European Journal of Medicinal Chemistry 44 (2009) 5103–5111

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and adrenolytic activity of 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxy phenoxy)ethylamino)propan-2-ol analogs and its enantiomers. Part 2

Grażyna Groszek [a,*], Agnieszka Nowak-Król [a], Tomasz Wdowik [a], Dariusz Świerczyński [b], Marek Bednarski [c], Monika Otto [c], Maria Walczak [d], Barbara Filipek [c,**]

[a] *Faculty of Chemistry, Rzeszów University of Technology, 6 Powstańców Warszawy Avenue, 35-959 Rzeszów, Poland*
[b] *Institute of Physical Chemistry, Polish Academy of Sciences, 44/52 Kasprzaka, 01-224 Warszawa, Poland*
[c] *Laboratory of Pharmacological Screening, Jagiellonian University Medical College, 9 Medyczna, 30-689 Kraków, Poland*
[d] *Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, 9 Medyczna, 30-689 Kraków, Poland*

ARTICLE INFO

Article history:
Received 17 January 2009
Received in revised form
12 July 2009
Accepted 16 July 2009
Available online 21 July 2009

Keywords:
Synthesis
Enantiomers
α_1-, α_2- and β_1-Adrenoceptor antagonists
Cardiovascular and spasmolytic activities

ABSTRACT

The synthesis of (2*RS*)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and (2*RS*)-1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and its enantiomers, analogs of 1-(1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol ((*RS*)-**9**) is described. Compounds were tested for electrographic, antiarrhythmic, hypotensive and spasmolytic activities as well as for α_1-, α_2- and β_1-adrenoceptors binding affinities. The antagonist potency of the new compounds was compared with carvedilol and (*RS*)-**9**.

1. Introduction

β-Adrenolytic drugs have an established position in cardiovascular disorders' treatment. Since development of this class of drugs in the late 50s of XX century, they are administered in the therapy of hypertension, coronary artery disease, arrhythmia, myocardial infarct and since several years in heart failure [1]. However, some adverse side effects, such as proatherosclerotic actions, peripheral circulatory and respiratory disturbances, erectile dysfunction, hypoglycemia during diabetic therapy, provocation of coronary vasospasm, and impaired quality of life, have limited their clinical use. β-Blockers, on the other hand, may increase the risk of severe hypoglycemia in patients with diabetes and hypertension because they blunt autonomic warning symptoms of hypoglycemia. The concern that β-blockers may mask hypoglycemia has militated against their use in patients with diabetes. Recently, much attention is being paid to β-blockers that possess vasodilator actions produced through different mechanisms, such as release of nitric oxide (NO), antioxidant action, β_2-agonistic action, Ca^{2+} entry blockade and α_1-blockade [2].

In the last decade, a new generation of β-blockers with additional α-adrenoceptor blocking activity was introduced to 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. They have also beneficial effects on the regular circulation in contrast to classic β-blockers [3].

Carvedilol, (2*RS*)-1-(9*H*-carbazol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol, is a non-selective β-adrenoceptor blocking agent which is most commonly used for treating primary hypertension and stable angina pectoris because of its vasodilating action [4,5]. Although originally used to treat hypertension, subsequent studies have revealed that carvedilol has efficacy in many other disease processes. Carvedilol improves the symptom profile in patients with heart failure and stable angina pectoris, reduces secondary cardiac events after myocardial infarction, decreases experimental infarct size after myocardial ischemia and reperfusion injury, and favorably reduces portal pressures in experimental models of cirrhosis [5,6]. Extensive investigations have revealed that this agent significantly improves the morbidity of the patients suffering from chronic heart failure [7].

* Corresponding author. Tel.: +48 17 8651751; fax: +48 17 8543655.
** Corresponding author. Tel.: +48 12 6205531; fax: +48 12 6205552.
E-mail addresses: ggroszek@prz.edu.pl (G. Groszek), mffilipe@cyf-kr.edu.pl (B. Filipek).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.07.012

Seeking for structures with potential circulatory activity, we have focused our attention on the carvedilol analogue, compound (*RS*)-**9** (Fig. 1). The synthesis of this compound (racemate and enantiomers) was described in Part 1 of the series [8]. (*RS*)-**9** has the characteristic structural fragment of each β_1-adrenergic blocking agent, namely aminopropan-2-ol moiety. In addition, it contains indole moiety instead of the carbazole one, which is specific for carvedilol.

In this part, we report on the preparation of two analogs of (*RS*)-**9**: (2*RS*)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and (2*RS*)-1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol, as well as their enantiomers. They have methoxy group introduced to indole aromatic ring into position 5 or 7 (Fig. 2). We also present the results of in vitro binding affinity to α_1-, α_2 and β_1-adrenergic receptors, antiarrhythmic and hypotensive activities of these compounds.

2. Chemistry

The synthesis of target compounds is outlined in Fig. 2.

In the designed synthetic pathway, indole **5** was the key intermediate. The procedure we used was similar to that described by Fukuyama et al. [9], but we had to modify it to obtain compound **5b**. It comprised: (i) change of protective group from acetyl to benzyl for compound **3b**; (ii) modification of Knoevenagel reaction conditions, step (d), leading to product **4**; and (iii) reductive elimination, step (e), to obtain indole derivative **5**. For last two steps, we applied the procedure as described in Part 1 [8]. Products **7** were obtained both as racemic mixtures and as individual enantiomers. To confirm, that deprotection of hydroxyl group took place in *ortho* position relative to aldehyde group in substrate **1**, X-ray analysis was made for single crystals of compounds **3b** and **7a**. The crystal structures are illustrated by the ORTEP diagrams in Figs. 3 and 4.

3. Pharmacological results

3.1. Radioligand receptor binding assay for α- and β-adrenergic receptors

The affinity of compounds **7a** and **7b** and their enantiomers to the catecholamines binding side of α_1-, α_2- and β_1-adrenoceptors was measured as a rate of specific displacement of [^{3}H]Prazosin, [^{3}H]Clonidine and [^{3}H]CGP12177 at the concentrations of 0.2 nM, 2 nM and 0.2 nM, respectively. Compound **7a** and its enantiomers inhibited [^{3}H]Prazosin binding with K_i ranging from 60.9 to 168.8 nM, [^{3}H]Clonidine binding with K_i ranging from 108.8 to 277.2 nM and [^{3}H]CGP12177 binding with K_i ranging from 59.3 to 530.6 nM to cortical α_1-, α_2- and β_1-adrenoceptors, respectively. Compound **7b** and its enantiomers displaced [^{3}H]Prazosin and [^{3}H]Clonidine from cortical binding sites in low concentration range ($K_i = 11.3$–45.8 nM and 43–60.9 nM, respectively). These compounds also displaced [^{3}H]CGP12177 from its binding sites in upper concentrations ($K_i = 0.89$–3.4 μM). The shape of the curves suggested competitive binding. The results are summarized in Table 1.

3.2. Effect on normal electrocardiogram (ECG) in vivo in rats

The effect on ECG intervals and heart rate was determined for all compounds in the same dose of 1 mg kg^{-1}.

Racemic mixture and both enantiomers of compound **7a** administered iv decreased heart rate by 10–15% up to 15 min after administration. Compounds (*RS*)-**7a** and (*S*)-**7a** prolonged P–Q interval and only enantiomer *S* of compound **7a** slightly prolonged QRS complex (Table 2). Racemic mixture of compound **7b** slightly prolonged QRS, whereas enantiomer *R* of compound **7b** considerably changed the ECG pattern by significantly decreasing the number of cardiac beats per minute and prolonged P–Q interval and QRS complex. Racemic mixture and enantiomer *R* of compound **7b** did not significantly affect the normal ECG in anesthetized rats.

3.3. Effect on adrenaline-induced arrhythmia in rats

In anesthetized rats, iv injections of adrenaline (20 μg kg^{-1}) caused sinus bradycardia (100%), atrioventricular disturbances, ventricular and supraventricular extrasystoles (100%) which led to 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, a dose producing a 50% inhibition of premature ventricular beats, in the adrenaline-induced arrhythmia are presented in Table 3. The compounds administered 15 min before adrenaline prevented and/or reduced in a statistically significant manner the number of premature ventricular beats. Compound **7a** and its enantiomers exhibited important antiarrhythmic effects with ED_{50} values ranging between 0.38 and 0.53 mg kg^{-1}. The second tested compound **7b** and its enantiomers diminished the occurrence of extrasystoles and reduced mortality with ED_{50} values ranging between 0.16 and 0.38 mg kg^{-1}.

3.4. Influence on blood pressure in rats

Hypotensive activity of compounds **7a** and **7b** (as racemic mixture and both enantiomers) was determined after iv administration to normotensive anesthetized rats.

After iv administration hypotensive activity was balanced for each form of compound **7a**. Both enantiomers and racemic mixture significantly decreased the systolic (12–25%) and diastolic (13–30%) blood pressures throughout the whole observation period in the range of doses 0.25–1.0 mg kg^{-1}. In the lowest dose (0.125 mg kg^{-1}) of compound **7a** and its enantiomers the hypotensive activity disappeared (Fig. 5).

Compound **7b** as a racemic mixture in the range of doses 0.25–1.0 mg kg^{-1} decreased the systolic blood pressure (7–18%) throughout the whole observation period and the diastolic blood pressure (8–19%). In the lower dose (0.125 mg kg^{-1}) this compound

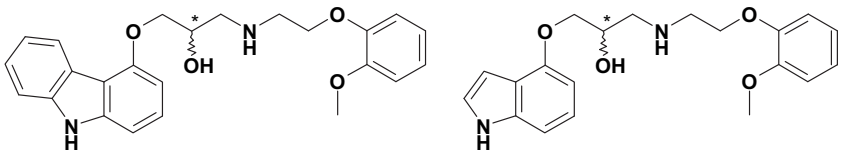

Fig. 1. Chemical structure of carvedilol and (*RS*)-**9**.

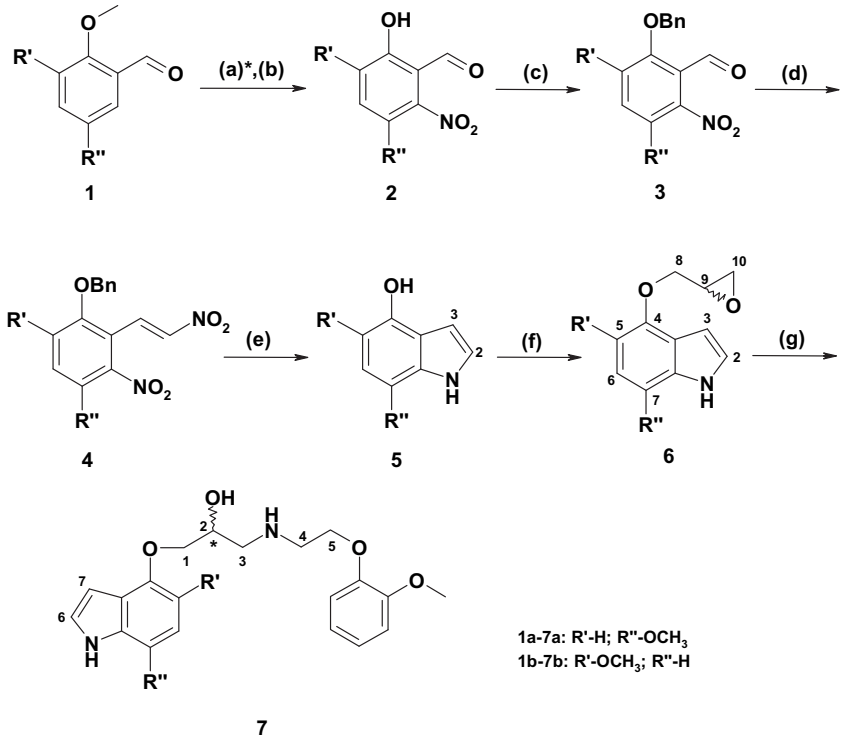

Fig. 2. Synthetic pathway: (a) fuming HNO_3, AcOH, rt.; (*) two extra regioisomers of nitro derivative were obtained for **1a** and **1b** in *para* and *meta* positions to aldehyde group, respectively, (b) BBr_3, CH_2Cl_2, for **1a** −40 °C → rt., and −78 °C for **1b**; (c) benzyl chloride, K_2CO_3, KI, CH_3CN; (d) CH_3NO_2, K_2CO_3, 18-crown-6 ether, 1,4-dioxane, 12 °C → rt, then Ac_2O, NaOAc, 100 °C; (e) H_2, Pd/C, EtOH–AcOH; (f) (+/−)-epichlorohydrin, ~3% NaOH aq., 1,4-dioxane; (g) (2-methoxyphenoxy)ethylamine, CH_3CN, 80 °C.

decreased the systolic and diastolic blood pressures only in 30 and 10 min after administration, respectively. Both enantiomers of compound **7b** displayed similar hypotensive activity. These compounds significantly decreased the systolic blood pressure in the range of doses 0.125–1.0 mg kg^{-1} and the diastolic blood pressure in the doses 1.0 and 0.5 mg kg^{-1} throughout the whole observation period. In the lower doses the hypotensive activity becomes weaker and disappeared in the doses 0.0625 and 0.0312 mg kg^{-1}, respectively for enantiomer *R* and enantiomer *S* of compound **7b** (Fig. 6).

3.5. Influence on isolated rabbit ileum

Compound **7a**, as a racemic mixture and enantiomer *S*, only given at doses 10^{-5} M and 10^{-6} M statistically significantly decreased the amplitude of contractions of isolated rabbit small intestine (of about 10–57%) but none of the tested doses of compound **7a** influenced on the frequency of contractions of isolated rabbit small intestine.

Compound **7b**, as a racemic mixture and both enantiomers, at the highest dose 10^{-5} M statistically significantly decreased the frequency, but only both enantiomers of compound **7b** significantly diminished the amplitude of contractions of isolated rabbit small intestine (of about 21–22%). In the lower doses compound **7b** had no influence on the frequency and amplitude of contractions of isolated rabbit small intestine.

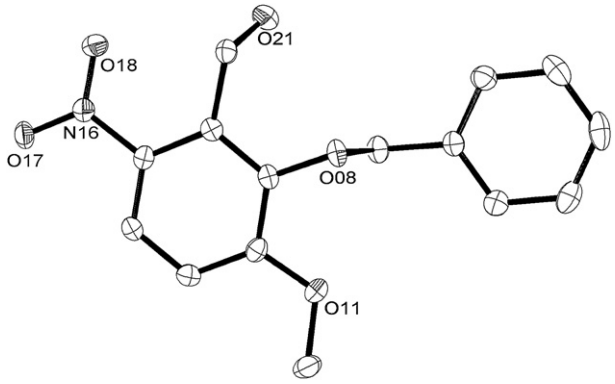

Fig. 3. ORTEP diagram of 2-(benzyloxy)-3-methoxy-6-nitrobenzaldehyde (**3b**) with the hydrogens omitted for clarity. Summary of data CCDC 295392.

4. Discussion

The aim of our study was to evaluate the cardiovascular activity of new compounds: **7a**, (2*RS*)-1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol, and **7b**, (2*RS*)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol as racemic mixtures and pairs of enantiomers. They are methoxy derivatives of compound (*RS*)-**9**, the chemical structure of which is related to carvedilol, non-selective β-antagonist with α_1-adrenolytic activity. Carvedilol has adjunctive pharmacologic properties, including α_1-blocking, antioxidant, anti-inflammatory

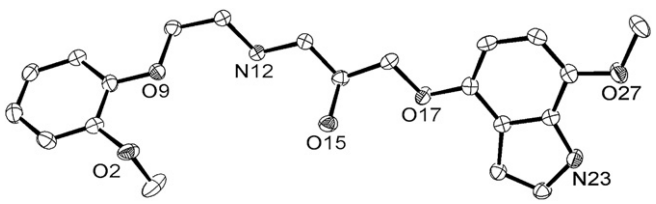

Fig. 4. ORTEP diagram of (2*RS*)-1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol (**7a**) with the hydrogens omitted for clarity. Summary of data CCDC 290726.

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 [nM] ± SEM	[^{3}H]CGP12177 K_i [nM] ± SEM
(*RS*)-**7a**	75.8 ± 2.7	180.8 ± 3.7	125.0 ± 5.2
(*R*)-**7a**	60.9 ± 1.6	108.8 ± 2.3	530.6 ± 24.8
(*S*)-**7a**	168.8 ± 7.2	277.2 ± 13.2	59.3 ± 5.8
(*RS*)-**7b**	21.1 ± 1.7	56.9 ± 0.7	1600 ± 200
(*R*)-**7b**	45.8 ± 6.8	43.0 ± 1.8	3400 ± 600
(*S*)-**7b**	11.3 ± 0.3	60.9 ± 0.2	886.7 ± 46.9
(*RS*)-**9**	89.8 ± 9.5	1.4 ± 0.4 μM	3.0 ± 0.6
Carvedilol	2.2 ± 0.2[a]	3.4 ± 0.9 μM	0.81 ± 0.06[a]

[a] Ref. [10].

Table 3
Prophylactic antiarrhythmic activity in anesthetized rats.

Compound	ED_{50} iv (mg kg^{-1})
(*RS*)-**7a**	0.45 (0.11–1.74)
(*R*)-**7a**	0.38 (0.10–1.46)
(*S*)-**7a**	0.53 (0.26–1.10)
(*RS*)-**7b**	0.38 (0.24–0.60)
(*R*)-**7b**	0.16 (0.06–0.37)
(*S*)-**7b**	0.30 (0.16–0.57)
(*RS*)-**9**	0.34 (0.23–0.51)
Carvedilol	0.25 (0.12–0.53)
Propranolol	1.05 (0.64–1.73)[a]

[a] Ref. [11].

antiendothelin, antiproliferative, and antiarrhythmic activities that appear to underlie the outcomes demonstrated in experimental models and clinical trials [12]. Compound (*RS*)-**9** described in Part 1 [8], possesses an antiarrhythmic and hypotensive activity and β- and α-adrenolytic mechanism of action.

Pharmacological properties of compounds **7a** and **7b** and their enantiomers were investigated in comparison with carvedilol and (*RS*)-**9**.

Table 2
Effects of an iv injection of the investigated compounds in dose of 1 mg kg^{-1} on heart rate and ECG intervals in anesthetized male Wistar rats (60 mg of thiopental kg^{-1} ip).

Compound	Parameters	Time of observation (min)			
		0	1	5	15
(*RS*)-**7a**	P–Q (ms)	54.4 ± 2.0	58.8 ± 0.9[b]	59.4 ± 0.4[c]	57.3 ± 1.1
	QRS (ms)	22.0 ± 1.0	24.2 ± 1.2	23.0 ± 0.8	22.7 ± 0.5
	Q–T (ms)	85.8 ± 2.5	87.1 ± 2.3	79.2 ± 3.4	79.2 ± 3.1
	Beats/min	332.0 ± 7.4	297.3 ± 4.5[d]	292.2 ± 6.8[d]	300.0 ± 7.6
(*R*)-**7a**	P–Q (ms)	54.8 ± 1.3	58.5 ± 1.4	59.7 ± 2.8	58.0 ± 2.2
	QRS (ms)	23.2 ± 0.5	22.7 ± 0.5	23.3 ± 0.6	24.5 ± 0.8
	Q–T (ms)	87.2 ± 2.8	89.2 ± 2.3	88.0 ± 2.2	81.7 ± 2.7
	Beats/min	292.3 ± 7.6	267.4 ± 9.7	259.0 ± 9.8[b]	255.2 ± 11.6[b]
(*S*)-**7a**	P–Q (ms)	51.2 ± 2.2	58.5 ± 2.1[b]	56.7 ± 2.4	53.3 ± 1.6
	QRS (ms)	22.4 ± 0.6	25.0 ± 0.8[a]	24.5 ± 0.8[b]	22.5 ± 0.5
	Q–T (ms)	81.2 ± 3.4	78.8 ± 5.4	94.8 ± 3.4	83.0 ± 3.9
	Beats/min	324.2 ± 11.0	292.0 ± 9.3[b]	292.3 ± 8.5[b]	275.9 ± 7.3[d]
(*RS*)-**7b**	P–Q (ms)	44.6 ± 2.5	48.7 ± 2.3	51.3 ± 1.3	48.0 ± 2.7
	QRS (ms)	26.7 ± 1.1	31.3 ± 1.7[a]	27.3 ± 1.3	26.0 ± 1.3
	Q–T (ms)	108.0 ± 5.8	111.4 ± 6.5	107.3 ± 6.5	118.0 ± 5.9
	Beats/min	396.7 ± 16.6	376.3 ± 13.8	372.9 ± 15.3	364.7 ± 13.8
(*R*)-**7b**	P–Q (ms)	41.3 ± 1.7	45.3 ± 1.3	42.7 ± 1.6	42.0 ± 1.3
	QRS (ms)	23.3 ± 1.1	28.0 ± 1.3[b]	28.7 ± 1.7[c]	28.7 ± 0.8
	Q–T (ms)	104.7 ± 2.3	106.7 ± 2.4	106.0 ± 1.9	113.3 ± 1.8[b]
	Beats/min	412.3 ± 5.8	395.5 ± 4.5[a]	403.2 ± 7.3	387 ± 2.1[c]
(*S*)-**7b**	P–Q (ms)	44.7 ± 1.7	48.7 ± 2.5	46.0 ± 1.6	46.0 ± 1.9
	QRS (ms)	27.3 ± 1.3	26.0 ± 1.3	28.7 ± 0.8	28.0 ± 0.8
	Q–T (ms)	110.0 ± 5.5	107.3 ± 5.6	111.3 ± 7.1	118. ± 4.30
	Beats/min	393.0 ± 16.2	388.1 ± 15.3	381.0 ± 13.6	367.6 ± 10.8
(*RS*)-**9**	P–Q (ms)	45.7 ± 2.0	47.2 ± 1.6	48.1 ± 2.4	46.2 ± 1.7
	QRS (ms)	26.4 ± 1.3	28.4 ± 1.2	24.9 ± 1.3	26.9 ± 1.1
	Q–T (ms)	72.6 ± 1.7	69.2 ± 1.1	69.0 ± 1.6	72.8 ± 1.3
	Beats/min	304.9 ± 8.9	298.7 ± 7.2	294.5 ± 7.7	287.3 ± 7.9
Carvedilol	P–Q (ms)	50.0 ± 3.2	50.0 ± 3.2	55.0 ± 5.5	54.6 ± 3.0
	QRS (ms)	21.2 ± 0.8	22.0 ± 0.6	22.8 ± 1.0	23.2 ± 0.8
	Q–T (ms)	72.0 ± 3.1	74.4 ± 2.8	68.0 ± 3.7	74.0 ± 2.4
	Beats/min	356.7 ± 21.0	345.2 ± 17.7	340.3 ± 14.8	320.4 ± 10.2

Values are the mean ± SEM of 6 experiments. Statistical analyses were performed using a one-way ANOVA test.
[a] $p < 0.05$.
[b] $p < 0.02$.
[c] $p < 0.01$.
[d] $p < 0.001$.

The compound **7a** as a racemic mixture and both enantiomers had high affinity to adrenergic receptors in rat cerebral cortex. For α-adrenoceptors (α_1 and α_2) there were no essential differences between enantiomers. The greatest discrepancy was observed in affinity to β_1-adrenoceptor. Enantiomer *S* had 10-fold greater affinity to β_1-adrenoceptors than enantiomer *R* of compound **7a**.

Compound **7b** did not display large differences (maximally four times) in adrenoceptor affinity between enantiomers.

These results, especially in the case of compound **7a**, give evidence about the relationship of spatial configuration and affinity to β-adrenoceptors and a lack of such a relationship in the case of α-adrenoceptors. The same results were observed for enantiomers of carvedilol and other β-adrenergic antagonists as well as enantiomers of (*RS*)-**9**, tested before [8,13].

The antiarrhythmic effects of the novel compounds were examined on rats using the model of adrenaline-induced arrhythmia. Compounds **7a** and **7b** and their enantiomers administrated iv 15 min before arrhythmogen, prevented or attenuated the symptoms of adrenaline-induced arrhythmia. The antiarrhythmic activity of the tested compounds was similar, but slightly weaker than that of carvedilol or compound (*RS*)-**9**. All tested compounds were more active than propranolol in this test. Antiarrhythmic activity of pure β-adrenergic blocking agents is known for years. Also, carvedilol possesses an antiarrhythmic effect. The electrophysiological properties of carvedilol, and application in antiarrhythmic therapy were described previously [7,8,14].

Hypotensive activity of the investigated compounds was determined after their iv administration to normotensive anesthetized rats. All forms of compound **7a** diminished the systolic and diastolic blood pressures throughout the whole observation period at a range of doses 0.25–1.0 mg kg^{-1}. In the lower dose the hypotensive effect disappeared. Each enantiomeric form of compound **7b** displayed similar hypotensive effect. Compound **7b** (*R*, *S* and *RS*) decreased the systolic blood pressure in the range of doses 0.125–1.0 mg kg^{-1} and in a less extent, the diastolic blood pressure. The hypotensive effect is probably due to adrenoceptor blockade (α_1-in arteries and β_1-in heart). In radioligand binding studies we found the difference only in affinity of enantiomers of compound **7a** to β_1-adrenoceptor. No difference in affinity of isomers of **7a** to α_1-adrenoreceptors was observed. In our research we did not find essential differences in hypotensive activity between enantiomers of compounds **7a** and **7b** after iv administration. In opposite to carvedilol and (*RS*)-**9**, tested before [8], there was no relationship between the spatial configuration and hypotensive activity.

In the experiment on isolated rabbit ileum, the compounds **7a** and **7b**, only in the highest dose of 10^{-5} M influenced the amplitude and/or frequency of isolated rabbit small intestine. Weak spasmolytic activity of the tested compounds seems to confirm that hypotensive activity is caused by α-adrenolytic activity, but not by spasmolytic one.

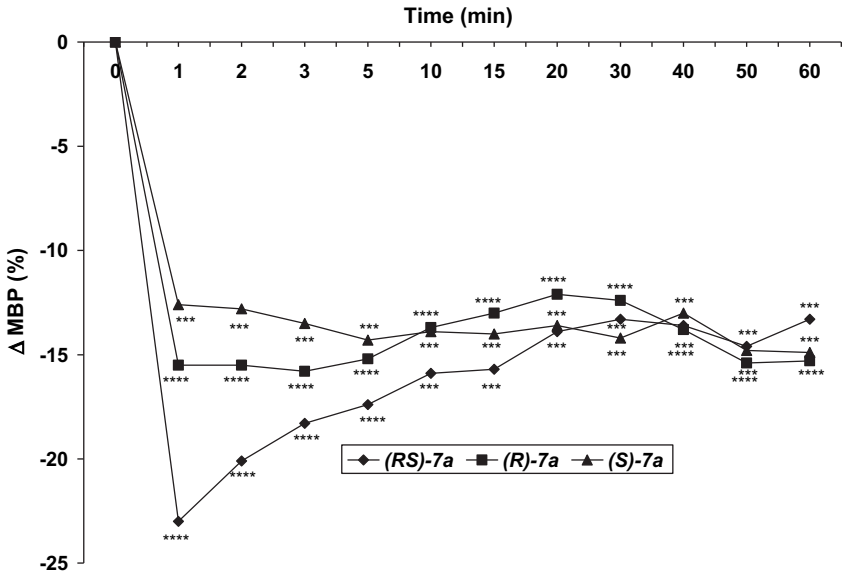

Fig. 5. Changes in mean blood pressure after iv administration of compound **7a** and its enantiomers in the dose of 0.25 mg kg^{-1}. Statistical analyses were performed using a one-way ANOVA test: $^{***}p < 0.01$, $^{****}p < 0.001$.

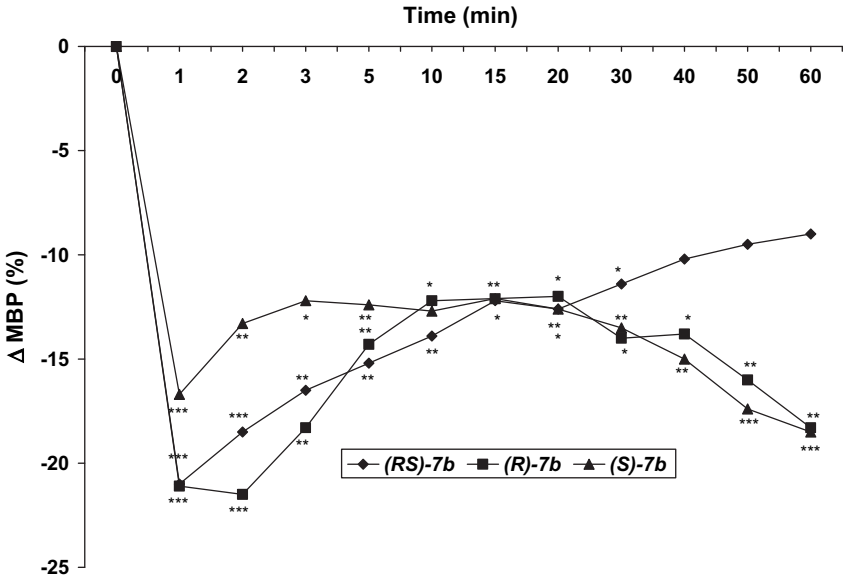

Fig. 6. Changes in mean blood pressure after iv administration of compound **7b** and its enantiomers in the dose of 0.125 mg kg^{-1}. Statistical analyses were performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.002$, $^{***}p < 0.01$.

5. Conclusion

The results of this study confirmed that (2*RS*)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and (2*RS*)-1-(7-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol and their enantiomers possess α_1-, α_2- and β_1-adrenolytic, antiarrhythmic and hypotensive activities. The results suggest that the antiarrhythmic and hypotensive effects of the compounds are related to their adrenolytic properties, but the pharmacological effects of the tested compounds and their enantiomers were qualitatively weaker than those of carvedilol and (*RS*)-**9**. The introduction of the methoxy group in 5 or 7 position of indole moiety of compound (*RS*)-**9** attenuates the activity of the tested compounds relative to compound (*RS*)-**9**.

6. Experimental protocol

6.1. Chemistry

6.1.1. General

Melting points were determined on a Boëtius apparatus and are uncorrected or on DSC Mettler Toledo Instrument 822^{e}. ^{1}H and ^{13}C NMR spectra were recorded on Bruker or Varian (500, 400 or 200 MHz) instruments. Chemical shifts are expressed in ppm (δ) referred to TMS, coupling constants (J) are in Hz. IR and UV spectra were recorded on Perkin–Elmer and Hewlett Packard 8453 instruments, respectively. IR spectra were recorded in KBr pallets and wavenumbers are expressed in cm^{-1}. Elemental analysis was done on AE 1108 Carlo Erba apparatus. Mass spectra were obtained

on an AMD-604 or Mariner Spec spectrometers. The X-ray structures were determined on the 'KappaCCD' (Nonius) diffractometer. Optical rotations were obtained on a Jasco P-1020 or P-2000 apparatus. TLC plates precoated with silica gel 60 F_{254} was used for monitoring, and silica gel 230–400 mesh was used for flash column chromatography (both from Merck).

6.1.2. Materials

(+/−)-1-Chloro-2,3-epoxypropane, was purchased from Aldrich Chemicals; 99.9% of (*S*)-(+) and 99.8% (*R*)-(−)-1-chloro-2,3-epoxypropane with optical purity 99.0% and 99.9% ee, respectively, were provided by Chemos GmbH. Fuming nitric acid, $d = 1.52$, 2,3-dimethoxybenzaldehyde and 2,5-dimethoxybenzaldehyde, boron tribromide solution 1.0 M in dichloromethane, were obtained from Fluka Chemicals.

Solvents were distilled and dried if required. Other common materials were commercial products.

(2-Methoxyphenoxy)ethylamine was obtained according to literature [15].

[^{3}H]Prazosin, [^{3}H]Clonidine and [^{3}H]CGP12177 were supply by Perkin–Elmer.

Reference compound, carvedilol, was submitted by Pharmaceutical Research Institute, Warsaw, Poland.

*6.1.3. 6-Hydroxy-3-methoxy-2-nitrobenzaldehyde (**2a**)*

2,5-Dimethoxybenzaldehyde, **1a**, (20 g; 0.12 mol) was dissolved in acetic acid (450 mL). Reaction mixture was cooled to 10 °C (water/ice), and then fuming nitric acid (95 mL; 144.4 g; 2.3 mol) was added dropwise during 25 min while vigorously stirring. Afterward reaction mixture was allowed to warm to room temperature and kept for 45 min. The yellow suspension was poured to mixture of water and crushed ice (2 L). The precipitate was filtered off in vacuum and rinsed excessively with water and dried in the air. The mixture of two compounds was obtained; 3,6-dimethoxy-2-nitrobenzaldehyde and 2,5-dimethoxy-4-nitrobenzaldehyde (detected by TLC). Part of compound 3,6-dimethoxy-2-nitrobenzaldehyde was separated from reaction mixture via crystallization from dichloromethane. Purification of the remaining residue by flash chromatography on silica gel (300 g; eluent: hexane/ethyl acetate, 1:1) furnished:

- 3,6-dimethoxy-2-nitrobenzaldehyde as orange crystals (19.25 g; 76%); m.p. 171 °C (DSC) (dichloromethane), m.p. lit. 171–172 °C [16]. ^{1}H NMR (200 MHz, $CDCl_3$): 3.93 (s, 3H, OC*H*$_3$); 4.02 (s, 3H, OC*H*$_3$); 7.18 (d, $J = 9.4$, 1H, Ar*H*); 7.36 (d, $J = 9.4$, 1H, Ar*H*); 10.43 (s, 1H, C*H*O). IR: 716.9; 826.1; 949.9; 1187.3; 1293.5; 1433.6; 1494.8; 1541.8; 1688.8 (C═O) and
- 2,5-dimethoxy-4-nitrobenzaldehyde was obtained as yellow crystals (4.47 g, 18%), crystallized from ethyl acetate, m.p. 174.5–175.5 °C, m.p. lit. 163–165 °C (Ethanol) [17].

3,6-Dimethoxy-2-nitrobenzaldehyde (7.1 g; 0.034 mol) was dissolved in dichloromethane (500 mL). The mixture was cooled to −50 °C (acetone/dry ice), then boron tribromide solution in dichloromethane (1 M; 34 mL; 0.034 mol) was added dropwise. The reaction mixture turned deep dark blue, and then allowed to warm up to room temperature. After 2 h reaction was completed (monitoring on TLC: toluene/methanol, 5:1). Water (400 mL) was poured into to the reaction mixture and stirred for ca. 20 min. The organic layer was separated and washed with water and brine, than dried over anhydrous magnesium sulfate. After evaporating solvent to dryness, crude product was obtained as a solid. Crystallization from dichloromethane/hexane afforded phenol **2a** as olive crystals (5.65 g, 85%), m.p. 99.5–101 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 3.91 (s, 3H, OC*H*$_3$); 7.14 (d, $J = 9.3$, 1H, Ar*H*); 7.35 (d, $J = 9.3$, 1H, Ar*H*); 9.84 (s, 1H, O*H*); 11.15 (s, 1H C*H*O). ^{13}C NMR (125 MHz, $CDCl_3$): 57.7 (O*C*H$_3$); 111.3; 121.5; 123.3; 141.2; 143.5; 155.8; 191.9 (*C*HO). IR: 714.0; 733.6; 810.6; 835.5; 1179.2; 1285.4; 1363.6; 1472.1; 1528.9; 1662.5. EIMS (70 eV) *m*/*z* [%]: 197 (M^+, 100); 179 (M^+-H_2O, 7); 149 (33); 137 (18); 136 (17); 135 (27); 134 (44); 121 (80); 120 (19); 119 (15); 108 (47); 107 (41); 106 (43); 95 (11); 93 (30); 92 (31); 79 (39); 65 (80). HRMS for $C_8H_7NO_5$ (M^+); calcd: 197.0324, found: 197.0327.

*6.1.4. 6-(Benzyloxy)-3-methoxy-2-nitrobenzaldehyde (**3a**)*

The compound **2a** (5.65 g, 0.03 mol) was dissolved in acetonitrile (200 mL). Then potassium carbonate (4.1 g, 0.03 mol) was added and stirred for 10 min in room temperature. Benzyl chloride (6 mL, 6.6 g, 0.05 mol) and potassium iodide (50 mg) were added. The reaction mixture was carried out under reflux for 2.5 h, (TLC monitoring; hexane/ethyl acetate, 3:1). Cooled reaction mixture was poured to water (1.75 L) and precipitate was filtered off in vacuum and dried in the air. The product **3a** was obtained as a fine crystalline solid (7.65 g; 93%). The compound **3a** was suitable for upcoming transformation. For physical and spectral analyses the product was recrystallized from dichloromethane/hexane, and afforded pale yellow fine crystalline powder, m.p. 146–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.85 (s, 3H, OC*H*$_3$); 5.19 (s, 2H, OC*H*$_2$Ph); 7.16 (d, $J = 9.35$, 1H, Ar*H*); 7.25 (d, $J = 9.35$, Ar*H*); 7.36–7.42 (m, 5H, Ar*H*); 10.42 (s, 1H, C*H*O). ^{13}C NMR (100 MHz, $CDCl_3$): 57.1 (O*C*H$_3$); 71.8 (O*C*H$_2$Ph); 115.9; 116.7; 119.9; 127.4 (2*C*); 128.7; 128.9 (2*C*); 135.2; 138.5; 144.7; 154.3; 186.0 (*C*HO). IR: 733.4; 813.8; 952.3; 1090.9; 1189.9; 1273.4; 1284.5; 1434.1; 1451.0; 1489.8; 1499.5; 1541.9; 1690.9. EIMS (70 eV) *m*/*z* [%]: 287 (M^+, 2); 92 (8); 91 (100); 79 (1); 65 (6). HRMS for $C_{15}H_{13}NO_5$ (M^+); calcd: 287.0794, found: 287.0792.

*6.1.5. 2-(Benzyloxy)-3-methoxy-6-nitrobenzaldehyde (**3b**)*

Conversion of 2,3-dimethoxybenzaldehyde to derivative **3b** was performed according to procedure described in Sections 6.1.3 and 6.1.4. Nitration of 2,3-dimethoxybenzaldehyde afforded a mixture of 5- and 6-nitro derivatives. They were separated by a cumbersome nuisance chromatography on silica gel (100-fold more of stationary phase to weight of product mixture; eluent: hexane/ethyl acetate, 1:1). Intermediate 2,3-dimethoxy-5-nitrobenzaldehyde was obtained with yield of 46%, m.p. 117–118 °C (EtOAc), and intermediate 2,3-dimethoxy-6-nitrobenzaldehyde with yield of 36%, m.p. 107.5–109 °C (EtOAc), lit. 108–109 °C [18].

2,3-Dimethoxy-6-nitrobenzaldehyde was subjected to selective monodemethylation, and afforded 2-hydroxy-3-methoxy-6-nitrobenzaldehyde, **2b**, with yield of 98%, m.p. 103–105.5 °C (EtOAc/hexane), lit. 92.5–93.5 °C [19]. The latter was converted to product **3b** with yield of 90%, m.p. 97–99 °C (dichloromethane/hexane), lit.108 °C (benzene/petrol ether) [20]. ^{1}H NMR (500 MHz, $CDCl_3$): 4.00 (s, 3H, OC*H*$_3$); 5.06 (s, 2H, OC*H*$_2$Ph); 7.05 (d, $J = 9.2$, 1H, Ar*H*); 7.32–7.41 (m, 5H, Ar*H*); 7.93 (d, $J = 9.2$, Ar*H*); 10.17 (s, 1H, C*H*O). ^{13}C NMR (125 MHz, $CDCl_3$): 56.6 (O*C*H$_3$); 76.8 (O*C*H$_2$Ph); 112.6; 121.7; 128.5 (2*C*); 128.6; 128.8 (2*C*); 130.6; 136.0; 139.5; 145.7; 158.6; 188.4 (*C*HO). IR: 702.9; 751.7; 1076.9; 1215.0; 1237.0; 1271.8; 1327.6; 1337.6; 1477.7; 1510.9; 1575.1; 1713.3; 2876.5; 2966.6; 3106.7. UV (EtOH), (nm), λ_{max}: 326 ($\varepsilon = 7500$), ($c = 0.21$ mg/10 mL). Crystal data: $C_{15}H_{13}NO_5$, MW 287.26, triclinic, *P*-1, $Z = 2$, Calculated density = 1.437 Mg/m^3, $a = 8.5710(3)$ Å, $b = 8.7110(2)$ Å, $c = 9.5770(4)$ Å, $\alpha = 82.678(2)^\circ$, $\beta = 77.641(2)^\circ$, $\gamma = 72.264(2)^\circ$, $V = 663.78(4)$ Å^3, $T = 100(2)$ K, $\lambda_{[MoK\alpha]} = 0.71073$ Å, $R_1 = 3.17\%$, $wR_1 = 8.59\%$, crystal size 0.2 × 0.2 × 0.2 mm^3, light green.

*6.1.6. 1-(Benzyloxy)-4-methoxy-3-nitro-2-(2-nitrovinyl)benzene (**4a**)*

Potassium carbonate (3.8 g, 0.028 mol) was suspended in 1,4-dioxane (100 mL). Then, nitromethane (7.5 mL; 8.48 g; 0.14 mol) was

added and stirred for 10 min at room temperature. The reaction mixture was cooled to 12 °C and kept for 1 h. Later, compound **3a** (3.94 g; 0.014 mol) was added followed by 18-crown-6 ether (305 mg; 1.16 mmol). The progress of the reaction was monitored by TLC (toluene/methanol, 9:1). After 24 h reaction was completed. Solid from the reaction mixture was filtered off, and then acetic anhydride (13 mL; 0.14 mol) was added to the filtrate followed by sodium acetate (200 mg) and heated at 100 °C. The progress of the reaction was monitored by TLC (toluene/methanol, 9:1). After 2 h reaction was completed, and then the reaction mixture was poured into water with ice (1.5 L). The yellow precipitate was filtered off and dried in the air. The crude product **4a** was crystallized from dichloromethane/hexane to yield yellow crystals (3.71 g; 82%), m.p. 158–161 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 3.85 (s, 1H, OCH_3); 5.21 (s, 2H, OCH_2Ph); 7.12 (br s, 2H, Ar*H*); 7.37–7.43 (m, 5H, Ar*H*); 7.74 (d, $J = 13.5$, 1H, =C*H*); 7.91 (d, $J = 13.5$, 1H, =C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 57.0 (OCH_3); 71.9 (OCH_2Ph); 112.5; 115.0; 116.5; 127.2; 127.4 (2*C*); 128.8; 129.0 (2*C*); 134.9; 142.2; 142.4; 144.8; 151.7. IR: 802.2; 952.3; 962.3; 1027.3; 1035.2; 1275.0; 1287.4; 1336.6; 1517.7; 1539.3; 1635.3. EIMS (70 eV) *m*/*z* [%]: 330 (M^+, <1); 193 (3); 91 (100); 65 (7). HRMS for $C_{16}H_{14}N_2O_6$ (M^+); calcd: 330.0852, found: 330.0843.

6.1.7. 2-(Benzyloxy)-1-methoxy-4-nitro-3-(2-nitrovinyl)benzene (**4b**)

The compound **4b** was obtained as described in Section 6.1.6. The crude product **4b** was crystallized from EtOAc/hexane, and afforded red-orange crystalline solid of compound **4b** (87%), m.p. 118–120 °C, lit. 119–120 °C (Methanol) [20]. ^{1}H NMR (500 MHz, $CDCl_3$): 4.04 (s, 3H, OCH_3); 5.03 (s, 2H, OCH_2Ph); 7.07 (d, $J = 9.2$, 1H, Ar*H*); 7.27–7.28 (m, 2H, Ar*H*); 7.34–7.35 (m, 3H, Ar*H*); 7.47 (d, $J = 13.6$, 1H, =C*H*); 7.95 (d, $J = 13.6$, 1H, =C*H*); 7.97 (d, $J = 9.2$, 1H, Ar*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 56.6 (OCH_3); 75.8 (OCH_2Ph); 112.3; 121.7; 122.6; 128.7 (2*C*); 128.8 (2*C*); 129.0; 130.0; 135.5; 141.9; 142.3; 146.3; 157.5. IR: 747.6; 953.5; 1085.5; 1277.9; 1343.1; 1465.0; 1519.9; 1570.5; 1641.8; 2944.8; 3092.4. UV (CH_3CN), (nm), λ_{max}: 278 ($\varepsilon = 13{,}700$), ($c = 0.17$ mg/10 cm^3).

6.1.8. 7-Methoxy-1H-indol-4-ol (**5a**)

The compound **4a** (4.1 g, 0.012 mol) was suspended in a mixture of ethanol (110 mL) and acetic acid (13 mL) and stirred with Pd/C (10%, 0.33 g) under an atmosphere of hydrogen (1 bar) at room temperature for 8 h. The progress of the reaction was monitored by TLC (hexane/acetone, 4:1). The catalyst was collected by filtration through Celite 545, and washed with dichloromethane (200 mL). Water (100 mL) was added to the filtrate and neutralized with saturated solution of sodium bicarbonate. The organic layer was separated and washed with water. Then, organic layer was dried over anhydrous sodium sulfate and solvent was evaporated to dryness. The crude product, oil, was purified by column chromatography on silica gel (20 g, eluent: hexane/acetone, 4:1). Compound **5a** was obtained as a pale pink solid (1.63 g, 80%), later was recrystallized from dichloromethane/petroleum ether, and afforded colorless fine crystals, m.p. 121.5–124 °C. ^{1}H NMR (500 MHz, acetone-d_6): 3.84 (s, 1H, OCH_3); 6.32 (d, $J = 8.1$, 1H, Ar*H*); 6.43 (d, $J = 8.1$, 1H, Ar*H*); 6.56 (t, $J = 2.6$, 1H, 3-H); 7.15 (t, $J = 2.6$, 1H, 2-H); 7.77 (s, 1H, O*H*); 10.1 (br s, 1H, N*H*). ^{13}C NMR (125 MHz, acetone-d_6): 55.9 (OCH_3); 100.2; 102.8; 103.0; 120.7; 123.5; 129.0; 141.3; 145.5. IR: 729.0; 1024.0; 1072.7; 1086.8; 1262.0; 1453.1; 1497.0; 1521.8; 3229.7; 3420.5. EIMS (70 eV) *m*/*z* [%]: 163 (M^+, 100); 148 (82); 120 (19); 92 (11); 91 (3); 65 (7). HRMS for $C_9H_9NO_2$ (M^+); calcd: 163.0633, found: 163.0641.

6.1.9. 5-Methoxy-1H-indol-4-ol (**5b**)

Indole derivative **5b** was obtained as described in Section 6.1.8. After work-up the crude product was purified by column chromatography on silica gel and crystallized from dichloromethane/hexane and afforded colorless crystals of indole **5b** (yield 58%), m.p.152–154 °C, lit. 129–132 °C [9]. ^{1}H NMR (500 MHz, $CDCl_3$): 3.91 (s, 3H, OCH_3); 5.87 (s, 1H, O*H*); 6.62–6.64 (m, 1H, 3-H); 6.88 (dd, $J = 8.6$ and 0.75, 1H, Ar*H*); 6.91 (d, $J = 8.6$, 1H, Ar*H*); 7.13 (t, $J = 2.8$, 1H, 2-H); 7.99 (br s, 1H, N*H*). ^{13}C NMR: 58.4 (OCH_3); 99.4; 102.0; 109.7; 117.7; 124.2; 133.2; 138.5; 138.8.

6.1.10. 7-Methoxy-4-(oxiran-2-ylmethoxy)-1H-indole (**6a**) and 5-methoxy-4-(oxiran-2-yl methoxy)-1H-indole (**6b**)

Indole derivatives **5a** and **5b** were converted to epoxide derivatives as described in literature [8,21]. The products **6a** and **6b** were obtained as an amorphic solid with yield of 69% and 86%, respectively.

Product **6a** was recrystallized from acetone/hexane, m.p. 96–98 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 2.78 (dd, $J = -5.0$ and 2.7, 1H, 10-H); 2.90 (dd, $J = -5.0$ and 4.2, 1H, 10-H); 3.40–3.43 (m, 1H, 9-H); 3.90 (s, 1H, OCH_3); 4.1 (dd, $J = -11.1$ and 5.4, 1H, 8-H); 4.28 (dd, $J = -11.1$ and 3.4, 1H, 8-H); 6.4 (d, $J = 8.3$, 1H, Ar*H*); 6.48 (d, $J = 8.3$, 1H, Ar*H*); 6.64 (dd, $J = 3.1$ and 2.3, 1H, 3-H); 7.09 (t, $J = 2.8$, 1H, 2-H); 8.42 (br s, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 44.9; 50.4; 55.6 (OCH_3); 69.5; 100.4; 100.8; 101.4; 120.3; 122.6; 127.7; 141.5; 146.5. IR: 717.7; 767.3; 1081.9; 1253.2; 1359.0; 1454.3; 1523.0; 3350.7. Anal. for $C_{12}H_{13}O_3N$ [%]; calcd: C-65.74; H-5.98; N-6.39, found: C-65.70; H-6.07; N-6.39.

Product **6b** was recrystallized from ethyl acetate, m.p. 48–50 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 2.70 (dd, $J = -5.0$ and 2.7, 1H, 10-H); 2.83–2.85 (m, 1H, 10-H); 3.38–3.41 (m, 1H, 9-H); 3.88 (s, 3H, OCH_3); 4.18 (dd, $J = -11.5$ and 5.9, 1H, 8-H); 4.39 (dd, $J = -11.5$ and 3.55, 1H, 8-H); 6.60–6.61 (m, 1H, 3-H); 6.90 (d, $J = 8.7$, 1H, Ar*H*); 7.04 (d, $J = 8.7$, 1H, Ar*H*); 7.10 (t, $J = 2.8$, 1H, 2-H); 8.29 (br s, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 44.8; 50.8; 58.3 (OCH_3); 73.9; 99.5; 106.6; 111.6; 123.0; 125.0; 133.1; 140.5; 144.8. IR: 733.5; 1023.0; 1040.3; 1091.4; 1212.1; 1238.6; 1330.8; 1354.1; 1492.8; 1579.2; 2830.8; 2931.6; 3326.0. UV (EtOH), (nm), λ_{max}: 271 ($\varepsilon = 7400$), ($c = 0.28$ mg/10 mL). Anal. for $C_{12}H_{13}NO_3$ [%]; calcd: C-65.74; H-5.98; N-6.39, found: C-65.73; H-5.88; N-6.41.

6.1.11. (2RS)-1-(7-Methoxy-1H-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol ((RS)-**7a**) and (2RS)-1-(5-methoxy-1H-indol-4-yloxy)-3-(2-(2-methoxyphenoxy) ethylamino)propan-2-ol ((RS)-**7b**)

The compounds **6a** and **6b** were converted to final products **7a** and **7b** as described in literature [8].

The compound (*RS*)-**7a** was obtained as a solid (yield 46%). Crystallization from acetone afforded colorless crystals, m.p. 133–133.5 °C. ^{1}H NMR (500 MHz, acetone-d_6): 2.74 (br s, 2H, O*H* and N*H* aliph.); 2.85 (dd, $J = -12.0$ and 6.7, 1H, 4-H); 2.97 (dd, $J = -12.0$ and 4.1, 1H, 4-H); 3.01 (m, 2H, 3-H); 3.78 (s, 3H, OCH_3); 3.86 (s, 3H, OCH_3); 4.05–4.10 (m, 5H, 1,2,5-H); 6.39 (d, $J = 8.2$, 1H, Ar*H*); 6.49 (d, $J = 8.2$, 1H, Ar*H*); 6.53 (dd, $J = 3.0$ and 2.2, 7-H); 6.84–6.91 (m, 2H, Ar*H*); 6.94 (dd, $J = 7.7$ and 1.9, Ar*H*); 6.97 (dd, $J = 7.7$ and 1.9, 1H, Ar*H*); 7.16 (m, 1H, 6-H); 10.19 (br s, 1H, N*H*). ^{13}C NMR (125 MHz, acetone-d_6): 49.8; 53.4; 55.9 (OCH_3); 56.2 (OCH_3); 69.9; 70.0; 72.1; 100.5; 100.7; 102.1; 113.4; 115.4; 121.6; 121.7; 122.2; 123.7; 128.7; 142.4; 147.9; 149.8; 151.1. IR: 750.4; 783.6; 1090.7; 1104.4; 1262.7; 1503.0; 1523.7; 1595.8; 3320.1. EIMS (70 eV) *m*/*z* [%]: 386 (M^+, 37); 225 (12); 224 (85); 180 (28); 164 (12); 163 (100); 162 (15.); 148 (19); 100 (15); 91 (2); 86 (12); 65 (2); 56 (28); 44 (22). HRMS for $C_{21}H_{26}N_2O_5$ (M^+): calcd 386.1842, found 386.1847. Crystal data: $C_{21}H_{26}N_2O_5$, MW 385.24, orthorhombic, *Pccn*, $Z = 8$, Calculated density = 1.309 Mg/m^3, $a = 12.6350(3)$ Å, $b = 33.5010(6)$ Å, $c = 9.2360(15)$ Å, $V = 3909.5(6)$ Å^3, $T = 100(2)$ K, $\lambda_{[MoK\alpha]} = 0.71073$ Å, $R_1 = 3.94\%$, $wR_1 = 9.98\%$, crystal size = $0.1 \times 0.1 \times 0.2$ mm^3, colorless.

The compound (*RS*)-**7b** was obtained chromatographically pure as a lightly brown oil (yield 77%). ^{1}H NMR (500 MHz, $CDCl_3$): 2.82

(m, 2H, 4-H); 3.02 (t, $J = 5.4$, 2H, 5-H); 3.80 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 4.03–4.04 (m, 1H, 2-H); 4.08–4.14 (m, 3H, 1,3-H); 4.27 (dd, $J = -10.4$ and 3.5, 1H, 1-H); 6.52 (br s, 1H, 7-H); 6.85 (d, $J = 8.75$, 1H, Ar*H*); 6.85–6.88 (m, 4H, Ar*H*); 7.02 (d, $J = 8.75$, 1H. Ar*H*); 7.08 (t, $J = 2.7$, 1H, 6-H); 8.79 (br s, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): 48.8; 51.6; 55.8 (OCH_3); 58.2 (OCH_3); 68.7; 69.2; 76.1; 99.2; 106.7; 110.9; 111.9; 114.1; 120.9; 121.5; 122.7; 125.1; 133.4; 140.3; 144.6; 148.3; 149.7. IR (film): 735.8; 1024.0; 1082.6; 1122.9; 1217.3; 1248.6; 1331.1; 1453.4; 1503.6; 1591.8; 2927.1; 3309.4, 3350.3. HRMS (ESI$^+$) for $C_{21}H_{27}N_2O_5$ ($[M+H]^+$); calcd: 387.1915, found: 387.1917.

*6.1.12. (2S)-1-(7-Methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol ((S)-**7a**) and (2S)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol ((*S*)-**7b**)*

(*S*)-(+)-4-(2,3-Epoxypropoxy)-1*H*-indole derivatives of **6** were obtained as described in Section 6.1.10 using (*R*)-(−)-1-chloro-2,3-epoxypropane in lieu of racemic mixture. For intermediate (*S*)-**6a** the yield was 76%, and product was obtained chromatographically pure as oil. Indole derivative (*S*)-**6a** was submitted to addition reaction with (2-methoxyphenoxy)ethylamine as described for compound (*RS*)-**7** in Section 6.1.11, and furnished product (*S*)-**7a** as a solid with yield of 77%, m.p. 97–98 °C (dichloromethane), $[\alpha]_D^{25} = -4.3°$ ($c = 1.005$ in MeOH).

Compound (*S*)-**6b** was obtained with yield of 82%, as colorless crystalline solid m.p. 60–61 °C (EtOAc), $[\alpha]_D^{25} = +11.25°$ ($c = 1.03$ in $CHCl_3$). In contrast to (*S*)-**7a**, derivative (*S*)-**7b** was obtained as sensitive to moisture oil, with yield of 82%.

*6.1.13. (2R)-1-(7-Methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol ((R)-**7a**) and (2R)-1-(5-methoxy-1*H*-indol-4-yloxy)-3-(2-(2-methoxyphenoxy) ethylamino)propan-2-ol ((R)-**7b**)*

(*R*)-(−)-4-(2,3-Epoxypropoxy)-1*H*-indole derivatives of **6** were obtained as described in Section 6.1.10 using (*S*)-(+)-1-chloro-2,3-epoxypropane in lieu of racemic mixture. For intermediate (*R*)-**6a** the yield was 77%, and the form of product was chromatographically pure oil. Indole derivative (*R*)-**6a** was submitted to addition reaction with (2-methoxyphenoxy)ethylamine as described for compound (*RS*)-**7** in Section 6.1.11, and furnished product (*R*)-**7a** as a solid with yield of 62%, m.p. 96–97 °C (dichloromethane), $[\alpha]_D^{25} = +4.6°$ ($c = 1.01$ in MeOH).

Compound (*R*)-**6b** was obtained with yield of 78%, as colorless crystalline solid m.p. 59.5–60.5 °C (EtOAc), $[\alpha]_D^{25} = -10.36°$ ($c = 1.15$ in $CHCl_3$). In contrast to (*R*)-**7a**, derivative (*R*)-**7b** was obtained with yield of 82% as foam sensitive to moisture.

*6.1.14. Enantiomeric purity of (S)-**7a**,**b** and (R)-**7a**,**b***

Enantiomeric purity of (*S*)-**7a** and (*R*)-**7a**, and (*R*)-**7b** and (*S*)-**7b** was assessed using liquid chromatography tandem mass spectrometry (ESI-LC/MS/MS) method equipped with an electrospray ionization interface. Mass spectrometric detection was achieved by an Applied Biosystems MDS Sciex API 2000 triple quadrupole mass spectrometer (Concord, Ontario, Canada). The instrument was coupled to an Agilent 1100 (Agilent Technologies, Waldbronn, Germany) LC system. The MS/MS detector was operated at unit resolution in the multiple reactions monitoring mode (MRM), monitoring the transition of the protonated molecular ions to the product ions *m*/*z*: 387.3 → 224.3 and 387.4 → 180.3 for (*RS*)-**7a** and (*RS*)-**7b**, respectively.

The separation of the enantiomers of (*S*)-**7a** and (*R*)-**7a** was carried out on a normal phase an amylose tris(3,5-dimethylphenylcarbamate) Chiralpak AD column (10 μm, 250 × 4.6 mm, Daicel Chemical Industry, Tokyo, Japan) with a gradient elution, using as a mobile phase a mixture of ethanol and hexane with an addition of 0.1% of ammonium acetate and pumped through the system at a flow rate of 1 mL min^{-1}. The retention times of (*R*)-**7a** and (*S*)-**7a** were 6.6 min, and 8.5 min, respectively. The studied isomers were well resolved and the ratio of their peak areas was used to estimate the enantiomeric purity. On the basis of the results obtained, a final purity of the selected enantiomers was 99.0% and 99.9% ee respectively.

Finally, in the case of enantiomers (*S*)-**7b** and (*R*)-**7b** their separation was done on the chiral stationary reversed phase, cellulose tris(3,5-dimethylphenylcarbamate) Chiralcel OD-RH column (5 μm, 150 × 4.6 mm, Daicel Chemical Industry, Tokyo, Japan). The mobile phase consisted of methanol with an addition of 0.1% of ammonium acetate was pumped through the system at a flow rate of 1 mL min^{-1}. The retention times of (*R*)-**7b** and (*S*)-**7b** were 3.7 min, and 4.3 min, respectively. The separation of the selected compounds gives enantiomers with a final purity of 99.0% and 99.9% ee respectively.

6.2. Pharmacology

6.2.1. Animals

The experiment was carried out on male Wistar rats (180–250 g) and male rabbits (2.5–3.0 kg). The animals were housed in constant temperature facilities exposed to 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 at a constant volume of 1.0 mL kg^{-1}. Control animals received the equivalent volume of solvent.

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

6.2.2. Reference compound

Carvedilol was used as a reference drug.

6.2.3. Statistical analysis

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

6.2.4. 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), [^{3}H]Clonidine (70.5 Ci mmol^{-1}, an α_2-adrenergic receptor) and [^{3}H]CGP12177 (48 Ci mmol^{-1} an β_1-adrenergic receptor) were used. The brains were homogenised in 20 vol of an ice-cold 50 mM Tris–HCl buffer (pH 7.6) and were centrifuged at 20,000 × *g* for 20 min (0–4 °C). The cell pellet was resuspended in the Tris–HCl buffer and centrifuged again. Radioligand binding assays were performed in plates (MultiScreen/Millipore). 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), [^{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), [^{3}H]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 fibre 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 equation [22].

6.2.5. Effect on normal electrocardiogram (ECG)

Electrocardiographic measurement was carried out using the Ascard B5 Eco apparatus, standard lead II, and paper speed 50 mm s^{-1}. The tested compounds were administered intravenously in a dose of 1.0 mg kg^{-1}. The ECG recording was carried out immediately before and 1, 5 and 15 min after administration of the tested compounds. The effect of the compounds on rat ECG recording was calculated according to Cambridge et al. [23].

6.2.6. Prophylactic antiarrhythmic activity in a model of adrenaline-induced arrhythmia according to Szekeres and Papp [24]

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 or orally 60 min before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and the inhibition of rhythm disturbances in comparison with the control group (ventricular bradycardia, atrioventricular block, ventricular tachycardia or ventricular fibrillation). The cardiac rhythm disturbances were recorded for 15 min after adrenaline injection. ECGs were analyzed according to the guidelines of the Lambeth Convention [25] on ventricular premature beats (VBs), bigeminy, salvos (less than four successive VBs), ventricular tachycardia (VT, four or more successive VBs) and ventricular fibrillation (VF).

6.2.7. 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 of 1.0–0.003 mg kg^{-1} into the caudal vein or given per os in a single dose of 1.0–0.03 mg kg^{-1} after a 5 min stabilization period at a volume equivalent to 1.0 mL kg^{-1}.

6.2.8. Influence on isolated rabbit ileum

The influence on isolated rabbit ileum of the investigated compounds on the smooth muscle was investigated on the rabbit small intestine, according to Magnus' method [26] modified and described by Orisadipe et al. [27]. The white rabbits were sacrificed by cervical dislocation and the small intestine was immediately removed, and cut into strips about 3–4 cm long. The isolated strips were incubated in Krebs solution at 37 °C and aerated with carbogen in special laboratory dishes. The isolated strip of the intestine was placed in a test glass tube with the Krebs solution constantly aerated by carbogen. After 1 hour incubation period, during which the physiological saline solution was changed every 15 min, the influence of the investigated compounds on spontaneous contractions of the rabbit ileum was evaluated. The contraction of the intestine was recorded on a TZ-4100 line recorder via isometric Harvard transducer, at the muscle load of 1 g. The influence of every single dose was recorded for 5 min.

Acknowledgements

This project was supported by Polish Ministry of Scientific Research and Information Technologies, Grant no. 2 P05F 010 28.

References

[1] G.S. Panjrath, F.H. Messerli, Prog. Cardiovasc. Dis. 49 (2006) 76–87.
[2] M. Thamer, N.F. Ray, T. Taylor, Clin. Ther. 21 (1999) 1387–1400;
N. Toda, Pharmacol. Ther. 100 (2003) 215–234.
[3] H. Marona, N. Szkaradek, M. Kubacka, M. Bednarski, B. Filipek, M. Cegła, E. Szneler, Arch. Pharm. (Weinheim) 341 (2008) 90–98.
[4] D. McTavish, D. Campoli-Richards, E.M. Sorkin, Drugs 45 (1993) 232–258.
[5] C.J. Dunn, A.P. Lea, A.J. Wagstaff, Drugs 54 (1997) 161–185.
[6] D.A. Sica, Am. J. Cardiol. 98 (2006) 1–4;
CAPRICORN Investigators, Lancet 357 (2001) 1358–1390;
H. Brunvand, G. Liu, X.L. Ma, T.L. Yue, R.R. Ruffolo, G.Z. Feuerstein, Eur. J. Pharmacol. 356 (1998) 193–198;
L. Han-Chieh, H. Yi-Tsau, W. Hung-Chi, Y. Ying-Ying, L. Tzung-Yan, W. Ying-Wen, H. Ming-Chih, L. Shou-Dong, J. Gastroenterol. 41 (2006) 361–368.
[7] J. Kikuta, M. Ishii, K. Kishimoto, Y. Kurachi, Eur. J. Pharmacol. 529 (2006) 47–54.
[8] G. Groszek, M. Bednarski, M. Dybała, B. Filipek, Eur. J. Med. Chem. 44 (2008) 809–817.
[9] Y. Fukuyama, Ch. Iwatsuki, M. Kodama, M. Ochi, K. Kataoka, K. Shibata, Tetrahedron 54 (1998) 10007–10016.
[10] K. Pönicke, I. Heinroth-Hoffmann, O. Brodde, J. Pharmacol. Exp. Ther. 301 (2002) 71–76.
[11] A. Siemieniuk, H. Szałkowska-Pągowska, S. Lochyński, K. Piątkowski, B. Filipek, J. Krupińska, R. Czarnecki, T. Librowski, S. Biała, Pol. J. Pharmacol. Pharm. 44 (1992) 557–593.
[12] H. Huang, J. Shan, X.H. Pan, H.P. Wang, L.B. Qian, Q. Xia, Diabetes Res. Clin. Pract. 75 (2007) 7–13;
S.L. Kopecky, Am. J. Cardiol. 98 (2006) 1115–1119;
M. Reiter, Prog. Cardiovasc. Dis. 47 (2004) 11–33;
P.A. Poole-Wilson, K. Swedberg, J.G. Cleland, Lancet 362 (2003) 7–13;
Y.S. Kim, M.S. Kim, H. Ha, J. Park, H.J. Kim, K. Park, Surg. Today 32 (2002) 230–235.
[13] B. Dulin, W.T. Abraham, Am. J. Cardiol. 93 (2004) 3B–6B;
O.E. Brodde, M.C. Michel, Pharmacol. Rev. 51 (1999) 651–689;
R.R. Ruffolo, M. Gellai, J.P. Hieble, R.N. Willette, A.J. Nichols, Eur. J. Clin. Pharmacol. 38 (1990) 82–88.
[14] B. Deng, X. Yu, S. Kuang, W. Zhang, Z. Zhou, K. Zhang, W. Qian, Z. Shan, M. Yang, S. Wu, S. Lin, Life Sci. 80 (2007) 665–671;
C. Karle, V. Kreye, W. Zhang, D. Thomas, K. Röcki, S. Kathöfer, J. Kiehn, Cardiovasc. Res. 49 (2001) 361–370;
J. Cheng, R. Niwa, K. Kamiya, J. Toyama, I. Kodama, Eur. J. Pharmacol. 376 (1999) 189–201.
[15] M.S. Gibson, R.W. Bradshaw, Angew. Chem. Int. Ed. 7 (12) (1968) 919–930.
[16] P. Cotelle, J.P. Catteau, Synth. Commun. 22 (14) (1992) 2071–2076.
[17] C.A. Howe, A. Howe, C.R. Hamel, H.W. Gibson, R.R. Flynn, J. Chem. Soc. (1965) 795–797.
[18] J.B. Press, E.G. Deegan, J.J. McNally, J.R. Roberts, J. Heterocycl. Chem. 23 (1986) 1821–1828.
[19] S. Kato, T. Morie, J. Heterocycl. Chem. 33 (1996) 1171–1178.
[20] M. Julia, P. Manoury, M.C. Voillaume, Bull. Soc. Chim. Fr. (1965) 1417–1423.
[21] S.M. Tejani-Butt, D.J. Brunswick, J. Med. Chem. (1986) 1524–1527.
[22] Y.C. Cheng, W.H. Prudoff, Biochem. Pharmacol. 22 (1973) 3099–3108.
[23] G.W. Cambridge, J.F. Parsons, R. Stafford, in: R. inBudden, D.K. Detweiler, G. Zbinden (Eds.), The Rat Electrocardiogram in Pharmacology and Toxicology, Pergamon Press, Oxford, New York, Toronto, 1981, pp. 135–138.
[24] L. Szekeres, G. Papp, in: J. Schmier, O. Eichler (Eds.), Handbook of Experimental Pharmacology, Springer-Verlag, Berlin, Heidelberg, New York, 1975.
[25] M.J. Walker, M.J. Curtis, D.J. Hearse, R.W. Campbell, M.J. Janse, D.M. Yellon, S.M. Cobbe, S.J. Coker, J.B. Harness, D.W. Harron, A.J. Higgins, D.G. Julian, M.J. Lab, A.S. Manning, B.J. Northover, J.R. Parratt, R.A. Riemersma, E. Riva, D.C. Russel, D.J. Sheridan, E. Winslow, B. Woodward, Cardiovasc. Res. 22 (1988) 447–455.
[26] R. Magnus, Versuche am überlebenden Dünnalarm von Säugetieren, Pflügers Arch. 102 (1904) 123–151.
[27] A. Orisadipe, S. Amos, A. Adesomoju, L. Binda, M. Emeje, J. Okogun, C. Wamebe, K. Gamaniel, Biol. Pharm. Bull. 24 (2001) 364–367.