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Original article

Synthesis and adrenolytic activity of 1-(1*H*-indol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol and its enantiomers. Part 1

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Abstract

The synthesis of (2*RS*)-1-(1*H*-indol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol ((*RS*)-**9**) and its enantiomers has been described and tested for electrocardiographic, antiarrhythmic, hypotensive and spasmolytic activities as well as for α_1-, α_2- and β_1-adrenoceptors' binding affinities. All compounds significantly decrease systolic and diastolic blood pressure, and possess antiarrhythmic activity and affinity to α_1-, α_2- and β_1-adrenoceptors. The results suggest that the antiarrhythmic and hypotensive effects of these compounds are related to their adrenolytic but not spasmolytic properties.

Keywords: α_1-, α_2-, β_1-Adrenoceptor antagonist; Antiarrhythmic, hypotensive and spasmolytic activities; Synthesis

1. Introduction

Stimulation of β-adrenoceptors in brain and periphery promotes the release of neurotransmitters which increase sympathetic nervous system activity. Stimulation of β_1-adrenoceptors in the sinoatrial node and in the myocardium increases heart rate and force of contraction. Stimulation of β-adrenoceptors in the kidney promotes renin release and the activity increase of renin–angiotensin–aldosterone system. The overall effect of stimulation of these receptors increases cardiac output, peripheral vascular resistance and causes an increase in aldosterone-mediated sodium and water retention [1].

Treatment with β-adrenergic blocking agents antagonizes all these effects resulting in blood pressure reduction and cardiac output decrease. Some β-blockers, such as labetalol and carvedilol also block effects of peripheral α-adrenoceptors. Carvedilol: [1-(9*H*-carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol] [2] (Fig. 1) is a selective α_1- and non-selective β-adrenoceptor antagonist, which decreases peripheral vascular resistance, systolic and diastolic blood pressure, moderately slows down the heart rhythm but does not have a significant effect on cardiac output. Carvedilol possesses antioxidant and antiproliferative effects on vascular smooth muscle, decreases insulin-resistance without influence on lipid and glucose metabolism and improves endothelial function [3–6]. Hence it is favourable for carvedilol, hemodynamic and metabolic profiles and great efficacy in treatment of hypertension, and heart failure encouraged us to look for new drugs with similar properties and mechanism of action. As a result of research we designed and obtained a new compound of chemical name 1-(1*H*-indol-4-yloxy)-3-{2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol, (*RS*)-**9** [7]. This compound has structure fragments of carvedilol and pindolol's structures that are linked by aminopropanol moiety. Most of the clinically available β-adrenergic blocking agents possess in their structures aminopropanol moiety **A** (Fig. 2).

This paper reports the synthesis and results of preliminary pharmacological testing of a new analogue of carvedilol and its enantiomers. The newly synthesized compounds were tested

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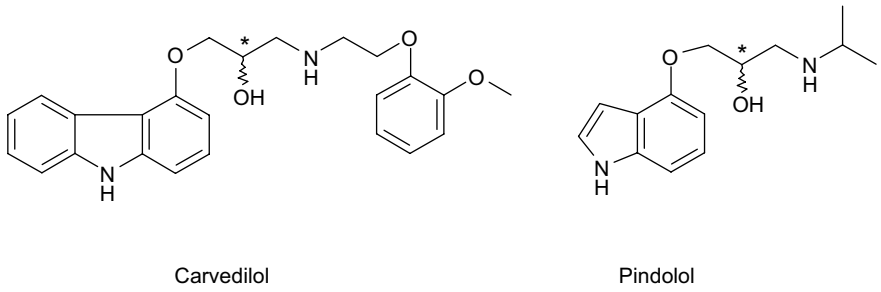

Fig. 1. Chemical structure of carvedilol and pindolol.

for electrocardiographic, antiarrhythmic and hypotensive activities as well as for α_1-, α_2- and β_1-adrenoceptors' binding affinities.

2. Chemistry

The synthesis of target compound is outlined in Figs. 3 and 4.

The synthesis of compound (*RS*)-**9** was achieved with the use of commercially available cheap substrate, guaiacol **1** and 3-nitrophenol **4**. The substrate **1** was transformed into bromoderivative **2** by method described elsewhere [8]. After that, appropriate primary amine **3** was obtained through known Gabriel synthesis [9]. The amine **3** is sensitive to air and that is why it should be stored under nitrogen or argon. For the synthesis of indole derivative **7** a method described by Mąkosza et al. was used [10]. The derivative **7** was obtained by applying vicarious nucleophilic substitution (VNS) of hydrogen to aromatic nitro derivative **5**. Then compound **6** was subjected to reductive 1,2-elimination and compound **7** was prepared. Deprotection of hydroxyl group took place in that condition. Phenol derivative **7** was converted to epoxide **8** using procedure described by Tejani-Butt and Brunswick [11]. The ring-opening addition reaction of epoxide **8** to amine **3** was performed according to our original procedure and the free base of compound (*RS*)-**9** as a racemic mixture was afforded. Also, enantiomers of compound (*RS*)-**9** were obtained. The *R*(−) or *S*(+)-epichlorohydrin was used to obtain enantiomers of epoxide **8** in procedure similar to described for racemic mixture. Then, enantiomers of epoxide **8** in addition reaction to amine **3** were converted to enantiomer *S* or *R* of compound (*RS*)-**9**. Configuration was established according to the Cahn–Ingold–Prelog convention [12]. Finally, three compounds: racemic mixture (*RS*)-**9** and its enantiomers (*R*)-**9** and (*S*)-**9** were obtained.

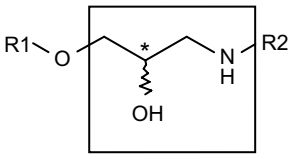

Fig. 2. **A** moiety.

3. Pharmacological results

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

The affinity of compound (*RS*)-**9** and its 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 concentration of 0.2, 2 and 0.2 nM, respectively. Compound (*RS*)-**9** and their enantiomers inhibited [^{3}H]prazosin binding with K_i ranging from 56.7 to 89.8 nM, [^{3}H]clonidine binding with K_i ranging from 365.5 to 1400 nM and [^{3}H]CGP12177 binding with K_i ranging from 2.0 to 143 nM to cortical α_1-, α_2- and β_1-adrenoceptors, respectively. The shape of curves suggests competitive binding. The results are given in Table 1.

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

Effects on ECG intervals and heart rate were determined for all compounds in the same dose of 1 mg kg^{-1}. Only compound (*S*)-**9** administered iv decreased heart rate by 5.6–9.6% in 1, 5 and 15 min after administration with no significant influence on P–Q, Q–T intervals and QRS complex (Table 2). Compounds (*RS*)-**9** and (*R*)-**9** 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) and therapeutic indexes of prophylactic antiarrhythmic activity in the adrenaline-induced arrhythmia are listed in Table 3. These compounds administered 15 min before adrenaline prevented and/or reduced in a statistically significant manner the number of premature ventricular beats. Compound (*RS*)-**9** and its enantiomers exhibited

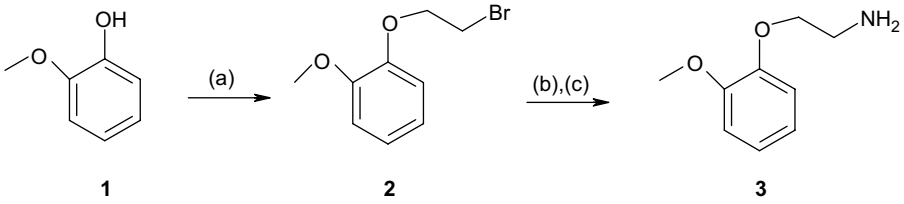

Fig. 3. (a) 1,2-Dibromoethane, NaOH; (b) potassium phthalimide, DMF; (c) NH_2–$NH_2 \cdot H_2O$.

important antiarrhythmic effects with ED_{50} values ranging between 0.059 and 0.43 mg kg^{-1} and with therapeutic indexes ranging 47–613.

All tested compounds retained antiarrhythmic activity after po administration. The range of their ED_{50} values was 0.44–1.28 mg kg^{-1}.

3.4. Influence on blood pressure in rats

Hypotensive activity of compound (*RS*)-**9** and both enantiomers was determined after iv and po administrations in normotensive anesthetized rats. After iv administration, the highest hypotensive activity was observed for compound (*S*)-**9**. It significantly decreased the systolic (15–34%) and diastolic (14–40%) blood pressure throughout the whole observation period in the range of doses 0.125–1 mg kg^{-1}. In the lowest dose (0.0625 mg kg^{-1}), compound (*S*)-**9** decreased systolic blood pressure (8–13%) throughout whole observation period and diastolic blood pressure (8–16%) only in the first few minutes after administration. The compound (*RS*)-**9** after iv administration significantly decreased systolic and diastolic blood pressure (10–32%) in the range of doses 0.25–1 mg kg^{-1}. Compound (*R*)-**9** after iv administration showed hypotensive activity only in dose of 1 mg kg^{-1} (Fig. 5).

After po administration all tested compounds maintained their hypotensive activity (Fig. 6). (*RS*)-**9** and (*S*)-**9** decreased systolic and diastolic blood pressure in doses ranging between 0.125–1 and 0.062 mg kg^{-1}, respectively. Compound (*R*)-**9** did not show hypotensive activity after po administration.

3.5. Influence on blood pressor response in rats

To examine the mechanism of the hypotensive effects of these compounds, we studied their influence on the pressor responses to epinephrine, norepinephrine and methoxamine (Figs. 7–9). All tested compounds, given in the dose of

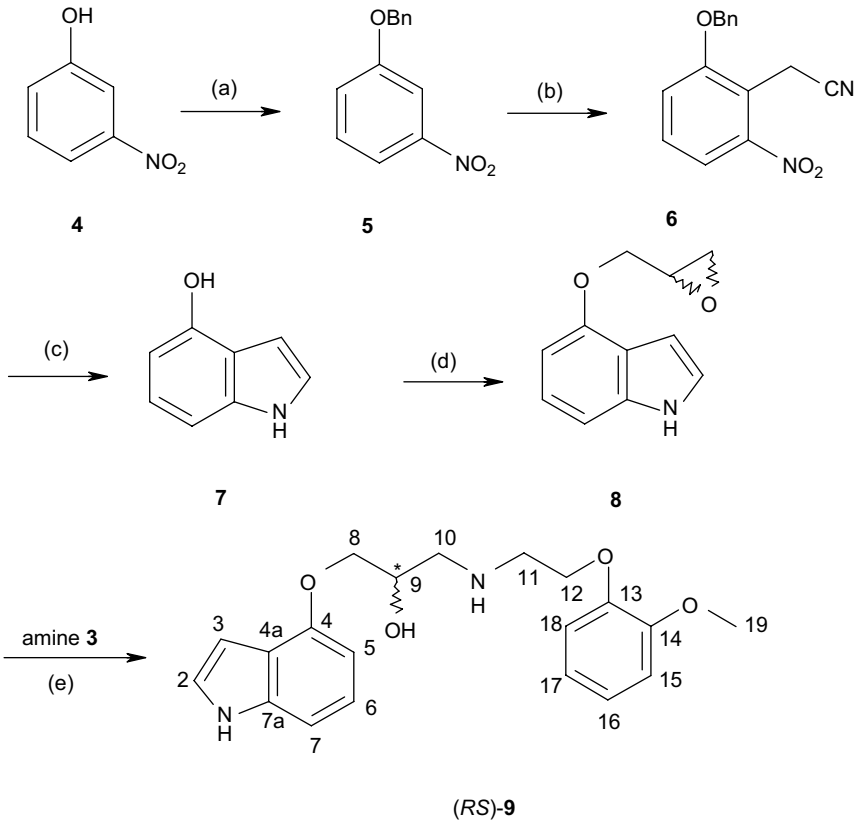

Fig. 4. Synthetic pathway: (a) benzyl chloride, K_2CO_3, CH_3CN, KI; (b) *t*-BuOK, *p*-Cl–Ph–OCH_2CN, DMF, −20 °C; (c) H_2, Pd/C, EtOH/AcOH; (d) (±)-epichlorohydrin, 1 N NaOH aq., dioxane; (e) amine **3**, CH_3CN.

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*)-**9**	89.8 ± 9.5	1.4 ± 0.4 μM	3.0 ± 0.6
(*R*)-**9**	88.3 ± 5.8	467.3 ± 32.3	143 ± 16.5
(*S*)-**9**	56.7 ± 2.1	365.5 ± 28.2	2 ± 0.08
Carvedilol	2.2 ± 0.2[a]	3.4 ± 0.9 μM	0.81 ± 0.06[a]

[a] Ref. [5].

1 mg kg^{-1}, significantly antagonized the pressor response elicited by epinephrine and methoxamine. Compounds (*RS*)-**9**, (*S*)-**9** and carvedilol significantly reduced the pressor response to norepinephrine (Fig. 10).

3.6. Influence on isolated rabbit ileum

Only compound (*RS*)-**9** gave at the highest dose (10^{-5} M) statistically significant amplitude decrease of contractions of isolated rabbit small intestine of about 29%. At the lowest concentration (10^{-9} M), tested compound significantly increased frequency of contractions of isolated rabbit small intestine of about 8%. Compound (*R*)-**9** gave at the highest dose (10^{-5} M) statistically significant amplitude decrease of contractions of isolated rabbit small intestine of about 31%. Compound (*S*)-**9** had no influence on the frequency and amplitude of contractions of isolated rabbit small intestine.

3.7. Acute toxicity

The acute toxicity of compound (*RS*)-**9** and its two enantiomers was obtained in rats after intravenous administration according to Litchfield and Wilcoxon [15]. The LD_{50} values are given in Table 3. Compounds (*RS*)-**9** and (*R*)-**9** showed imperceptibly higher toxicity than carvedilol, while (*S*)-**9** showed a little lower toxicity than the reference compound.

Table 2
Effects of an iv injection of the investigated compounds in dose 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*)-**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
(*R*)-**9**	P–Q (ms)	48.5 ± 0.9	53.7 ± 1.7	50.7 ± 1.8	51.7 ± 3.1
	QRS (ms)	23.0 ± 1.5	22.3 ± 0.6	21.7 ± 0.8	21.7 ± 0.6
	Q–T (ms)	61.0 ± 3.2	63.0 ± 1.9	66.0 ± 3.2	62.8 ± 1.6
	Beats/min	299.3 ± 12.3	307.8 ± 10.8	310.2 ± 8.9	298.0 ± 9.0
(*S*)-**9**	P–Q (ms)	45.0 ± 3.5	52.3 ± 3.4	49.7 ± 3.3	48.0 ± 2.9
	QRS (ms)	26.0 ± 1.5	24.3 ± 2.2	24.3 ± 2.0	22.7 ± 1.3
	Q–T (ms)	69.7 ± 3.7	72.3 ± 2.3	76.3 ± 2.3	72.0 ± 2.0
	Beats/min	322.0 ± 4.7	297.2 ± 5.4[b]	291.3 ± 5.3[b]	304.2 ± 3.6[a]
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 six experiments. Statistical analyses were performed using a one-way ANOVA test.
[a] $p < 0.02$.
[b] $p < 0.01$.

Table 3
Prophylactic antiarrhythmic activity in anesthetized rats

Compound	ED_{50} iv (mg kg^{-1})	LD_{50} iv (mg kg^{-1})	IT iv	ED_{50} po (mg kg^{-1})
(*RS*)-**9**	0.34 (0.23–0.51)	20.0 (19.2–20.8)	59	0.44 (0.18 ± 1.10)
(*R*)-**9**	0.43 (0.28–0.65)	20.4 (16.7–24.5)	47	1.28 (0.56 ± 2.93)
(*S*)-**9**	0.059 (0.038–0.093)	36.2 (28.9–45.4)	613	0.61 (0.30 ± 1.27)
Carvedilol	0.25 (0.12–0.53)	25[a]	100	
Propanolol	1.05 (0.64–1.73)	39 (33.6–45.2)[b]	37	

[a] Ref. [13].
[b] Ref. [14].

4. Discussion

The aim of our study was to evaluate cardiovascular activity of a new compound – (*RS*)-**9** and its enantiomers which are similar to carvedilol that is a non-selective β-antagonist with α_1-adrenolytic activity and antioxidant, antiproliferative and antiendothelin activities [16–19].

This work includes synthesis and pharmacological studies of 1-(1*H*-indol-4-yloxy)-3-{2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol, (*RS*)-**9** and its enantiomers. It examines antiarrhythmic and hypotensive activities and α- and β-adrenolytic mechanisms of action. Pharmacological properties of compound (*RS*)-**9** and its enantiomers were investigated in comparison with carvedilol.

The method which can initially determine the direction of action of newly tested compounds is a radioligand binding assay. The compound (*RS*)-**9** 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 for enantiomers, but the greatest discrepancy was shown in affinity to β_1-adrenoceptor. (*S*)-**9** enantiomer had 70-fold greater affinity to β_1-adrenoceptors than (*R*)-**9** enantiomer. Unfortunately, none of the enantiomers had larger affinity to adrenoceptors than carvedilol. These results gave evidence of relationship between spatial configuration and affinity to β-adrenoceptors, and lack of this relationship in the case of α-adrenoceptors. The same results were observed for enantiomers of carvedilol and other β-adrenergic antagonists [20–22].

The antiarrhythmic effects of novel compounds were examined on rats using model of adrenaline-induced arrhythmia. The tested compound (*RS*)-**9** and its enantiomers given iv 15 min before arrhythmogen prevented or attenuated the symptoms of adrenaline-induced arrhythmia. (*S*)-**9** was the most active compound. Data reported in Table 3 suggest that all compounds show more beneficial therapeutic indexes than propanolol but only (*S*)-**9** has better therapeutic index than carvedilol. All tested forms of compound (*RS*)-**9** kept its antiarrhythmic activity after po administration but the differences were less noticeable. Carvedilol in dose 12 mg kg^{-1} po and lower did not prevent the adrenaline-induced rhythm

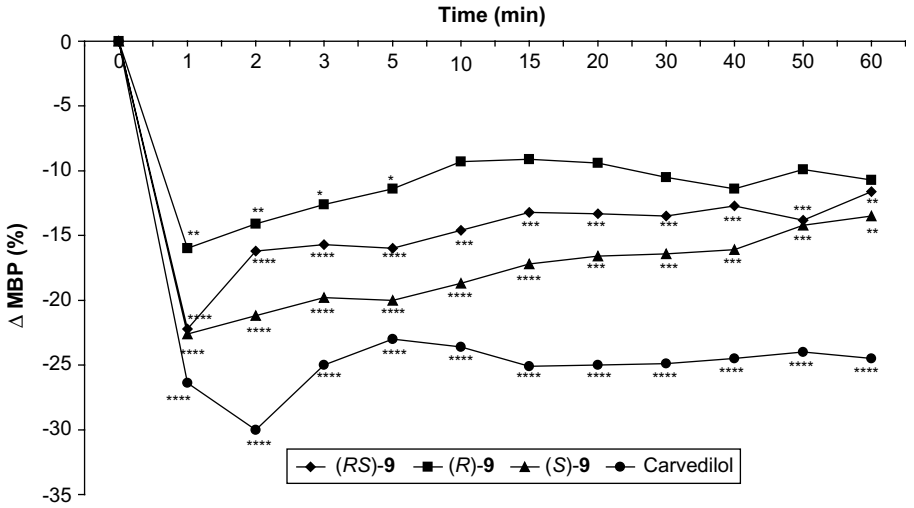

Fig. 5. Changes in mean blood pressure after iv administration of the tested compounds in the dose 0.25 mg kg^{-1}. Statistical analyses were performed using a one-way ANOVA test: *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, ****$p < 0.001$.

disturbances. Antiarrhythmic activity of pure β-adrenergic blocking agents is known for years. Carvedilol is known to exert an antiarrhythmic effect too. The electrophysiological properties of carvedilol include moderate prolongation of potential action duration and effective refractory period. It shows atrioventricular conduction and reduced dispersion of refractoriness. Carvedilol in a concentration range similar to concentrations achieved in clinical setting blocks potassium current such as: I_{Kr}, I_{Kur} (the rapid and ultra-rapid component of the delayed rectifier K^+ current) and, in higher concentration, I_{to} (the transient outward K^+ current) and L-type Ca^{2+} current I_{Ca}. Such balanced inhibition of K^+ and Ca^{2+} channels resulted in a moderate prolongation of action potential duration with minimal reverse frequency-dependence. This property would be beneficial in the treatment of ventricular tachyarrhythmias in patients with chronic heart failure or post-myocardial infarction [4,23–25].

Based on our results, we put forward a hypothesis that the antiarrhythmic effects of the tested compounds are related to the β_1-adrenoceptor blockade in heart tissue and to potassium and calcium channels' inhibition.

Hypotensive activity of the investigated compounds was determined after their iv and po administration to normotensive anesthetized rats. All compounds caused a significant hypotensive effect throughout the whole observation period. The most hypotensive effect was shown by compound (*S*)-**9** which significantly diminished blood pressure in dose 0.062 mg kg^{-1} after iv and po administration. In both routes of administration (iv and po) relationship between spatial configuration and hypotensive activity was kept. Carvedilol after iv administration was the most active one, but after po administration it was less effective than compound (*S*)-**9**.

The hypotensive effect is probably the result of adrenoceptors' blockade (α_1- in arteries and β_1- in heart). Both

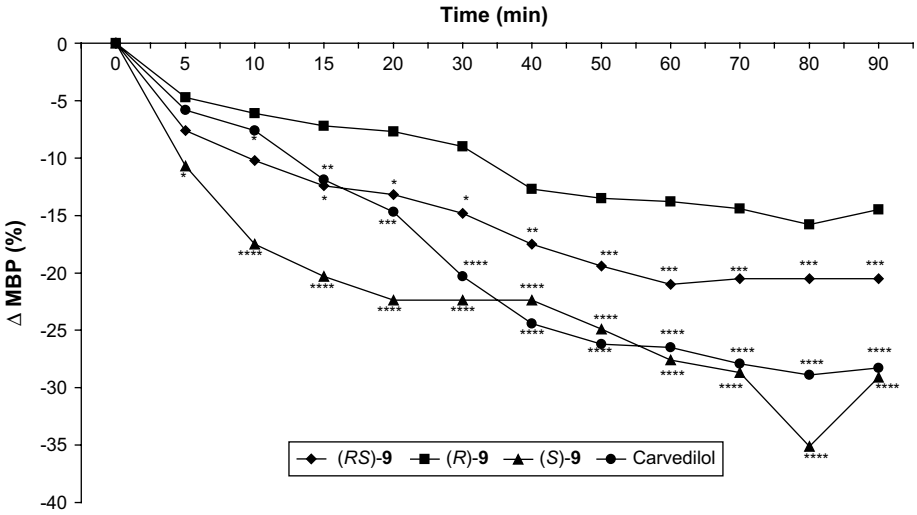

Fig. 6. Changes in mean blood pressure after po administration of the tested compounds in the dose 0.5 mg kg^{-1}. Statistical analyses were performed using a one-way ANOVA test: *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, ****$p < 0.001$.

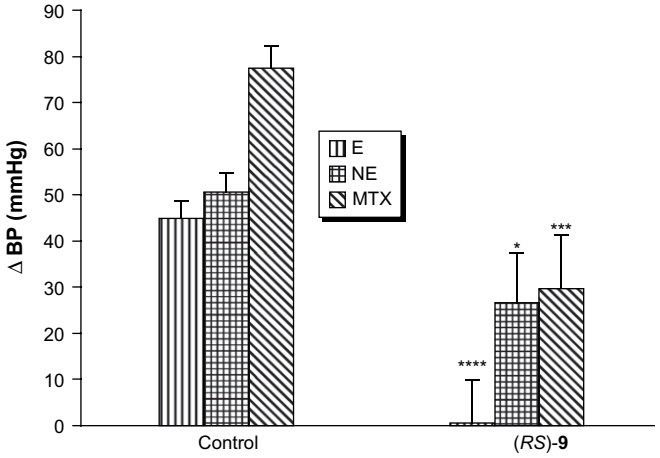

Fig. 7. Effect of (*RS*)-**9** on the blood pressor response to epinephrine, norepinephrine and methoxamine. All values represent the mean ± S.E.M. in 6–8 rats. Statistical analyses were performed using a one-way ANOVA test: $*p < 0.05$, $***p < 0.01$, $****p < 0.001$.

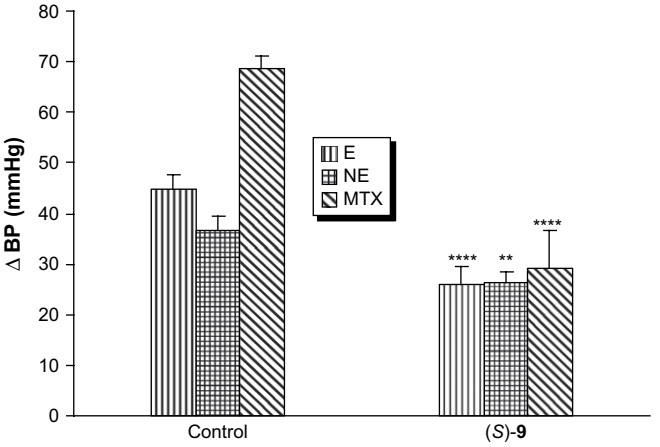

Fig. 9. Effect of (*S*)-**9** on the blood pressor response to epinephrine, norepinephrine and methoxamine. All values represent the mean ± S.E.M. in 6–8 rats. Statistical analyses were performed using a one-way ANOVA test: $**p < 0.02$, $****p < 0.001$.

enantiomers and racemic mixture (*RS*)-**9** similarly to carvedilol significantly antagonized the pressor responses to epinephrine and methoxamine. Compounds (*RS*)-**9**, (*S*)-**9** and carvedilol also significantly decreased the pressor response elicited by norepinephrine. These experiments with a non-selective agonist of α_1- and α_2-adrenoceptors such as epinephrine or norepinephrine and a selective agonist of α_1-adrenoceptor such as methoxamine suggest that hypotensive effects of the tested compounds were related to their adrenolytic properties. It is generally accepted that α_1-antagonists inhibit the pressor responses.

In the experiment on isolated rabbit ileum only compounds (*RS*)-**9** and (*R*)-**9**, in the highest concentrations (10^{-5} M), significantly decreased the contractions' amplitude without influence on the frequency of contractions of isolated rabbit small intestine. The most hypotensive compound (*S*)-**9** had no influence on the amplitude and frequency of contractions of isolated rabbit small intestine. No correlation between spasmolytic and hypotensive activities confirmed that hypotensive activity is caused by α-adrenolytic but not spasmolytic activity.

5. Conclusion

The results confirmed that 1-(1*H*-indol-4-yloxy)-3-{2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol and its enantiomers possess α_1-, α_2- and β_1-adrenolytic, antiarrhythmic and hypotensive activities. Research suggests that the antiarrhythmic and hypotensive effects of tested compounds are related to their adrenolytic properties. Most of the pharmacological effects of compound (*RS*)-**9** and its enantiomers, especially enantiomer (*S*)-**9**, were qualitatively similar or weaker to those of carvedilol.

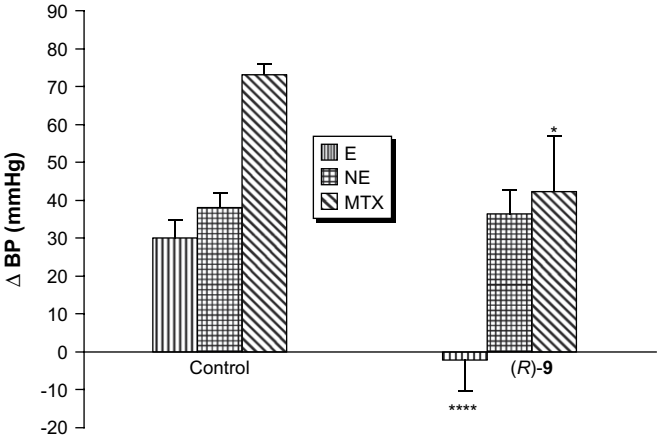

Fig. 8. Effect of (*R*)-**9** on the blood pressor response to epinephrine, norepinephrine and methoxamine. All values represent the mean ± S.E.M. in 6–8 rats. Statistical analyses were performed using a one-way ANOVA test: $*p < 0.05$, $****p < 0.001$.

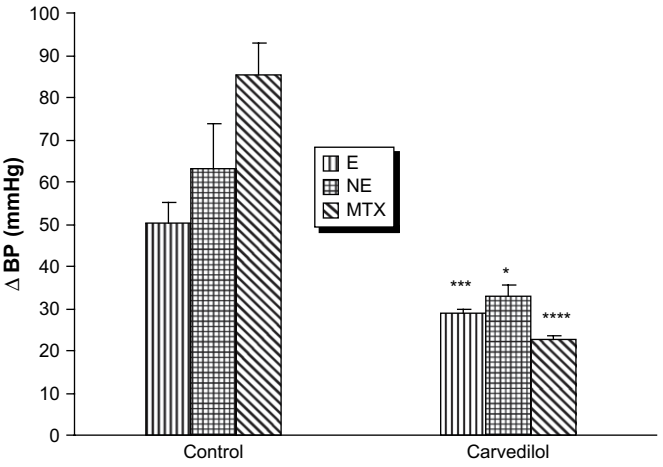

Fig. 10. Effect of carvedilol on the blood pressor response to epinephrine, norepinephrine and methoxamine. All values represent the mean ± S.E.M. in 6–8 rats. Statistical analyses were performed using a one-way ANOVA test: $*p < 0.05$, $***p < 0.01$, $****p < 0.001$.

6. Experimental protocol

6.1. Chemistry

6.1.1. General

Melting points were determined on a Boëtuis apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini (300 MHz) and Bruker (500 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. Mass spectra were obtained on an AMD-604 spectrometer. Optical rotations were obtained on a Jasco P-2000 apparatus. TLC plates were silica gel 60 F_{254} Merck and silica gel Merck 70–230 mesh was used for column chromatography.

6.1.2. Materials

Guaiacol, 1,2-dibromoethane, (±)-1-chloro-2,3-epoxypropane, benzyl chloride, and *t*-BuOK were purchased from Aldrich Chemicals; >98.5% of *S*(+) and *R*(−)-1-chloro-2,3-epoxypropane, potassium phthalimide and 10% Pd/C were obtained from Fluka Chemicals. 3-Nitrophenol was submitted by POCH Chemicals.

(4-Chlorophenoxy)acetonitrile was obtained according to literature [26].

Solvents were distilled and dried if required, and other materials were commercial.

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

6.1.3. 2-(2-Methoxyphenoxy)ethylamine (**3**)

Guaiacol **1** (44.25 cm^3; 0.4 mol), water (145 cm^3), 1,2-dibromoethane (69 cm^3; 0.8 mol) and NaOH aq. (20%, 70 cm^3; 0.48 mol) were heated together for 48 h at 80 °C temperature. Organic phase was separated; aqueous phase was shaken with methylene chloride (2 × 150 cm^3). The combined organic phase was washed with 10% NaOH aq. and water and then solvent was evaporated. Oily residue was chromatographed on silica gel using hexane/ethyl acetate mixture as an eluent. Product **2** was obtained as colourless crystals; 69.8 g (75% yield), m.p. 39–40 °C. Lit. m.p. 38–41 °C [27].

The mixture of bromide **2** (5 g; 0.0216 mol), potassium phthalimide (4.26 g; 0.023 mol), 18-crown-6 ether (0.15 g; 6.25×10^{-4} mol) in DMF (20 cm^3) was heated for 5 h at 50 °C temperature. The reaction mixture was poured into water (170 cm^3), stirred for 2 h, precipitate was filtered off, washed with water and dried on air afforded *N*-[2-(2-methoxyphenoxy)ethylo]phthalimide, 5.69 g (88% yield); m.p. 102–108 °C, lit. m.p. 102–104.5 °C [27].

A mixture of phthalimide derivative (1 g; 3.37 mmol), water (10 cm^3) and hydrazine hydrate (100%, 1.5 cm^3; 0.03 mol) was stirred for 5 h at room temperature. Product was extracted with chloroform (2 × 20 cm^3), dried over potassium carbonate and evaporated to leave colourless solid pure amine **3**, 0.48 g (86% yield), meltable at room temperature. ^{1}H NMR ($CDCl_3$) δ: 1.72 (s, 2H, NH_2); 3.01 (t, 2H, $J = 5.3$, CH_2–NH_2); 3.76 (s, 3H, OCH_3); 3.96 (t, 2H, $J = 5.3$, O-CH_2); 6.84 (s, 4H, ArH).

6.1.4. 4-(2,3-Epoxypropoxy)-1H-indole (**8**)

3-Nitrophenol, **4**, was converted to benzyl protected derivative **5** with yield of 96%, and then transformed to intermediate **6** with yield of 53%. M.p. 93–94 °C (EtOH), lit. 92–94 °C (EtOH) and ^{1}H NMR spectra for compound **6** were according to literature [27]. Then, compound **6** was submitted to reductive cyclization to give 1*H*-indol-4-ol, **7**, with yield of 78%; m.p. 94–97 °C (cyclohexane), lit. m.p. 95–97 °C (cyclohexane) [28].

Indole derivative **7** was treated with (±)-epichlorohydrin in the presence of stoichiometric quantity of base in dioxane and water to give the epoxide **8** with yield of 83% as a colourless solid. Crystallization from ethyl acetate afforded colourless crystals: m.p. 64–66 °C, lit. m.p. 65–67 °C [28]. ^{1}H NMR (acetone-d_6) δ: 2.75 (2d, 1H, $J = 2.85$ and 5.19, CH_2O); 2.84 (2d, 1H, $J = 4.26$ and 5.19, CH_2O); 3.35–3.40 (m, 1H, CHO); 3.99 (2d, 1H, $J = 6.15$ and 11.34, OCH_2CH); 4.40 (2d, 1H, $J = 2.85$ and 11.34, OCH_2CH); 6.51 (2d, 1H, $J = 0.93$ and 7.56, CHCHN); 6.54–6.56 (m, 1H, CHCHN); 6.96–7.06 (m, 2H, ArH); 7.20–7.22 (m, 1H, ArH); 10.22 (br s, 1H, NH).

6.1.5. (2RS)-1-(1H-Indol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol, ((RS)-**9**)

The epoxide **8** (0.945 g, 5 mmol) was dissolved in acetonitrile (5 cm^3). After that amine **3** (2.65 g, 15.8 mmol) was added and the reaction mixture was heated at 80 °C temperature. The progress of the reaction was monitored by TLC (toluene/methanol 4:1). After 7 h reaction was completed, salicylic aldehyde (3 cm^3) was added and the reaction mixture was stirred for 10 min at room temperature. Then, the reaction mixture was subjected to column chromatography on silica gel (50 g, eluent toluene/methanol 99.5:0.5–97:3). Compound (*RS*)-**9** was obtained as a solid (1.03 g, 58%). Crystallization from dichloromethane afforded fine colourless crystals, m.p. 68–70 °C. ^{1}H NMR (500 Hz, $CDCl_3$) δ: 2.53 (br s, 2H, OH, NH); 2.90–3.00 (m, 2H, H-10); 3.06–3.09 (m, 2H, H-11), 3.82 (s, 3H, H-19), 4.12–4.18 (m, 5H, H-8, 9 and 12), 6.52 (br d, 1H, $J = 7.70$, H-3); 6.62–6.63 (m, 1H, H-2); 6.86–6.88 and 6.90–6.92 (2m, 7H, ArH), 8.17 (br s, 1H, NH). ^{13}C NMR (125 Hz, $CDCl_3$) δ: 45.5 (C-11), 48.6 (C-10), 52.6 (C-19), 65.3, 65.9, 67.4 (C-8, 9 and 12), 96.7 (C-2), 97.9 (C-3), 101.5 (C-7), 109.1 (C-15), 111.8 (C-18), 115.7 (C-4a), 117.7, 118.6, 119.3, 119.4 (C-5, 6, 16 and 17), 134.2 (C-7a), 145.2 (C-14), 146.9 (C-13), 149.2 (C-4). IR (KBr, cm^{-1}) ν: 744.2 (Ar), 1125 (ether, OCH_2), 1252.5 (ether, O–Ar), 1505.6 and 1589.1 (C=C, Ar), 3005.5 and 3369.8 (NH, OH). HRMS (ESI) *m/z* for $C_{20}H_{25}N_2O_4$ $(M+H)^+$: found 357.1816, calcd 357.1809 UV (96%, EtOH) (nm) λ_{max}: 217 ($\varepsilon = 27\,000$); 266 ($\varepsilon = 11\,450$), ($c = 0.4$ mg/10 cm^3).

6.1.6. (2S)-1-(1H-Indol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol, ((S)-**9**)

(*S*)-(+)-4-(2,3-Epoxypropoxy)-1*H*-indole was obtained according to procedure described in Section 6.1.4 using *R*(−)-1-chloro-2,3-epoxypropane instead their raceme mixture, with yield of 75% as colourless solid after solvent evaporation: m.p. 81–83 °C and $[\alpha]_D^{24} = +10.6°$ ($c = 1.0$ in $CHCl_3$).

Then, optical active indole derivative was submitted to addition reaction with amine **3** according to procedure described for compound (*RS*)-**9**, Section 6.1.5, and gave product (*S*)-**9** as a foam solid with yield of 50%, $[\alpha]_D^{23} = -6.3^\circ$ ($c = 1.1$ in $CHCl_3$). Very hygroscopic and must be stored in argon atmosphere.

6.1.7. (2R)-1-(1H-Indol-4-yloxy)-3-{[2-(2-methoxyphenoxy) ethyl]amino}propan-2-ol, ((R)-9)

(*R*)-(−)-4-(2,3-Epoxypropoxy)-1*H*-indole was obtained as described above, using *S*(+)-1-chloro-2,3-epoxypropane, with yield of 69% as colourless solid after solvent evaporation: m.p. 83–84 °C and $[\alpha]_D^{24} = -9.8^\circ$ ($c = 1.0$ in $CHCl_3$).

The product (*R*)-**9** was obtained as a foam solid with yield of 45%, $[\alpha]_D^{23} = +4.7^\circ$ ($c = 1.2$ in $CHCl_3$). Very hygroscopic and must be stored in argon atmosphere.

6.1.8. Enantiomeric purity of (R)-9 and (S)-9

Enantiomeric purity of (*R*)-**9** and (*S*)-**9** was assessed by chiral HPLC, column of Chiracel OD-RH (4.6 × 150 mm, 5 μm, Daicel Chemical Industries, Tokyo) with isocratic elution using a mobile phase of acetonitrile/water (35:65) with 0.01% formic acid at flow rate of 600 μl min^{-1}. Detection was achieved by an Applied Biosystems AMS Sciex (Concord, Ontario) API 2000 triple quadrupole mass spectrometer. The retention times of (*R*)-**9** and (*S*)-**9** were 8.22 and 12.64 min, respectively. The isomers were well resolved and the ratio of the peak areas was used to estimate enantiomeric purity. (*R*)-**9** and (*S*)-**9** were consistently assayed at >98.5% enantiomeric purity.

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 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 or intragastrically at a constant volume of 1 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 ± S.E.M. The statistical significance was calculated using 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 $mmol^{-1}$, a β_1-adrenergic receptor) were used. The brains were homogenised in 20 volumes 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} to 10^{-4} M) of 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 propanolol − 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 assays were made in duplicate. The radioligand binding data were analysed using an interactive curve-fitting routine (GraphPAD/Prism,Version 3.0 − San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation [29].

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 was 50 mm s^{-1}. The tested compounds were administered intravenously in dose of 1 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. [30].

6.2.6. Prophylactic antiarrhythmic activity in a model of adrenaline-induced arrhythmia according to Szekeres and Papp [31]

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 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 analysed according to the guidelines of the Lambeth Convention [32] 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 anaesthetized 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 1–0.003 mg kg^{-1} into the cadual vein or given per os in a single dose 1–0.03 mg kg^{-1} after 5 min stabilization period at a volume equivalent to 1 ml kg^{-1}.

6.2.8. Influence on blood pressor response in rats

In separate series of experiments on anesthetized normotensive rats, the effect of studied compounds, given intravenously in dose of 1 mg kg^{-1}, on a pressor response to epinephrine (2 μg kg^{-1}), norepinephrine (2 μg kg^{-1}) and methoxamine (150 μg kg^{-1}) was investigated. Pressor response to norepinephrine, norepinephrine and methoxamine injected intravenously was obtained before and 5 min after the tested compounds (iv).

6.2.9. Influence on isolated rabbit ileum

The influence on isolated rabbit ileum of the investigated compounds was tested by the method according to Magnus [33], and modified and described by Orisadipe et al. [34]. 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 h 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.

6.2.10. Acute toxicity

The tested compounds were dissolved in a 0.9% saline and injected into the caudal vein of rat (1 ml kg^{-1}). Each dose was given to six animals. LD_{50} values were calculated according to the method of Litchfield and Wilcoxon [15] after a 24 h observation period.

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