (dd, *J* 12.12 and 8.15, 1H). ^{13}C NMR (CD_3OD): 155.9, 154.9, 154.6, 140.8, 140.2, 126.2, 124.9, 123.9, 121.2, 109.4, 108.1, 107.0, 102.2, 100.7, 72.8, 71.0, 70.5, 62.3, 57.5, 54.4, 50.4. EA [%] for $C_{21}H_{26}N_2O_5$, calculated: C 65.27, H 6.78, N 7.25; found: C 64.88, H 6.61, N 7.28.

(*R*)-**3b**: Yield 65%, mp 135–136°C (methylene chloride), $[\alpha]_D^{20} = +3.4°$ (0.99, MeOH).

(*S*)-**3b**: Yield 46%, mp 135–136°C (methylene chloride), $[\alpha]_D^{20} = -3.2°$ (0.99, MeOH).

*(2RS)-1-(2-(2,6-Dimethoxyphenoxy)ethylamino)-3-(1H-indol-4-yloxy)propan-2-ol, (RS)-**3c***
Yield 35% as colorless crystals from acetone, mp 113–114°C. IR (KBr): 3299, 3290, 3253, 2942, 2925, 2874, 2838, 1606, 1479, 1256, 1110, 1046, 749, 721. ^{1}H NMR ($CDCl_3$): 8.26 (brs, 1H), 7.10–7.08 (m, 1H), 7.06 (d, *J* 7.70, 1H), 7.00 (d, *J* 8.15, 1H), 6.52 (t, *J* 8.40, 1H), 6.65 (t, *J* 2.25, 1H), 6.56 (d, *J* 8.40, 2H), 6.54 (d, *J* 7.70, 1H), 4.20–4.11 (m, 5H), 3.82 (s, 6H), 3.01–2.98 (m, 1H), 2.94 (t, *J* 4.95, 2H) 2.90–2.87 (m, 1H), 2.20–1.80 (brs, 2H). ^{13}C NMR ($CDCl_3$): 153.6 (2*C*), 152.5, 137.3, 136.8, 123.7, 122.7, 122.6, 118.8, 105.2 (2*C*), 104.7, 100.8, 99.9, 72.6, 70.5, 68.5, 56.0 (2*C*), 51.7, 49.6. UV (EtOH), (nm), λ_{max}: 220 (lgε 4.76), (c 0.064 mg/10 mL). MS HR (ESI): for $C_{21}H_{27}N_2O_5$ ($[M+H]^+$) calculated: 387.1915; found: 318.1920. EA [%] for $C_{21}H_{26}N_2O_5$, calculated: C 65.27, H 6.78, N 7.25; found: C 65.26, H 6.69, N 7.19.

(*R*)-**3c**: Yield 45%, mp 111–112°C (methylene chloride), $[\alpha]_D^{20} = +2.18°$ (0.98, acetone).

(*S*)-**3c**: Yield 51%, mp 111–112°C (methylene chloride), $[\alpha]_D^{20} = -2.95°$ (0.98, acetone).

*(2RS)-1-(2-(3,4-Dimethoxyphenoxy)ethylamino)-3-(1H-indol-4-yloxy)propan-2-ol, (RS)-**3d***
Yield 62% as colorless crystals from methylene chloride, mp 131–132°C. IR (KBr): 3387, 3304, 2920, 1596, 1513, 1449, 1235, 1119, 1020, 749. ^{1}H NMR (CD_3OD): 7.08 (d, *J* 3.0, 1H), 7.02–6.97 (m, 2H), 6.82 (d, *J* 8.75, 1H), 6.55 (d, *J* 2.70, 1H), 6.53 (d, *J* 3.0, 1H), 6.49 (dd, *J* 8.75 and 2.70, 1H), 6.45 (dd, *J* 8.75 and 2.70, 1H), 4.25–4.17 (m, 1H), 4.15–4.12 (m, 1H), 4.07–4.04 (m, 3H), 3.76 and 3.74 (2s, 6H), 3.03–2.98 (m, 3H), 2.89–2.84 (dd, *J* 12.20 and 7.97, 1H). ^{13}C NMR (CD_3OD): 154.9, 153.6, 151.5, 144.9, 138.8, 123.9, 123.0, 120.0, 114.2, 105.7, 105.3, 102.2, 101.0, 100.0, 71.8, 69.8, 69.2, 57.0, 56.1, 53.5, 49.9. UV (EtOH), (nm), λ_{max}: 220 (lgε 4.73), (c 0.084 mg/10 mL). MS HR (ESI): for $C_{21}H_{27}N_2O_5$ ($[M+H]^+$) calculated: 387.1915; found: 318.1906. EA [%] for $C_{21}H_{26}N_2O_5$, calculated: C 65.27, H 6.78, N 7.25; found: C 65.15, H 6.67, N 7.20.

(*R*)-**3d**: Yield 30%, as oil.

(*S*)-**3d**: Yield 44%, as oil.

*(2RS)-1-(2-(3,5-Dimethoxyphenoxy)ethylamino)-3-(1H-indol-4-yloxy)propan-2-ol, (RS)-**3e***
Yield 86% as colorless crystals from methylene chloride/hexane, mp 121–123°C. IR (KBr): 3421, 3405, 3294, 3124, 2933, 1614, 1595, 1467, 1194, 1170, 741. ^{1}H NMR (CD_3OD): 7.08 (d, *J* 3.12, 1H), 7.02–6.98 (m, 2H), 6.54 (d, *J* 3.12, 1H), 6.50 (dd, *J* 6.22 and 2.22, 1H), 6.10 (d, *J* 2.10, 2H), 6.09–6.08 (m, 1H), 4.22–4.17 (m, 1H), 4.16–4.05 (m, 4H), 3.71 (s, 6H), 3.03 (t, *J* 5.45, 2H), 3.00 (dd, *J* 12.40 and 4.20, 1H), 2.86 (dd, *J* 12.40 and 8.02, 1H). ^{13}C NMR (CD_3OD): 163.2 (2*C*), 162.2, 153.7, 139.4, 124.1, 123.1, 120.3, 106.2, 101.3, 99.8, 94.6 (2*C*), 94.4, 72.1, 70.1, 68.2, 55.9 (3*C*), 53.8. EA [%] for $C_{21}H_{26}N_2O_5$, calculated: C 65.27, H 6.78, N 7.25; found: C 65.37, H 6.69, N 7.30.

(*R*)-**3e**: Yield, 70%, mp 107–108°C (methylene chloride/hexane), $[\alpha]_D^{20} = +6.7°$ (1.0, MeOH).

(*S*)-**3e**: Yield 70%, mp 105–106°C (methylene chloride/hexane), $[\alpha]_D^{20} = -6.6°$ (1.0, MeOH).

*(R)-1-((2-(2,6-Dimethoxyphenoxy)ethyl)-((S)-2-hydroxy-3-(1H-indol-4-yloxy)propyl))amino)-3-(1H-indol-4-yloxy)-propan-2-ol, by-product **4***
As colorless crystals from methylene chloride, mp 75–76°C. IR (KBr): 3405, 3106 (OH and NH), 2938, 2837 (C–H), 1597, 1588, 1510, 1478, 1363, 1245, 1109, 745. ^{1}H NMR (acetone-d_6): 10.17 (brs, 2H, N*H*), 7.17 (t, *J* 2.70, 2H), 7.03–6.96 (m, 5H), 6.65 (d, *J* 8.40, 2H), 6.57–6.56 (m, 2H), 6.52 (dd, *J* 7.38 and 0.63, 2H), 5.62 (s, 2H), 4.27–4.12 (m, 8H), 3.80 (s, 6H, $2 \times OCH_3$), 3.06–2.97 and 2.92–2.83 (2m, 8H). ^{13}C NMR (aceton-d_6): 154.5 (2*C*), 153.6 (2*C*), 138.7 (2*C*), 138.2, 124.4, 123.7, 123.5, 122.9 (2*C*), 119.0 (2*C*), 106.3 (2*C*), 105.5 (2*C*), 100.9 (2*C*), 99.9 (2*C*), 72.2 (2*C*), 71.3 (2*C*), 68.4 (2*C*), 59.9 (2*C*), 56.4 ($2 \cdot OCH_3$), 54.9. EA [%] for $C_{32}H_{37}N_3O_7 \cdot CH_2Cl_2$, calculated: C 60.00, H 5.95, N 6.36; found: C 59.34, H 5.78, N 6.27. UV (EtOH), (nm), λ_{max}: 220 (lgε = 4.98), (c = 0.058 mg/10 mL). $[\alpha]_D^{20} = +39.0°$ (1.005, $CHCl_3$).

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Animals

The experiment was carried out on male Wistar rats (180–250 g). The animals were housed in constant temperature facilities exposed to a 12:12 light–dark cycle and maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 6–8 animals each. The investigated compounds were administered intravenously or intragastrically at a constant volume of 1.0 mL · kg^{-1}. Control animals received an equivalent volume of solvent.

All procedures were conducted according to the guidelines of ICLAS (International Council on Laboratory Animals Science) and approved by The Local Ethic Committee on Animal Experimentation.

Statistical analysis

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

Adrenoceptor radioligand binding assay

The experiment was carried out on rat cerebral cortex. [^{3}H]Prazosin (19.5 Ci mmol^{-1}, an α_1-adrenergic receptor antagonist), [^{3}H]clonidine (70.5 Ci mmol^{-1}, an α_2-adrenergic receptor agonist), and [^{3}H]CGP12177 (48 Ci mmol^{-1}, a β_1-adrenergic receptor antagonist) were used. The brains were homogenized in 20 volumes of an ice-cold 50 mM Tris-HCl buffer (pH 7.6) and were centrifuged at 20000 × *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). The final incubation mixture (final volume 300 μL) consisted of 240 μL of the membrane suspension (10 mg protein in 1 mL of membrane suspension), 30 μL of [^{3}H]prazosin (0.2 nM), [^{3}H]clonidine (2 nM) or [^{3}H]CGP12177 (0.2 nM) solution, and 30 μL of the buffer containing seven to eight concentrations (10^{-11}–10^{-4} M) of the tested compounds. For measuring the unspecific binding, phentolamine, 10 μM (in the case of [^{3}H]prazosin), clonidine, 10 μM (in the case of [^{3}H] clonidine), and propranolol, −1 μM (in the case of [^{3}H] CGP12177) were applied. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed twice with the assay buffer and placed in scintillation vials with a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter. All the assays were made in duplicate. The radioligand binding data 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 [29] equation.

Prophylactic antiarrhythmic activity in a model of adrenaline-induced arrhythmia according to Szekeres and Papp [30]

Arrhythmia was evoked in thiopental (60 mg · kg^{-1}, *ip*) – anaesthetized rats by *iv* injection of adrenaline (20 μg · kg^{-1}). The tested compounds were administered intravenously 15 min before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and the inhibition of rhythm disturbances in comparison with the control group (ventricular bradycardia, atrioventricular block, ventricular tachycardia, or ventricular fibrillation). In anesthetized rats, *iv* injections of adrenaline (20 μg · kg^{-1}) caused sinus bradycardia (100%), atrioventricular disturbances, and ventricular and supraventricular extrasystoles (100%), which led to the death of approximately 50% of animals. The cardiac rhythm disturbances were recorded for 15 min after adrenaline injection. ECGs were analyzed according to the guidelines of the Lambeth Convention [31] on ventricular premature beats (VBs), bigeminy, salvos (less than four successive VBs), ventricular tachycardia (VT, four or more successive VBs), and ventricular fibrillation (VF).

Influence on blood pressure in rats

Male Wistar normotensive rats were anesthetized with thiopental (50–75 mg kg^{-1}, *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). The studied compounds were injected in a single dose into the caudal vein after a 15 min stabilization period at a volume equivalent to 1 mL · kg^{-1}.

Molecular modeling

Ligand docking to the models of G protein-coupled receptors involved adrenergic α_{1A}-receptor homology models and β_1-receptor crystal structure. The homology models have been obtained by applying the well-validated method [32], which has been used in successful modeling of several receptor models [33, 34].

The novel homology models of human α_{1A}-adrenergic receptor were built on the basis of β_2-adrenergic receptor crystal structure (PDB ID: 2RH1). Sequence alignment between target receptor (UniProt database accession number P35348) and the template were performed by hhsearch tool via GeneSilico Metaserver [34]. The acceptable overall sequence identity (31% according to hhsearch tool) authorized the use of 2RH1 for α_{1A}-homology modeling [35]. The artificial fragments replacing the third intracellular loop (ICL3) in the protein crystal structure were removed and short loops were created by joining Leu230 and Lys263. The crude receptor models were obtained using SwissModel [36], and were validated by processing in Protein Preparation Wizard [37]. Ligand-based binding site optimization, performed using induced fit docking (IFD) workflow [38] and docking-based validation, were carried out using various chemical classes of high affinity α_{1A}-receptor ligands. This procedure resulted in a variety of conformational models that served as molecular targets in docking studies.

The β_1-adrenergic receptor model has been developed on the basis of an experimental structure of the receptor co-crystallized with carvedilol (PDB ID: 4AMJ) [39]. The structure (chain B) has been refined using default settings of the Protein

Preparation Wizard. Water molecules and hetero groups other than carvedilol were deleted and the whole system was minimized (OPLS_2005). The model has been tested throughout docking studies involving β_1-receptor ligands. The obtained consistent binding modes of the reference compounds of experimentally proven affinity, verified the accuracy of both the crystal-based and the homology models.

Ligand structures were optimized using LigPrep tool. Glide XP flexible docking procedure was carried out using default parameters. H-bond constraint, as well as centroid of a grid box for docking studies were located on Asp3.32.

Glide, induced fit docking, LigPrep, and Protein Preparation Wizard were implemented in Small-Molecule Drug Discovery Suite (Schrödinger, Inc.), which was licensed for Jagiellonian University Medical College.

This project was supported by Polish Ministry of Science and Higher Education, National Science Center, grant no. UMO-2011/01/D/NZ4/01735. The authors gratefully acknowledge Faculty Laboratory of Spectrometry of Faculty of Chemistry, Rzeszow University of Technology for the NMR measurements.

The authors have declared no conflicts of interest.

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[10] Crystal, Data, C.H.N.O.x. CH_2Cl_2. MW 660.59, triclinic, P1, Z = 1, Calculated density = 1.281 mg/mm-^{3}, a = 8.3905 (3) Å, b = 10.0215(4) Å, c = 10.3015(5) Å, a = 93.783(4)°, b = 93.740(3)°, g = 97.765(3)°, V = 853.98(6) Å3, T = 293 (2) K, $l_{[CuKaKa]}$ = 1.5418 Å R1 = 6.29%, wR2 = 4.46%, crystal size 0.5612 × 0.2302 × 0.1808 mm^3, colorless.
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