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Original article

Synthesis, antiarrhythmic, and antihypertensive effects of novel 1-substituted pyrrolidin-2-one and pyrrolidine derivatives with adrenolytic activity

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Abstract

A series of 1-substituted pyrrolidin-2-one and pyrrolidine derivatives were synthesised and tested for electrocardiographic, antiarrhythmic, and antihypertensive activity as well as for α_1- and α_2-adrenoceptors binding affinities. Among the newly synthesised derivatives several compounds with 3-(4-arylpiperazin-1-yl)propyl moiety displayed strong antiarrhythmic (**7a**–**12a**) and antihypertensive (**7a**–**11a**) activities. Compound **11a**, 1-[2-acetoxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one, was the most potent in this series. The pharmacological results and binding studies suggest that their antiarrhythmic and hypotensive effects may be related to their α-adrenolytic properties, and that those properties depend on the presence of the 1-phenylpiperazine moiety with a methoxy- or chloro- substituent in the *ortho* position in the phenyl ring. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: *N*-(4-Arylpiperazin-1-yl)propylpyrrolidin-2-one and pyrrolidine derivatives; Antiarrhythmic; Antihypertensive; α-Adrenoceptors blocking activity

1. Introduction

In the search for new antiarrhythmics among derivatives of pyrrolidin-2-one, we obtained **MG-1**, 1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]pyrrolidin-2-one [1–3] (Fig. 1) which became a lead structure for further investigations. **MG-1** attenuated or prevented the adrenaline- and barium chloride-induced arrhythmia, statistically diminished arrhythmias associated with coronary artery occlusion and reperfusion in the isolated rat's hearts, demonstrated potent local anaesthetic properties and depressed the depolarisation phase of the action potential of cardiac cells [2]. According to Vaughan Williams classification [4], **MG-1** could be considered as a class Ia antiarrhythmic drug. Moreover, **MG-1** showed strong hypotensive/antihypertensive effect and affinity for α_1- and α_2-adrenoceptors [5].

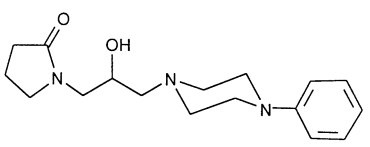

Fig. 1. Chemical structure of **MG-1**.

Previously, we have described the synthesis and the antiarrhythmic and antihypertensive activities of a series of 1-substituted pyrrolidin-2-one and pyrrolidine derivatives [5]. Some of them slightly decreased the heart rate, prolonged P–Q, Q–T intervals and QRS complex. Compound 1-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]pyrrolidine possessed potent antiarrhythmic activity, slight hypotensive properties and affinity for α_1- and α_2-adrenoceptors. Our earlier investigations suggested that the antiarrhythmic effect of **MG-1** may be related to their α-adrenolytic properties.

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It has been known that non-selective α-blocking agents (phentolamine, prazosine) in relatively high doses diminished or prevented reperfusion arrhythmias and cardiac arrhythmias caused by adrenaline intoxication [6–9]. On the other hand, blocking the action of adrenergic neurotransmitters on the α_1-adrenoceptor is a well-known approach for the clinical treatment of hypertension [10,11]. It was also recently reported that α_1-adrenergic receptors mediate both vascular and lower urinary tract tone. Several non-subtype selective α_1-adrenergic receptors antagonists, such as prazosin and terazosin, are used to treat both hypertension and benign prostatic hyperplasia (BPH) [12–14].

Taking into consideration the pharmacologically interesting properties shown by 1-substituted pyrrolidin-2-one derivatives, we decided to continue our studies directed toward the synthesis of the products with potential α-adrenolytic activity.

In this paper, we report the synthesis and pharmacological results of a new series of **MG-1** derivatives. Thus, the 1-phenylpiperazine moiety was replaced by an arylalkylamine group such as benzylamine or 2-chloro, 4-methoxy, 3,4-dimethoxy substituted benzylamine; the phenyl ring of **MG-1** was replaced by pyrimidine, diphenylmethyl group or 2-chloro-, 4-chloro-, 2-methoxyphenyl. Using previously structure–activity relationships as a guide, we have chosen novel derivatives of lead structure for further modifications within hydroxy group and pyrrolidin-2-one ring. The newly synthesised compounds (in form of salts) were tested for antiarrhythmic, and antihypertensive activity as well as for α_1- and α_2-adrenoceptors binding affinities.

2. Chemistry

1-(2,3-Epoxypropyl)pyrrolidin-2-one was used as a starting material for synthesis of new 1-substituted pyrrolidin-2-one or pyrrolidine derivatives. The aminolysis of 1-(2,3-epoxypropyl)pyrrolidin-2-one with benzyl or substituted benzylamines such as: 2-chloro-, 3-methoxy- or 3,4-dimethoxybenzylamine gave relevant 1-[(3-benzylamino)-2-hydroxypropyl]pyrrolidin-2-one derivatives (**1**–**4**). However, the similar reaction with *N*-substituted piperazine (2-chlorophenyl-, 2-methoxyphenyl-; pyrimidine or diphenylmethyl) led to obtain relative 1-[2-hydroxy-3-(4-arylpiperazin-1-yl)propyl]pyrrolidin-2-one (**5**–**9**). The yields of that reactions were in range from 59 to 76%. Compounds were isolated as a free bases and a water soluble hydrochloric salt. The course of reactions is presented at Fig. 2. Compounds **7** and **8** were chosen for farther synthesis as they are the most active derivatives in pharmacological investigations. Acetylation of compounds **7** and **8** enabled to obtain appropriate acetoxy derivatives such as 1-[2-acetoxy-3-[4-(2-methoxyphenyl)- or 2-acetoxy-3-[4-(2-chlorophenyl)piperazin-1-yl]propyl]pyrrolidin-2-one. The above compounds were isolated as a water soluble hydrochloride salt (**10a**, **11a**). Reduction of **7** with lithium aluminium hydride (LAH) led to 1-[2-hydroxy-3-[4-(2-methoxyphenyl)-piperazin-1-yl]propyl]pyrrolidine (**12**), which was isolated as a maleic acid salt (**12a**). The course of reaction is presented in Fig. 3. The structures of these new compounds were established by elemental analysis and spectral data [15].

3. Pharmacology

In the present study, several pharmacological tests were carried out to assess the effect on normal electrocardiogram (ECG), antiarrhythmic, hypotensive and α-adrenergic blocking activities of novel pyrrolidin-2-one and pyrrolidine derivatives.

The effect on ECG intervals and the heart rate (the number of cardiac beats per minute) was determined for all compounds in a dose of 1/10 and 1/5 LD_{50}. The novel pyrrolidin-2-one derivatives (**1a**–**5a** and **12a**) in these two doses, and compounds **6a**, **8a** and **10a** in the dose of 1/10 LD_{50} did not significantly affect the normal ECG (not included in Table 1). Electrocardiographic experiments showed that compounds **7a**, **9a** and **11a** affected the normal ECG in a dose of 1/10 LD_{50}, i.e. significantly decreased the number of cardiac beats per minute (**7a**, **9a**), prolonged P–Q intervals (**11a**) and QRS complex (**9a**), (Table 1). On the other hand compounds **7a** and **11a** administered in a dose of 1/5 LD_{50} induced disturbances of cardiac rhythm. Compounds **6a**, **8a**–**10a** changed the ECG pattern in a dose of 1/5 LD_{50} significantly decreased the number of cardiac beats per minute (**9a**), prolonged P–Q (**6a**) and QT (**6a**, **8a**, **9a** and **10a**) intervals and QRS complex (**6a**). The electrocardiographic changes observed after administration of these compounds were similar to those seen after administration of **MG-1** and quinidine [5].

All compounds were tested in two arrhythmia's models (prophylactic and therapeutic) using adrenaline- and barium chloride-induced arrhythmia [16]. The ED_{50} values and therapeutic index of prophylactic antiarrhythmic activity are presented in Table 2. Among the studied compounds, only compounds **7a**–**12a** inhibited adrenaline-induced arrhythmia in the anaesthetised rat by more than 50% after intravenous dosing. These compounds also significantly reduced mortality. Compounds **7a**, **8a** and **11a**, given orally 60 min before adrenaline, also diminished the number of extrasystoles by 50% or more in rats and decreased or prevented mortality of these animals. The data reported in Table

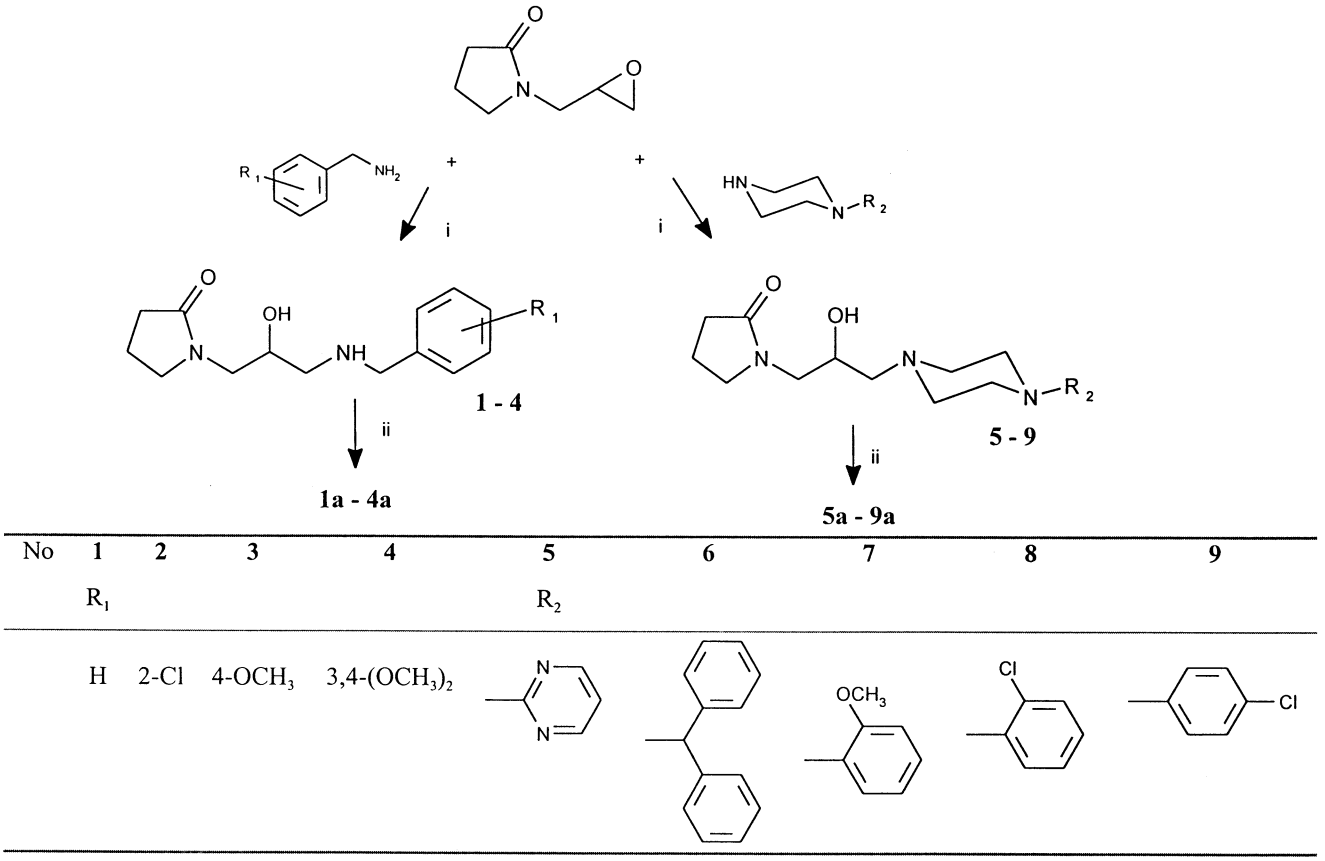
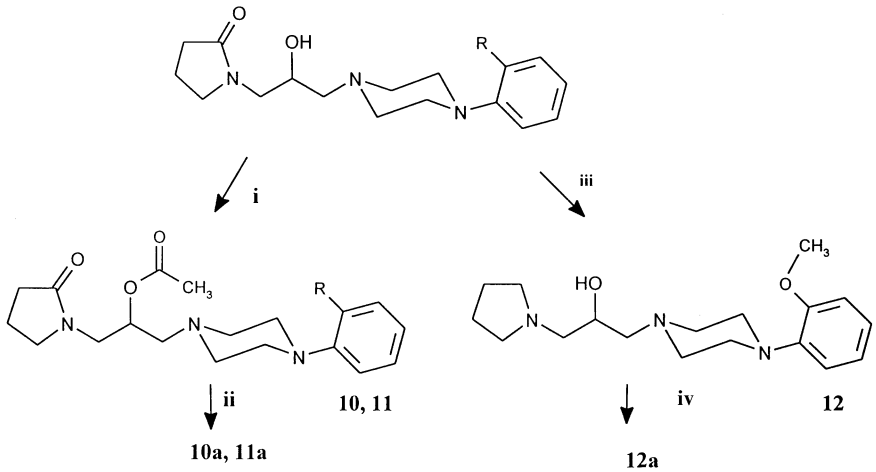


Fig. 2. Reagents and conditions: (i) *n*-propanol, reflux, 12 h; (ii) HCl, anhydrous ethanol.



No	10	11
R	-Cl	$-OCH_3$

Fig. 3. Reagents and conditions: (i) $(CH_3CO)_2O$, pyridine, 50 °C, 5 h; (ii) ethanol solution HCl gas; (iii) LAH, THF, room temperature; (iv) ethanol solution $(HCCOOH)_2$.

2 suggest that special attention should be paid to compound **11a**, which showed more profitable therapeutic index than **MG-1**. The other compounds possessed therapeutic index a little more profitable (**8a**) or worse (**7a**, **9a**, **10a**, **12a**) than that of reference compound. Compounds **7a**, **8a** and **11a** possessed prophy-

lactic antiarrhythmic properties also after oral application, but this effect was weaker than that given in the reference compound **MG-1**. These compounds administered 15 min before barium chloride reduced the number of premature ventricular beats, prevented in a statistically significant manner occurrence of ventricular fibrillation and protected the rats against fatal consequence of barium chloride administration. The ED_{50} (a dose producing a 50% inhibition of premature ventricular beats) and LD_{50}/ED_{50} are presented in Table 2. The ED_{50} values after intravenous administration were similar (**7a**), about 1.6 times lower (**8a**), or about 1.5 times higher (**11a**) than that of **MG-1**. Compounds **9a**, **10a** and **12a** did not change the occurrence of disturbances in this barium chloride-induced model of arrhythmia.

Compounds **7a**–**12a** were evaluated for therapeutic antiarrhythmic activity. Compounds **7a**, **8a** and **12a**, administered intravenously at the peak of adrenaline-induced arrhythmia converted the cardiac arrhythmias into regular sinus rhythms in more than 50% animals. The therapeutic antiarrhythmic activity data are shown in Table 3. From the data presented it follows that compounds **7a**, **8a** and **12a** were about 2–4 times less effective than that of reference compound. Similarly to **MG-1**, these compounds did not abolish cardiac arrhythmias caused by barium chloride intoxication.

Hypotensive activity of compounds **1a**–**12a** was determined after iv administration to normotensive anaesthetised rats. The results are presented in Fig. 4. The studied compounds injected intravenously in doses cor-

Table 1
Effects of an intravenous injection of the investigated compounds on heart rate and ECG intervals in anaesthetised male Wistar rats (60 mg thiopental kg^{-1} intraperitoneally)

Compound	Dose (mg kg^{-1})	Parameters	Time of observation (min)			
			0	1	5	15
6a	14	beats per minute	364.5 ± 10.5	320.2 ± 22	333.2 ± 29.5	325.2 ± 23.5
		P–Q (ms)	52.5 ± 2.5	55.0 ± 2.9	55.0 ± 2.9	65.0 ± 2.9 ***
	1/5 LD_{50}	QRS (ms)	17.0 ± 0.9	19.0 ± 0.6 *	18.5 ± 0.5	19.0 ± 0.6 *
		Q–T (ms)	70.0 ± 4.1	88.0 ± 4.8 ***	85.0 ± 2.9 **	88.0 ± 2.5 ***
7a	14.5	beats per minute	401.3 ± 13	347 ± 14	336 ± 21.7	327 ± 29.9 *
		P–Q (ms)	53.3 ± 3.3	63.3 ± 3.3	56.7 ± 3.4	63.3 ± 3.3
	1/10 LD_{50}	QRS (ms)	20.7 ± 0.7	20.7 ± 0.7	20.7 ± 0.7	20.7 ± 0.8
		Q–T (ms)	76.7 ± 3.4	83.3 ± 3.0	86.7 ± 3.3	83.3 ± 3.3
8a	16	beats per minute	378 ± 19.7	343.5 ± 10.5	378 ± 19.7	359.2 ± 27.8
		P–Q (ms)	62.5 ± 2.5	65.0 ± 2.9	62.5 ± 2.5	67.5 ± 4.8
	1/5 LD_{50}	QRS (ms)	19.5 ± 0.5	20.5 ± 0.5	20.5 ± 0.5	20.5 ± 0.5
		Q–T (ms)	75.0 ± 2.9	82.5 ± 2.5	85.0 ± 2.9 *	85.0 ± 2.9 *
9a	10	beats per minute	402 ± 15.6	384.2 ± 20.3	360.2 ± 16.5	345.7 ± 18 *
		P–Q (ms)	55.0 ± 2.9	55.0 ± 2.9	55.7 ± 3.3	61.2 ± 1.2
	1/10 LD_{50}	QRS (ms)	18.5 ± 0.5	18.5 ± 0.5	19.7 ± 0.6	20.2 ± 0.2 *
		Q–T (ms)	77.5 ± 2.5	83.0 ± 2.4	77.5 ± 2.5	82.7 ± 2.4
	20	beats per minute	388.8 ± 13.5	353.5 ± 8.6 *	354 ± 12.1 *	324.5 ± 5 ****
		P–Q (ms)	50.0 ± 4.1	55.0 ± 2.9	57.5 ± 7.5	62.5 ± 2.5
	1/5 LD_{50}	QRS (ms)	17.5 ± 0.9	24.0 ± 3.5	24.0 ± 3.5	24.0 ± 3.5
		Q–T (ms)	75.0 ± 2.9	88.0 ± 2.5 ***	90 ± 0.2 ****	88 ± 2.5 ***
10a	24	beats per minute	378 ± 20.1	343 ± 11	350 ± 19.6	351 ± 26.7
		P–Q (ms)	55.0 ± 3.0	55.0 ± 3.0	56.0 ± 3.2	58.0 ± 3.6
	1/5 LD_{50}	QRS (ms)	18.2 ± 0.6	18.5 ± 0.5	19.6 ± 0.7	19.6 ± 0.9
		Q–T (ms)	76.0 ± 3.0	86.0 ± 2.1 *	87.0 ± 2.2 *	87.0 ± 2.2 *
11a	35	beats per minute	364.5 ± 10.5	335.2 ± 15.4	367.5 ± 22.8	335.2 ± 15.4
		P–Q (ms)	50.2 ± 2.5	58 ± 2.5 ***	60 ± 0.2 ****	60 ± 0.2 ****
	1/10 LD_{50}	QRS (ms)	19.0 ± 0.6	20.5 ± 0.5	20.5 ± 0.5	20.5 ± 0.5
		Q–T (ms)	80.2 ± 0.2	82.5 ± 2.5	82.5 ± 2.5	85.0 ± 2.9

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

Table 2
Prophylactic antiarrhythmic activity in anaesthetised rats

Compound	Route	Adrenaline-induced arrhythmia		Barium chloride-induced arrhythmia	
		ED_{50} (mg kg^{-1})	LD_{50}/ED_{50}	ED_{50} (mg kg^{-1})	LD_{50}/ED_{50}
7a	iv	12 (9.7–14.8)	12.1	11 (7.9–15.3)	13.2
	po	151 (75.9–300.5)	5.3	–	–
8a	iv	2.7 (1.95–3.7)	29.6	7.1 (4.7–10.6)	11.3
	po	65.5 (32.5–132)	5.3	–	–
9a	iv	13.5 (7.9–22.9)	7.4	–	–
	po	–	–	–	–
10a	iv	8.8 (4.9–15.9)	13.6	–	–
	po	–	–	–	–
11a	iv	4.9 (2.5–6.9)	83.3	17.5 (10.3–29.7)	20
	po	110 (68.3–177)	6.7	–	–
12a	iv	12 (9.1–24.7)	12.1	–	–
	po	–	–	–	
MG-1	iv	7.6 (6.9–8.4)	21	11.3 (6.45–19.2)	14
	po	29.0 (21.3–39.4)	10	31.0 (18.8–51.1)	9
Propranolol	iv	1.05 (0.64–1.73)	37	–	–
	po	19.5 (14.5–26.1)	24	–	–
Quinidine	iv	8.7 (8.0–9.4)	6	–	–
	po	38.0 (33.6–42.9)	15.6	–	–

Each value was obtained from three experimental groups. Each group consisted of six animals. The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [17].

Table 3
Therapeutic antiarrhythmic activity in anaesthetised rats

Compound	Route of administration	Suppression of arrhythmia induced by adrenaline ED_{50} (mg kg^{-1})	Ratio LD_{50}/ED_{50}
7a	iv	9.5 (6.3–14.2)	15.3
8a	iv	10.5 (8.27–13.3)	7.6
12a	iv	21.0 (15.7–28.1)	6.9
MG-1	iv	5.2 (3.1–8.0)	31
Quinidine	iv	3.2 (1.7–6.0)	17

Each value was obtained from three experimental groups. Each group consisted of six animals. The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [17].

responding to 1/40–1/10 LD_{50} had no effect on the blood pressure (systolic and diastolic) (**1a**–**6a** and **12a**) or evoked directly after administration 44–15.6 (systolic) and 51–20.9% (diastolic) decreased in blood pressure, which persisted for ca. 10–60 min or more (**7a**). Their hypotensive effects were comparable to **MG-1** [5]. Compounds with a significant hypotensive effect after iv administration were also tested for hypotensive activity after intragastrically administration (**7a**–**11a**). Only compounds **7a**, **8a**, **9a** and **11a** given in a dose of 1/10 LD_{50}, significantly decreased the systolic (**7a**, **8a**, **9a**) and/or diastolic pressure (**7a**, **8a**, **9a**, **11a**) (Table 4).

To examine the mechanism of the hypotensive effects of these compounds, we studied their influence on the pressor responses to epinephrine, norepinephrine, methoxamine, tyramine and DMPP (Figs. 5–11). Compounds **6a**–**12a**, given intravenously in doses of 1/40–1/10 LD_{50}, significantly antagonised the pressor response elicited by epinephrine. As shown in Figs. 6 and 10, the most active compounds were **7a** and **11a** which blocked the pressor response up to dose 1/40 LD_{50}. Compounds **7a** (Fig. 6), **10a** (Fig. 9) (1/10 LD_{50}), and **11a** (Fig. 10) (1/40 LD_{50}) also statistically significant antagonised the pressor response to norepinephrine. Compounds **7a**, **8a**, **10a**, **11a** significantly reduced the pressor responses to methoxamine, tyramine and DMPP. Compound **6a** in a dose of 1/10 LD_{50} significantly increased the pressor response after methoxamine.

Binding studies presented in Table 5. Compounds **6a**–**12a** displaced [^{3}H]prazosin from cortical binding sites in low concentration range ($K_i = 0.27$–2.9 μM).

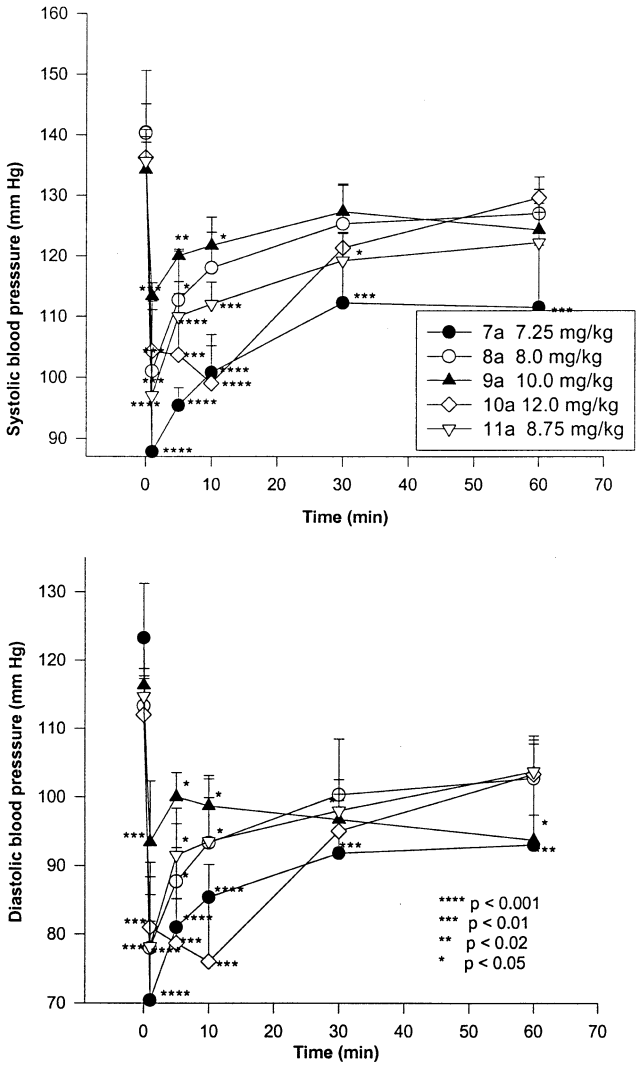


Fig. 4. Hypotensive activity of tested compounds in anesthezised normotensive rats.

The shape of the curves suggested competitive binding. Compounds **6a**–**8a** and **10a**–**12a** also displaced [^{3}H]clonidine from its binding sites in μM concentrations ($K_i = 5.3–80$). Only compound **8a** weakly displaced [^{3}H]-CGP$_{12177}$ from cortical binding sites.

The acute toxicity of the investigated compounds **1a**–**12a** was determined in mice after intravenous or intragastrically administration according to Litchfield and Wilcoxon [17], the LD_{50} values are presented in Table 6.

4. Discussion

In previous studies, we have demonstrated that the compound **MG-1** reduced significantly the blood pressure in normotensive and hypertensive anaesthetised rats, and reversed the pressor response to adrenaline. Preliminary pharmacological assessment revealed that derivatives of 3-[(4-arylpiperazin-1-yl)-propyl]pyrrolidin-2-one which contain *ortho*-chloro, *ortho*-methoxy or *para*-chloro substituent in the phenyl ring (**7a**–**11a**), similarly to **MG-1** displayed strong antiarrhythmic and antihypertensive activity. The replacement of the arylpiperazine ring with the benzylamine, substituted benzylamine group (**1a**–**4a**) or the phenylpiperazine ring with pirimidinepiperazine (**5a**) or diphenylmethylpiperazine (**6a**) group led to the lack of activity. Compound **12a**, obtained by reduction of **7** exhibited prophylactic and therapeutic antiarrhythmic activity in the adrenaline induced model of arrhythmia. The pyrrolidine derivative **12a** had no effect on the blood pressure. In order to further exploration structure–activity relationship, acylation of the hydroxy group in compounds **7** and **8** was effected to gave compounds **10a** and **11a**. These acylated derivatives showed both antiarrhythmic and hypotensive effects. Compound **11a** showed enhanced activity (about seven times higher) in comparison to the parent compounds when tested in the prophylactic antiarrhythmic activity. Among the hypotensive active derivatives of pyrrolidin-2-one **7a**–**11a**, compounds **7a** and **11a** with *ortho*-methoxyphenylpiperazine substituent were the most potent.

It is generally accepted that α_1-antagonist reversed the pressor response to epinephrine, depressed the pressor effect of methoxamine, tyramine and DMPP and potentiated the pressor response to norepinephrine, while α_2-antagonists antagonised the hypertensive effect of norepinephrine, reversed the hypertension induced by epinephrine, no changed or potentiated the pressor response to tyramine or DMPP [18–20]. Some of investigated compounds significantly antagonised the pressor response to epinephrine (**6a**–**12a**), norepinephrine (**7a**, **10a**, **11a**), methoxamine (**7a**–**11a**), tyramine and DMPP (**7a**–**11a**) or no changed the pressor responses to norepinephrine.

These in vivo experiments with a non-selective agonist of α_1 and α_2 such as epinephrine or norepinephrine and a selective agonist of α_1-adrenoceptor such as methoxamine suggest in agreement with previous results that hypotensive effects of the compounds **7a**–**11a** were related to their adrenolytic properties.

Compounds **6a**–**12a** were identified as ligands for the α_1-adrenoceptors. When the *ortho*-chloro or *ortho*-methoxy group was introduced into the phenyl ring of **MG-1**, the affinity increased, in agreement with reports showing that the introduction of these two substituents to the *ortho* position of the phenyl ring of the phenylpiperazine may produce potent α_1-adrenoceptor antagonists [21,22]. Compound **10a** was the most potent and selective compound in this series with 12-fold greater affinity than **MG-1**. Investigated compounds displayed lower affinity for α_2-adrenoceptors than for α_1-adrenoceptors. The α_2/α_1-selectivity ratio of compounds **7a**, **8a**, **10a** and **11a** were higher than that of

Table 4
Hypotensive activity of tested compounds in anaesthetised normotensive rats after orally administration in the dose corresponding

Compound	Dose (mg kg^{-1})	Blood pressure (mmHg ± S.E.M)	Before	Time of observation (min)		
				10	30	60
7a	80.0 po	systolic	147.3 ± 9.1	96.7 ± 9.2 **	97.0 ± 12.1 **	118.8 ± 17.0
		diastolic	123.3 ± 13.6	64.7 ± 3.2 ***	73.7 ± 15.5 **	90.3 ± 18.3
8a	34.8 po	systolic	132.4 ± 3.3	119.8 ± 3.1 *	124.0 ± 2.8	126.5 ± 3.5
		diastolic	111.0 ± 4.5	97.0 ± 2.2 *	99.2 ± 4.0	103.2 ± 4.3
9a	59.5 po	systolic	136.2 ± 2.0	127.4 ± 5.7	19.8 ± 4.0 **	127.6 ± 5.5
		diastolic	106.6 ± 4.6	95.0 ± 5.3 **	91.6 ± 2.2 ***	93.6 ± 3.6 *
10a	40.0 po	systolic	143 ± 1.4	140 ± 4.2	134 ± 2.8	129.0 ± 8.6
		diastolic	115 ± 8.2	112 ± 4.2	108 ± 8.3	115.0 ± 4.2
11a	73.3 po	systolic	150.0 ± 1.2	128.0 ± 12.0	108.3 ± 13.1	117.7 ± 10.3
		diastolic	134.7 ± 3.3	115.3 ± 9.9	93.7 ± 12.4 *	97.0 ± 12.3 *

The data were the means of 5–6 experiments ± S.E.M. Statistical analyses were performed using a one-way ANOVA test.
* $P<0.05$;
** $P<0.02$;
*** $P<0.01$.

MG-1. These investigations demonstrated that new derivatives of 1-[3-(4-phenylpiperazin-1-yl)propyl]-pyrrolin-2-one with *ortho*-substituent in the phenyl ring displayed higher affinity than **MG-1** for α_1-adrenoceptors.

5. Conclusions

In summary, we have synthesised several new analogues of **MG-1** with potent antiarrhythmic activity on adrenaline-induced arrhythmia (**7a**–**12a**) and hypotensive activity (**7a**–**11a**). Compound **11a** was the most potent in this series. The pharmacological results and binding studies suggested that the antiarrhythmic and hypotensive effects of these compounds were related to their adrenolytic properties, and those properties were due to the presence of the 1-phenylpiperazine moiety with a methoxy- or chloro- substituent in the *ortho* position in the phenyl ring. The mode of action and activity of compounds **7a**–**11a** were promising enough to continue further experiments.

6. Experimental

6.1. Chemistry

M.p. were determined in open glass capillaries on a Büchi 353 m.p. apparatus and are uncorrected. Elemental analyses (C, H, N) were determined within 0.4% of

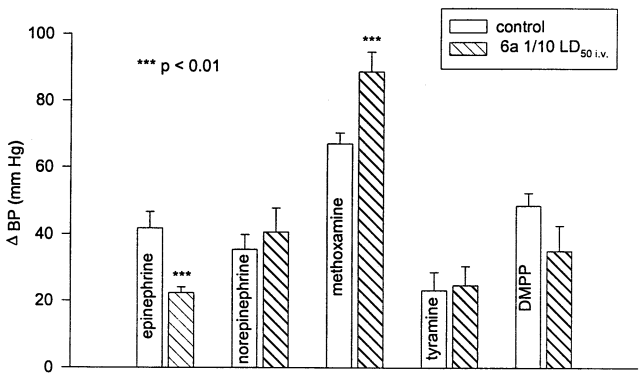


Fig. 5. The effect of **6a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

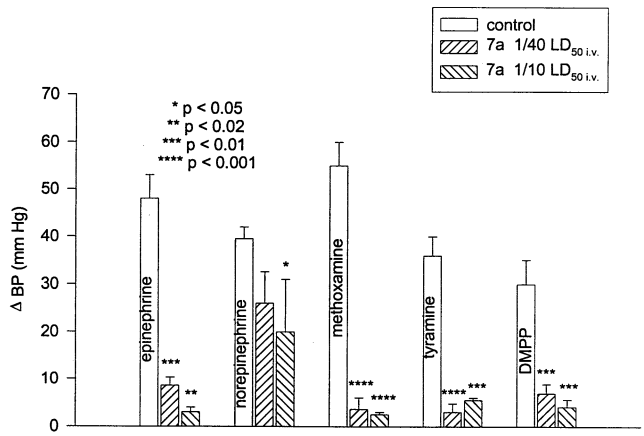


Fig. 6. The effect of **7a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

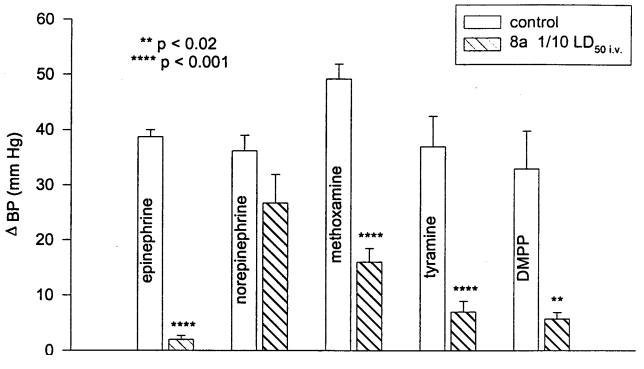


Fig. 7. The effect of **8a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

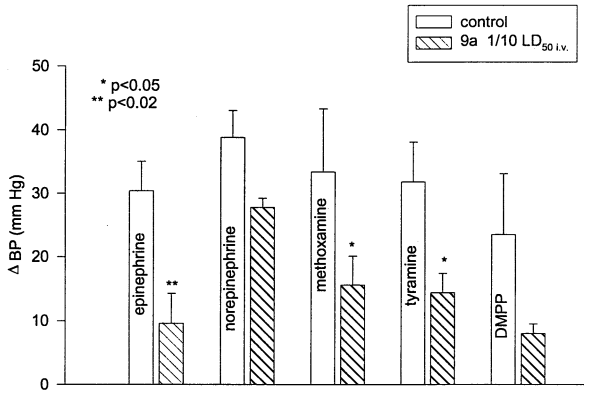


Fig. 8. The effect of **9a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

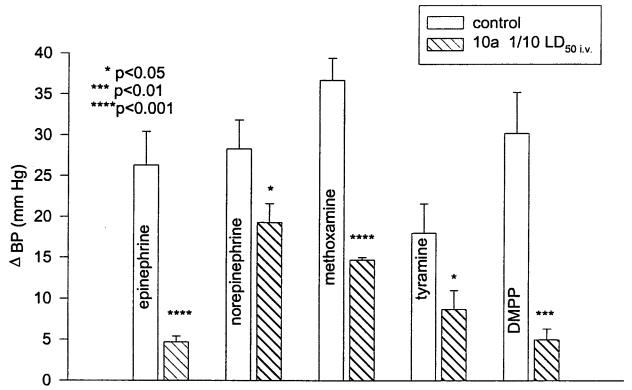


Fig. 9. The effect of **10a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

theoretical values. ^{1}H-NMR spectra were recorded on a Bruker spectrometer at 300 MHz in $CDCl_3$ or in [d_6]-DMSO with tetramethylsilane (TMS) as an internal standard. The mass spectra were taken at 70 eV with an LKB 2091 GCMS spectrometer. Thin-layer chromatography (TLC) was performed on Merck precoated (0.20 mm) silica gel 60 F_{254} plates, using: S_1-$CHCl_3$–C_3H_6O (1:1); S_2-$CHCl_3$–MeOH–AcOH (60:10:5); S_3-n-C_6H_{14}:anhydrous EtOH:Et_3N (7:2:1) or S_4-MeOH–25% NH_3 (98:2) as developing system. The plates were visualised with UV light or iodine solution (0.05 M in 10% HCl). Column chromatography was performed on silica gel 60 (70–230 mesh ASTM) using S_3 or S_4 as eluent.

*6.1.1. General procedure for the synthesis of 1-(2-hydroxy-3-substituted aminopropyl)pyrrolidin-2-one (**1a**–**9a**)*

A solution of 1.4 g (10 mmol) of 1-(2,3-epoxypropyl)pyrrolidin-2-one and 10 mmol of the corresponding amine in *n*-propanol (40 mL) was heated under reflux for 12 h. After evaporating the solvent, the residue was dissolved in EtOAc and cooled down. In the case of compounds **5**, **7**, **9** which crystallised in this condition, the further purification was done by crystallisation from a mixture of n-C_6H_{14}:acetyl acetate (1:5) (compound **7**) or by column chromatography using S_3 (**5**, **9**) as a solvent. The other compounds were purified by column chromatography using S_3 or S_4 as a

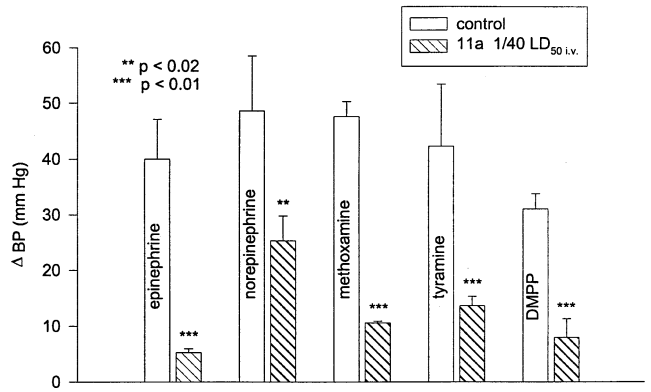


Fig. 10. The effect of **11a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

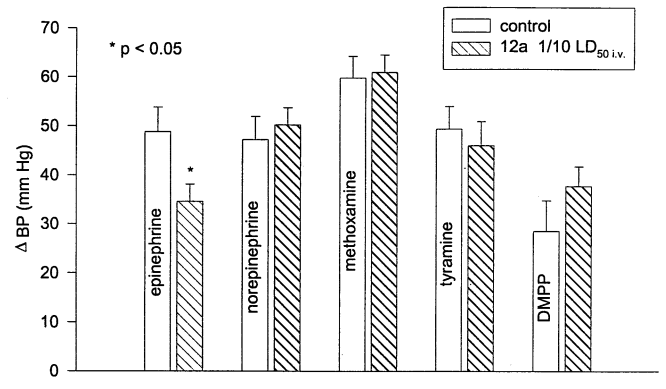


Fig. 11. The effect of **12a** on the blood pressure response to epinephrine, norepinephrine, methoxyamine, tyramine and DMPP. All values represent the mean ± S.E.M. from six rats.

Table 5
Affinity towards different α-adrenoceptor subtypes in rat cerebral cortex

Compound	[^{3}H]Prazosin (α_1 rec.) K_i (μM)	[^{3}H]Clonidine (α_2 rec.) K_i (μM)	[^{3}H]-CGP$_{12177}$ (β rec.) K_i (μM)	Ratio α_2/α_1
5a	>100	>100	>100	–
6a	0.27	5.3	ND	20
7a	1.3	40.5	>100	31
8a	0.27	16.2	54	60
9a	2.6	>100	>100	–
10a	0.16	11.2	ND	70
11a	1.3	80	>100	62
12a	2.9	16.9	>100	6
MG-1	1.9	29	>100	15

ND, not determined; each K_i value was obtained from three experiments.

solvent. The obtained oils were dissolved in anhydrous EtOH and then EtOH saturated with HCl gas was added until the mixture become acidic.

*6.1.1.1. 1-[3-(Benzylamino)-2-hydroxypropyl]pyrrolidin-2-one hydrochloride (**1a**).* Yield: 60.25%. Anal. Calc. for $C_{14}H_{20}N_2O_2$·HCl; M_r 283.83; m.p. 247–248 °C; TLC: 0.08 (S_1), 0.24 (S_2), 0.10 (S_4); MS (70 eV), *m/z* (70 eV) 248 [M^+] (0.34), 230 (0.53), 167 (0.18), 106 (0.50), 101 (24.71), 86 (100), 72 (4.07), 58 (35.31), 56 (4.59), 44 (10.85), 42 (7.46), 38 (6.42), 36 (19.10); ^{1}H-NMR ([d_6]-DMSO): $\delta = 1.99–2.14$ (m, $CH_2CH_2CH_2$, 2H), 2.32–2.40 (m, CH_2CO, NCH_2CH, 4H), 3.16–3.24 (m, $CHCH_2NH$, CH_2 benzyl, 4H), 3.51–3.58 (m, CH_2CH_2N, C*H*, 3H), 3.91 (s, O*H*, 1H), 5.28 (s, N*H*, 1H), 7.06–7.34 (m, arom., 5H).

*6.1.1.2. 1-[3-(2-Chlorobenzylamino)-2-hydroxypropyl]-pyrrolidin-2-one hydrochloride (**2a**).* Yield: 68.32%. Anal. Calc. for $C_{14}H_{19}ClN_2O_2$·HCl; M_r 319.23; m.p. 141.7–142.8 °C; TLC: 0.26 (S_1), 0.31 (S_2); MS (70 eV), *m/z* (%): 285 (0.3) [$M^+ + 2$], 283 (0.6) [M^+], 264 (1.75), 216 (0.84), 203 (3.10), 184 (4.04), 180 (10.12), 154 (50.04), 139 (95.30), 125 (100), 98 (16.01), 86 (21.83), 77 (3.63), 70 (5.90), 36 (17.24); ^{1}H-NMR ([d_6]-DMSO): $\delta = 1.95–2.09$ (m, $CH_2CH_2CH_2$, 2H), 2.32–2.40 (m, CH_2CO, NCH_2CH, 4H), 3.04–3.09 (d, $CHCH_2NH$, 2H), 3.26–3.32 (d, CH_2 benzyl, 2H), 3.51–3.58 (m, CH_2CH_2N, C*H*, 3H), 4.11 (s, O*H*, 1H) 5.36 (s, N*H*, 1H), 7.26–7.91 (m, arom., 4H).

*6.1.1.3. 1-[2-Hydroxy-3-(4-methoxybenzylamino)-propyl]pyrrolidin-2-one hydrochloride (**3a**).* Yield: 59.23%. Anal. Calc. for $C_{15}H_{22}N_2O_3$·HCl; M_r 314.81; m.p. 159.1–160.6 °C; TLC: 0.38 (S_1), 0.35 (S_2); MS (70 eV), *m/z* (%): 278 (0.44) [M^+], 260 (1.92), 162 (6.04), 157 (2.77), 150 (5.35), 139 (24.03), 136 (28.54), 128 (9.20), 121 (100), 98 (5.03), 91 (2.98), 86 (4.35), 77 (4.10), 70 (2.83), 36 (5.47); ^{1}H-NMR ([d_6]-DMSO: $\delta =$ 1.93–2.08 (qw, $CH_2CH_2CH_2$, 2H), 2.33–2.40 (m, CH_2CO, NCH_2CH, 4H), 3.19–3.22 (d, CH_2 benzyl, 2H), 3.23–3.26 (d, $CHCH_2NH$, 2H), 3.40–3.57 (m, CH_2CH_2N, C*H*, 3H), 3.73 (s, O*H*, 1H), 4.11 (s, OCH_3, 3H), 5.65 (s, N*H*, 1H), 6.87–6.93 (t, arom., 2H), 7.51–7.57 (t, arom., 2H).

*6.1.1.4. 1-[2-Hydroxy-3-(3,4-dimethoxybenzylamino)-propyl]pyrrolidin-2-one hydrochloride (**4a**).* Yield: 67.85%. Anal. Calc. for $C_{16}H_{24}N_2O_4$·HCl; M_r 344.84; m.p. 150–153 °C; TLC: 0.24 (S_2), 0.10 (S_3); MS (70 eV), *m/z* (%): 308 (2.39) [M^+], 209 (2.31) [$M^+ - H_2O$], 192 (8.48), 166 (37.80), 151 (100), 139 (21.93), 136 (25.97), 128 (7.71), 124 (7.13), 107 (8.89), 98 (4.29), 86 (5.70), 77 (4.35), 36 (10.68); ^{1}H-NMR ([d_6]-DMSO):

Table 6
Acute toxicity of the investigated compounds, according to Litchfield and Wilcoxon [17] *in mice*

Compound	Route	LD_{50} mg kg^{-1}
1a	iv	340 (315–367)
2a	iv	175 (160.6–190.7)
3a	iv	124 (96.8–158.7)
4a	iv	150 (133–169)
5a	iv	310 (269.5–356.5)
6a	iv	71 (60.7–83.1)
7a	iv	145 (118.8–176.9)
	po	800 (710–920)
8a	iv	80 (61.5–104)
	po	348 (274–442)
9a	iv	100 (76–132)
	po	595 (476–743.7)
10a	iv	120 (100.2–137.4)
	po	400 (312–476)
11a	iv	350 (324–384)
	po	733 (632–850)
12a	iv	145 (126–187.2)
	po	700 (610–897)
MG-1	iv	132 (102.3–170.3)
	po	290 (238.9–368.0)
Propranolol	iv	22
	po	471 (448–503)
Quinidine	iv	66.9 (63.6–70.3)
	po	594.0 (511–677)

The data are median lethal doses with 5% confidence limits in parentheses.

$\delta = 1.98–2.09$ (qw, $CH_2CH_2CH_2$, 2H), 2.32–2.40 (m, CH_2CO, NCH_2CH, 4H), 3.23–3.26 (m, $CHCH_2NH$, CH_2 benzyl, 4H), 3.50–3.53 (m, CH_2CH_2N, CH, 3H), 3.81 (s, OH, 1H), 3.86 (s, OCH_3, 3H), 3.94 (s, OCH_3, 3H), 5.58 (s, NH, 1H), 6.81–6.85 (d, arom., $J_{H–H} = 8.18$ Hz, 1H), 7.04–7.08 (d, arom., $J_{H–H\ arom.} = 8.18$ Hz, 1H), 7.35 (s, 1H, arom.).

*6.1.1.5. 1-[2-Hydroxy-3-[4-(2-pyrimidinyl)piperazin-1-yl]propyl]pyrrolidin-2-one (**5**).* Yield: 78.36%. Anal. Calc. for $C_{15}H_{23}N_5O_2$; M_r 305.38; m.p. 93.0–94.7 °C; TLC: 0.20 (S_2), 0.63 (S_4); MS (70 eV), *m/z* (%): 276 (2.61), 258 (0.81), 237 (0.73), 208 (0.72), 177 (100), 150 (0.63), 147 (29.39), 137 (1.39), 133 (4.84), 122 (33.22), 120 (7.39), 108 (6.85), 106 (2.39), 94 (1.06), 79 (5.83); ^{1}H-NMR ($CDCl_3$): $\delta = 1.93–2.06$ (qw, $CH_2CH_2CH_2$, 2H), 2.17–2.25 (m, CH_2CO, NCH_2CH, 4H), 3.17–3.20 (m, $CHCH_2NH$, CH_2 piper., 6H), 3.40–3.58 (m, CH_2CH_2N, CH, CH_2 piper., 7H), 3.79 (s, OH, 1H), 6.04–7.27 (m, arom., 3H); ^{13}C-NMR ($CDCl_3$): $\delta =$ 40.83, 52.64, 54.19 (CH_2 pyrrol.); 56.64, 69.53, 62.36 (propyl), 54.21, 62.36 (piper.), 111.53, 121.99, 129.23 (pyrimid.), 157.90 (carbonyl).

*6.1.1.6. 1-[2-Hydroxy-3-[4-(2-pyrimidinyl)piperazin-1-yl]propyl]pyrrolidin-2-one hydrochloride (**5a**).* Anal. Calc. for $C_{15}H_{23}N_5O_2 \cdot 2HCl$; M_r 378.38; m.p. 150.9–152.6 °C.

*6.1.1.7. 1-[2-Hydroxy-3-[4-(diphenylmethyl)piperazin-1-yl]propyl]pyrrolidin-2-one hydrochloride (**6a**).* Yield: 64.24%. Anal. Calc. for $C_{24}H_{31}N_3O_2 \cdot 2HCl$; M_r 466.45; m.p. 238.1–239.6 °C; TLC: 0.11 (S_2), 0.57 (S_3); MS (70 eV), *m/z* (%): 393 (5.35) [M^+], 251 (1.21), 226 (0.67), 208 (12.78), 194 (4.56), 167 (100), 152 (7.22), 142 (3.59), 128 (1.89), 98 (6.39), 77 (0.73), 70 (2.98), 36 (52.18); ^{1}H-NMR ([d_6]-DMSO): $\delta = 1.86–1.93$ (qw, CH_2CH_2-CH_2, 2H), 2.17–2.25 (m, CH_2CO, NCH_2CH, 4H), 2.45–2.58 (m, CH_2 piper., 4H), 3.19–3.22 (m, $CHCH_2NH$, CH_2 piper., 6H), 3.38–3.45 (m, CH_2CH_2-N, CH, 3H), 3.78 (s, OH, 1H), 4.21 (s, CH, 1H), 7.29–7.94 (m, arom., 10H); ^{13}C-NMR ([d_6]-DMSO): $\delta = 17.71$, 25.51, 38.25 (CH_2 pyrrol.), 30.31 (NCH_2CH), 39.34 ($CHOHCH_2N$), 39.91 (CH_2-$CHOHCH_2$), 40.75 (CH_2 piper.), 47.84 (CH_2 piper.), 63.55 (CH), 128.33, 128.82, 192.32, 136.15 (arom.), 174.79 (carbonyl).

*6.1.1.8. 1-[2-Hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one (**7**).* Yield: 76.26%. Anal. Calc. for $C_{18}H_{27}N_3O_3$; M_r 333.43; m.p. 97.7–95.6 °C; TLC: 0.37 (S_2), 0.32 (S_3); MS (70 eV), *m/z* (%): 333 (6.88) [M^+], 315 (10.17), 206 (21.16), 205 (100), 190 (25.14), 162 (9.15), 133 (4.09), 120 (4.87), 98 (3.49), 69 (19.93); ^{1}H-NMR ($CDCl_3$): $\delta = 1.99–2.11$ (qw, $CH_2CH_2CH_2$, 2H), 2.36–2.46 (m, CH_2CO, NCH_2CH, 4H), 2.61–2.66 (m, CH_2 piper., 4H), 2.78–2.88 (m, CH_2 piper., 4H), 3.08 (s, OCH_3, 3H), 3.15–3.26 (dd, $CHCH_2N$, 2H), 3.44–3.45 (d, OH, 1H), 3.51–3.63 (m, CH_2CH_2N, CH, 3H), 6.84–7.00 (m, arom., 4H); ^{13}C-NMR ($CDCl_3$): $\delta = 18.14$, 30.78, 49.29 (CH_2 pyrrol.), 46.98 (NCH_2CH), 50.63 ($CHOHCH_2N$), 66.05 ($CH_2CHOHCH_2$), 53.35 (CH_2 piper.), 55.35 (CH_2 piper.), 61 (OCH_3), 111.18, 118.09, 120.88, 141.03, 152.18 (arom.), 175.76 (carbonyl).

*6.1.1.9. 1-[2-Hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one hydrochloride (**7a**).* Yield: 76.26%. Anal. Calc. for $C_{18}H_{27}N_3O_3 \cdot 2HCl$; M_r 405.99; m.p. 208–209 °C.

*6.1.1.10. 1-[3-[4-(2-Chlorophenyl)piperazin-1-yl]-2-hydroxypropyl]pyrrolidin-2-one hydrochloride (**8a**).* Yield: 76.32%. Anal. Calc. for $C_{17}H_{24}N_3O_2Cl$; M_r 374.31; m.p. 202.5–203.9 °C; TLC: 0.46 (S_2), 0.77 (S_3); MS (70 eV), *m/z* (%): 339 (0.47) [$M^+ + 2$], 337 (1.32) [M^+], 319 (6.24), 221 (1.41), 211 (31.37), 209 (100), 194 (8.10), 181 (0.92), 171 (6.36), 166 (15.75), 138 (5.46), 128 (2.39), 111 (1.54), 98 (4.71), 70 (32.12), 36 (6.00); ^{1}H-NMR ([d_6]-DMSO): $\delta = 1.89–2.19$ (qw, $CH_2CH_2CH_2$, 2H), 2.23–2.50 (m, CH_2CO, NCH_2CH, 4H), 2.51 (m, CH_2 piper., 4H), 3.07–3.47 (m, CH_2, piper., $CHCH_2N$, OH, 7H), 3.57–3.70 (m, CH_2N, CH, 3H), 7.00–7.40 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): $\delta = 17.69$, 25.47, 40.33 (CH_2 pyrrol.), 38.24 (NCH_2CH), 47.54 ($CHOHCH_2N$), 63.30 ($CH_2CHOHCH_2$), 51.33 (CH_2 piper.), 54.06 (CH_2 piper.), 120.95, 124.76, 127.51, 128.25, 130.45, 147.43 (arom.), 174.58 (carbonyl).

*6.1.1.11. 1-[3-[4-(Chlorophenyl)piperazin-1-yl]-2-hydroxypropyl]pyrrolidin-2-one hydrochloride (**9**).* Yield: 60.53%. Anal. Calc. for $C_{17}H_{24}N_3O_2Cl$; M_r 337.85; m.p. 106.8–108.0 °C; TLC: 0.50 (S_2), 0.41 (S_3); MS (70 eV), *m/z* (%): 339 (2.95) [$M^+ + 2$], 337 (8.70) [M^+], 319 (6.14), 221 (1.80), 211 (32.51), 209 (100), 194 (7.75), 171 (20.42), 168 (10.16), 166 (23.07), 142 (2.81), 138 (10.98), 128 (5.49), 125 (2.89), 111 (4.63), 98 (7.68), 70 (54.37), 56 (6.84), 42 (7.05); ^{1}H-NMR ($CDCl_3$): $\delta = 2.00–2.11$ (qw, $CH_2CH_2CH_2$, 2H), 2.31–2.44 (m, CH_2CO, NCH_2CH, 4H), 2.43–2.61 (m, CH_2 piper., 4H), 2.73–2.83 (m, CH_2 piper., 4H), 3.22–3.44 (dd, $CHCH_2N$, 2H), 3.44–3.48 (d, OH, 1H), 3.51–3.64 (m, CH_2N, CH, 3H), 6.78–7.27 (m, arom., 4H); ^{13}C-NMR ($CDCl_3$): $\delta = 18.15$, 30.77, 49.13 (CH_2 pyrrol.), 46.93 (NCH_2CH), 49.33 ($CHOHCH_2N$), 66.20 (CH_2CHO HCH_2), 52.98 (CH_2 piper.), 61.13 (CH_2 piper.), 117.15, 124.50, 128.83, 149.66 (arom.), 175.73 (carbonyl).

*6.1.1.12. 1-[3-[4-(Chlorophenyl)piperazin-1-yl]-2-hydroxypropyl]pyrrolidin-2-one hydrochloride (**9a**).* Anal. Calc. for $C_{17}H_{24}N_3O_2Cl \cdot 2HCl$; M_r 410.85; m.p. 227.9–229.1 °C.

6.1.2. Synthesis of 1-[2-acetoxy-3-(4-arylpiperazin-1-yl)propyl]pyrrolidin-2-one ***10***, ***11***

6.1.2.1. General procedure. Five millimol of compound **7** or **8** was added to mixture of 10 mL Ac_2O and 1 mL of (10 mmol) Py. The mixture was heated under reflux at 50 °C for 5 h and then the solvents were evaporated under vacuum. The crude product was purified by column chromatography using S_4 as a solvent. The obtained oil was dissolved in anhydrous EtOH and then EtOH saturated with HCl gas was added until the mixture become acidic.

6.1.2.2. 1-[2-Acetoxy-3-[4-(2-chlorophenyl)piperazin-1-yl]propy]pyrrolidin-2-one hydrochloride ***(10a)***. Yield: 76.32%. Anal. Calc. for $C_{19}H_{26}N_3O_3Cl{\cdot}2HCl$; M_r 452; m.p. 202.5–203.948 °C; TLC: 0.37 (S_2), 0.73 (S_3); MS (70 eV), m/z (%): 381 (0.31) [$M^+ + 2$], 379 (0.31) [M^+], 363 (2.27), 319 (12.43), 239 (4.82), 211 (31.97), 209 (100), 194 (8.16), 184 (3.76), 171 (4.23), 168 (5.50), 166 (14.92), 154 (2.13), 138 (5.53), 124 (3.23), 111 (1.38), 98 (4.27), 77 (1.24), 70 (18.62), 56 (2.55), 36 (3.76); ^{1}H-NMR ($CDCl_3$): $\delta = 2.00$–2.11 (qw, $CH_2CH_2CH_2$, 2H), 2.18 (s, CH_3, 3H), 2.34–2.42 (m, CH_2CO, NCH_2CH, 4H), 2.94–3.64 (m, CH, CH_2CH_2N, CH_2 piper., 11H), 3.69–3.80 (m, $CHCH_2N$, 2H), 7.02–7.36 (m, arom., 4H).

6.1.2.3. 1-[2-Acetoxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrrolidin-2-one hydrochloride ***(11a)***. Yield: 76.32%. Anal. Calc. for $C_{20}H_{29}N_3O_4{\cdot}2HCl$; M_r 429.94; m.p. 176.6–178.2 °C; TLC: 0.52 (S_1), 0.67 (S_2), 0.75 (S_3); MS (70 eV), m/z (%): 375 [M^+] (9.48), 316 (4.78), 315 (20.58), 217 (4.66) 205 (100), 190 (25.74), 162 (16.75), 134 (6.28), 124 (5.75), 120 (7.37), 98 (4.78), 70 (21.93), 56 (5.66); ^{1}H-NMR ($CDCl_3$): $\delta = 1.86$–2.18 (qw, $CH_2CH_2CH_2$, 2H), 2.18 (s, CH_3CO, 3H), 2.22–2.26 (m, CH_2CO, NCH_2CH, 4H), 3.05–3.59 (m, CH, CH_2CH_2N, CH_2 piper., 11H), 3.67–3.79 (m, $CHCH_2N$, 2H), 3.79 (s, OCH_3, 3H), 6.86–7.10 (m, arom., 4H); ^{13}C-NMR ($CDCl_3$): $\delta = 17.75$, 30.32, 47.76 (CH_2 pyrrol.), 38.26 (NCH_2CH), 46.30 ($CHOHCH_2N$), 63.43 ($CH_2CHOHCH_2$), 51.18 (CH_2 piper.), 52.14 (CH_2 piper.), 55.54 ($COCH_3$), 59.16 (OCH_3), 112.25, 118.72, 120.93, 124.30, 138.34 (arom.), 151.90 (carbonyl ester) 174.20 (carbonyl).

6.1.3. Synthesis of 1-[2-hydroxy-3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrrolidine ***(12a)***

A suspension of 0.57 g (15 mmol) of LAH in dry THF (40 mL) was stirred at room temperature for 10 min. Then a solution of 1.16 g (5 mmol) of **7** in THF was added dropwise. The mixture was stirred overnight. The reaction was quenched by adding EtOAc and water. The organic phases were collected, dried with Na_2SO_4 and evaporated under vacuum. The obtained residue was purified by column chromatography using S_3 as a solvent. Free base was dissolved in anhydrous EtOH, and a solution of maleic acid in EtOH was added until the mixture become acidic.

Yield: 52.01%. Anal. Calc. for $C_{18}H_{29}N_3O_2$; M_r 435.45; m.p. 246–247 °C; TLC: 0.62 (S_4); MS (70 eV), m/z (%): 319 (7.71) [M^+], 239 (4.41), 212 (4.14), 209 (100), 196 (3.01), 194 (7.40), 168 (5.46), 166 (15.37), 138 (6.42), 98 (6.31), 79 (27.85), 72 (9.75), 70 (24.58), 52 (16.52); ^{1}H-NMR ([d_6]-DMSO): $\delta = 1.96$–2.03 (qw, CH_2CH_2, 4H), 2.41–2.75 (t, CH_2N, CH_2N, 4H), 2.97–3.33 (m, CH, OCH_3, NCH_2CH, CH_2 piper., 14H) 3.78–3.85 (d, $CHCH_2N$, 2H), 4.30 (s, OH, 1H) 6.83–7.28 (m, arom., 4H); ^{13}C-NMR ([d_6]-DMSO): $\delta = 22.49$, 47.41 (pyrrol.); 38.25 (NCH_2CH), 46.16 ($CHOHCH_2N$), 61.62 ($CH_2CHOHCH_2$), 52.07 (CH_2 piper.), 53.82 (CH_2 piper.), 59.14 (OCH_3); 111.96, 115.73, 118.21, 119.63, 120.87, 129.08 (arom.).

6.2. Pharmacology

6.2.1. Materials and methods

6.2.1.1. Compounds. Barium chloride (POCh, Poland), [^{3}H]Clonidine (Amersham), DMPP (dimethylphenylpiperazine, Sigma, Aldrich Chemie Gmbh), epinephrine (Adrenalinum hydrochloricum, Polfa), [^{3}H]CGP_{12177} (NEN), norepinephrine (Levonor, Polfa), methoxamine (Sigma, Aldrich Chemie Gmbh), [^{3}H]Prazosin (Amersham), tyramine (Sigma, Aldrich Chemie Gmbh), sodium heparin (Polfa), thiopental sodium (Biochemie Gmbh, Vienna).

6.2.1.2. Animals. The experiments were carried out on male albino Swiss mice (18–25 g) and male Wistar rats (180–250 g). Animals were housed in wire mesh cages in room at 20 ± 2 °C with natural light–dark cycles. The animals had free access to standard pellet diet and water, and were used after a minimum of 3 days acclimatisation to the housing conditions. Control and experimental groups consisted of 8–10 animals each.

6.2.1.3. Reference compound. **MG-1** was used as a reference compound.

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

6.2.2. The effect on normal electrocardiogram

Electrocardiographic investigations were carried out using Multicard 30 apparatus, standard lead II and paper speed of 50 mm s^{-1}. The tested compounds were administered intraperitoneally or intravenously in the

dose corresponding to 1/10–1/5 LD_{50} iv. The ECG record was made just before and 1.5 and 15 min after administration of compounds.

6.2.3. Antiarrhythmic activity

6.2.3.1. Prophylactic effect

Adrenaline-induced arrhythmia according to Szekeres [16]. The arrhythmia was evoked in rats anaesthetised with thiopental (60 mg kg^{-1}, ip) by iv injection of adrenaline (20 μg kg^{-1}). The tested compounds were administered intravenously 15 min or orally 1 h before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and inhibition of cardiac arrhythmia in comparison with the control group.

Barium chloride-induced arrhythmia according to Szekeres [16]. Barium chloride solution was injected into the caudal vein of rats (32 mg kg^{-1}, in a volume of 1 mL kg^{-1}). The tested compounds were given iv 15 min or po 1 h before arrhythmogen. The criterion of antiarrhythmic activity was a gradual disappearance of the arrhythmia and restoration of the sinus rhythm.

6.2.3.2. Therapeutic effect. The tested compound was administered intravenously at the peak of arrhythmia, immediately after administration of adrenaline or barium chloride.

6.2.4. Influence on the blood pressure

Male Wistar normotensive rats were anaesthetised with thiopental (50–75 mg kg^{-1}) by intraperitoneally injection. The right carotid artery was cannulated with polyethylene tub filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments). The studied compounds were injected in doses corresponding to 1/40–1/10 LD_{50} iv into the cadual vein, after a 5 min stabilisation period, in a volume equivalent to 1 mL kg^{-1}.

In separate series of experiments on anesthetised normotensive rats, the effect of studied compounds (1/40–1/10 LD_{50}) on the pressor response to epinephrine (2 μg kg^{-1}), norepinephrine (2 μg kg^{-1}), methoxamine (150 μg kg^{-1}), tyramine (200 μg kg^{-1}) and dimethylphenylpiperazine (100 μg kg^{-1}) was investigated. Pressor responses of epinephrine, norepinephrine, methoxamine, tyramine and DMPP injected intravenously were obtained before and 5 min after the tested compound.

6.2.5. α-Adrenoceptor radioligand binding assay

The experiment was carried out on the rat cerebral cortex. [^{3}H]Prazosin (22-Ci $mmol^{-1}$, α_1-adrenergic receptor) and [^{3}H]clonidine (25.5 Ci $mmol^{-1}$, α_2-adrenergic receptor) were used. The membrane preparation and the assay procedure were carried out according to the published procedure [23] with slight modifications. Briefly, rats' brains were homogenised at 0 °C in 20 vols. of 50 mM Tris–HCl buffer (pH 7.6). The homogenate was centrifuged at $1000 \times g$ (0 °C) for 10 min using a Polytron. The membranes were collected by centrifuration at $25{,}000 \times g$ for 30 min, resuspended in Tris–HCl buffer and centrifuged again. A 450 μL sample of the latter membrane suspension was incubated at 37 °C for 30 min with 50 μL of the buffer solution of the investigated compound (1 nM–100 μM) and 50 μL of a [^{3}H]prazosin (0.6 nM) or 50 μL of a [^{3}H]clonidine (2 nM) solution. The incubation was followed by a rapid vacuum filtration through Whatman GB/C glass filters, and then was washed two times with 5 mL of a cold buffer (50 mM Tris–HCl, pH 7.6) dried, immersed in 3 mL of a scintillation liquid. Radioactivity was measured in a Beckmann LS 3801 β-scintillation counter. For measuring unspecific binding, phentolamine (in the case of [^{3}H]prazosin) and clonidine (in the case of [^{3}H]clonidine) in a final concentration 10 μM were present.

6.2.6. Acute toxicity according to Litchfield and Wilcoxon [17]

The compounds, dissolved in 0.9% saline, were injected into the caudal vein (10 mL kg^{-1}) or administered intragastrically. Each dose was given to six animals. The LD_{50} values were calculated according to the method of Litchfield and Wilcoxon [17] after a 24 h observation period.

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