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Preliminary evaluation of pharmacological properties of some xanthone derivatives

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ABSTRACT

A series of xanthone derivatives were synthesized and examined for electrocardiographic, antiarrhythmic, hypotensive and anticonvulsant activities as well as for α_1- and β_1-adrenergic binding affinities. Among the investigated compounds, some of them exhibited significant antiarrhythmic and/or hypotensive activity. The data obtained via receptor binding assay are in agreement with pharmacological results and could explain antiarrhythmic and/or hypotensive activity of the newly synthesized structures.

1. Introduction

Cardiovascular diseases are caused by disorders of the heart and blood vessels, and include coronary heart disease, cerebrovascular disease, hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. Although, development of modern, effective therapies, cardiovascular diseases remains the number one cause of death, representing 30% of all global deaths. Hypertension and arrhythmia are the major factors for cardiovascular mortality, which reach nearly three quarter of them.[1,2] In the genesis of cardiac dysfunctions, an important role plays activation of sympathetic nervous system and from here adrenergic receptors antagonists have been widely accepted in treatment.[3,4] On the other hand, an ideal antiarrhythmic or hypotensive medicament does not exist. Treatment is associated with side-effects, such as tiredness, changes in mood, sleep disturbances, dry mouth, blurry vision or impotence. Thus, delicate balance between drug efficacy and unexpected adverse side effects is essential. It results in importance of searching for the new agents improving heart function with minimal side effects.

It is known that most classic β-blockers, classified as second class of antiarrhythmic drugs according to Vaughan Williams, contain an 1-aroxy-3-alkylamino-2-propanol group in their structure (propranolol, atenolol, metoprolol, acebutolol). Furthermore 1-aroxy-3-alkylamino-2-propanol group is structural element of some nonselective, third generation β-blockers with additional α-adrenolytic properties (carvedilol) revealing beneficial effect on lowering blood pressure.[5] Taking into account these facts, publications concerning cardiovascular properties of aminoalcanolic derivatives of xanthone[6–8] and our own experience,[9–11] herein we reported the results of in vivo evaluating of potential antiarrhythmic and hypotensive activity of selected xanthone derivatives.

It is known from the literature that the anticonvulsant activity has been demonstrated for compounds without any structural similarity to classic antiepileptic drugs (valproic acid, carbamazepine, lamotrigine, etc.). Significant example can be propranolol having an established indication in a variety of cardiovascular diseases and interestingly, potently preventing maximal electroshock (MES) seizures in rodents and raising the after discharge threshold in rats.[12] Among these compounds there are also aminoalcanols, for which a high anticonvulsant activity has been described for example 2-(3*N*-(*tert*-butylamino)-2-hydroxypropoxy)-9*H*-xanthen-9-one revealing protection index (PI) in maximal electroshock-induced seizures (mice, *i.p.*) ranging 4.5, which is comparable with that for Carbamazepine (PI = 4.9).[10] These knowledge prompted us to carry out additional tests evaluating neurotoxicity and potential antiepileptic properties of the newly

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synthesized compounds. Preliminary pharmacological and neurotoxicological assays have been provided by the ADD (Anticonvulsant Drug Development) program, Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health (Rockville, USA).

2. Results

2.1. The influence on the normal electrocardiogram

The effect on ECG intervals and the heart rate was determined for all compounds at the doses of 5 or 10 mg/kg body weight (Table 1). Electrographic experiments showed that compounds **4**, **5**, **6**, **8** significantly decreased the number of cardiac beats per minute—**4** by 13%, **5** by 20%, **6** by 15%, and **8** by 21%, besides compounds **4, 5** and **6** prolonged the PQ interval, especially in the first minute of the test (Figs. 1 and 2). Compounds **3** and **7** significantly decreased the number of cardiac beats per minute (24% and 30%) and prolonged the QT interval (17% and 13%) during all the time of observation (Fig. 3). The compound **1** diminished the heart rate and prolonged the PQ interval only in the first minute of the test (Fig. 3). The compound **2** did not significantly affect the normal ECG, only slightly prolonged the QT interval.

2.2. Antiarrhythmic activity

Prophylactic antiarrhythmic activity of the tested compounds was evaluated using anesthetized rats in the adrenaline-induced model of arrhythmia. Rapid intravenous injection of adrenaline at a dose of 20 μg/kg caused reflex bradycardia (100%), atrioventricular disturbances, extrasystoles (88%), which led to the death of approximately 76% of animals of the control group within 15 min of the observation.

The tested compounds, injected intravenously 15 min before adrenaline, diminished the occurrence of heart-rhythm disturbances.

Among the studied compounds, the strongest prophylactic antiarrhythmic activity was shown by compounds **4** and **7**. Their ED_{50} values (a dose producing a 50% inhibition of premature ventricular beats) are presented in Table 2. These data show that compound **4** was the most active in this test. This compound as well as **7** also prevented the appearance of other adrenaline-induced arrhythmia symptoms (bradycardia, blocks, bigeminy) and significantly reduced mortality. The compound **7** at the lowest dose in this test (1.25 mg/kg) still reduced mortality by 60% in comparison to the control group.

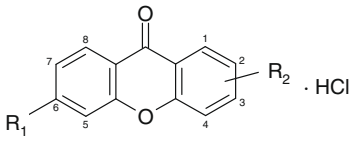
2.3. Influence on blood pressure

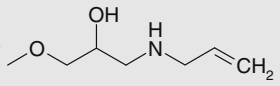
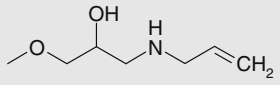
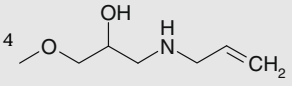
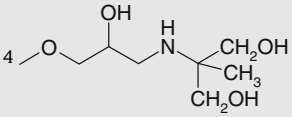
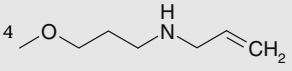
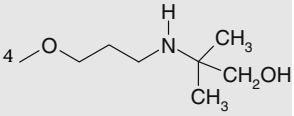
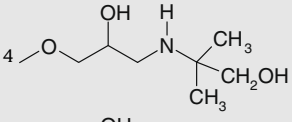
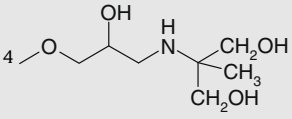
The investigated compounds were tested for hypotensive activity after intravenous administration at the doses of 10 mg/kg (**1, 2, 3, 5, 6, 7**) or 2.5 mg/kg (**4** and **8**). For active compounds, these doses were reduced until the hypotensive activity disappeared.

Intravenous injections of compound **7** at doses of 10–1.25 mg/kg significantly reduced the systolic and diastolic blood pressure in normotensive rats and that hypotensive effect lasted throughout the observation. At a lower dose (0.625 mg/kg), the compound **7** significantly reduced the blood pressure only in 60 min of the observation (data not shown). Compound **7** reduced systolic blood pressure by 27–17% and diastolic blood pressure by 33–19% at the lowest dose used in this test (1.25 mg/kg).

Compound **3** reduced the systolic blood pressure by 16–9% and diastolic blood pressure by 18–11% at a dose of 5 mg/kg. At a lower dose (2.5 mg/kg), the compound **3** did not have any significant influence on blood pressure (data not shown). Compound **1** revealed hypotensive activity only at a dose of 10 mg/kg; it reduced both systolic (22–10%) and diastolic (25–11%) blood pressure, which persisted for 60 min.The results for these compounds are presented in Figures 4 and 5. Compounds **2** and **6** at a dose of 10 mg/kg significantly influenced blood pressure only in 60 min of the test. Compound **5** (at a dose of 10 mg/kg) initially reduced systolic and diastolic pressure, but the pressure returned to the baseline value within next couple of minutes after administration. Compounds **4** and **8**, at a dose of 2.5 mg/kg, had no effect in this test (data not shown).

Table 1
Chemical structures of the tested compounds



Compound	R_1	R_2
1	H	
2	H	
3	H	
4	H	
5	H	
6	H	
7	Cl	
8	Cl	

2.4. Radioligand-binding assay

Compounds **3**, **4**, **7** and **8** displaced [^{3}H]CGP-12177 from cortical binding sites in low concentration range (K_i = 3.9–83.8 nM). Other compounds moderately inhibited [^{3}H]CGP-12177 binding with μM-range. All compounds inhibited [^{3}H]prazosin binding to cortical α_1-adrenoceptors with μM-range. The results are summarized in Table 2.

2.5. Anticonvulsant and neurotoxicity assay

Synthesized compounds were evaluated for anticonvulsant activity in the maximal electroshock (MES)- and subcutaneous

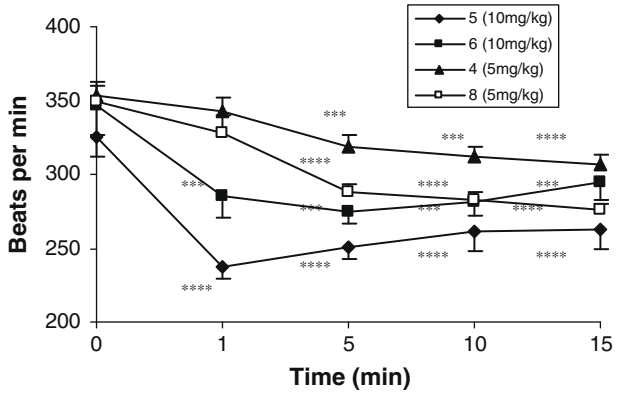


Figure 1. Effect of compounds **4**, **5**, **6** and **8** on electrocardiogram (cardiac beats per minute) in anaesthetized rats after iv administration. Statistical analyses were performed using a one-way ANOVA test: $^{***}p < 0.01$, $^{****}p < 0.001$.

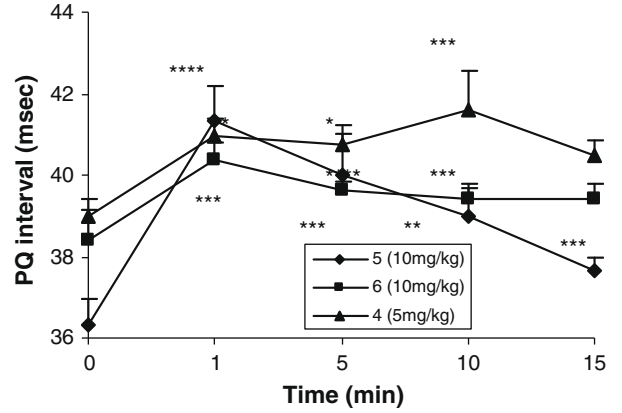


Figure 2. Effect of compounds **4**, **5** and **6** on electrocardiogram (PQ intervals per minute) in anaesthetized rats after iv administration. Statistical analyses were performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

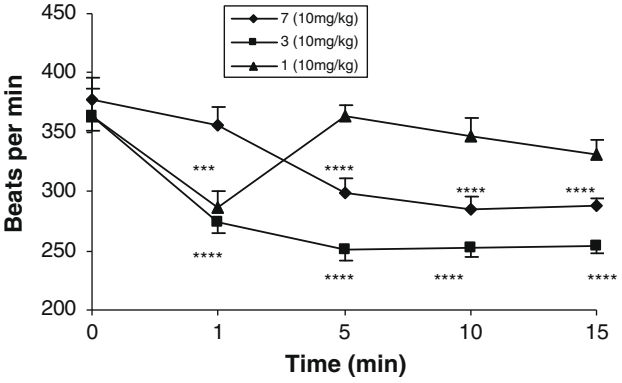


Figure 3. Effect of compounds **1**, **3** and **7** on electrocardiogram (cardiac beats per minute) in anaesthetized rats after iv administration. Statistical analyses were performed using a one-way ANOVA test: $^{***}p < 0.01$, $^{****}p < 0.001$.

pentylenetetrazole (ScMet)-induced seizures tests and for neurotoxicity (TOX) in the rotorod test in mice (*i.p.*). Among the tested compounds only **1** and **2** revealed some anti-MES activity (after 0.5 and 4 h of *i.p.* administration, mice) in the doses of 100 and 300 mg/kg (see Table 3). None of the tested compounds displayed anti-ScMet protection (data not published). On the other hand, most of the examined xanthone derivatives did not show neurotoxicity in the dose of 30 mg/kg (compounds **1**, **2**, **4**, **5**, **6**, **8**) as well

Table 2
K_i values for the inhibition of the binding of [^{3}H]prazosin and [^{3}H]CGP-12177 to α_1- and β_1-adrenoceptors and the prophylactic antiarrhythmic activity in adrenaline induced arrhythmia in anesthetized (thiopental 75 mg/kg *i.p.)* male Wistar rats (route of administration—*i.v.*)

Compound	$K_i \pm$ SEM[a] (μM)		ED_{50}[b] (mg/kg)
	α_1-Adrenoceptors	β_1-Adrenoceptors	
1	4.9 ± 1.1	30.9 ± 0.7	—
2	8.1 ± 1.6	44.4 ± 4.2	—
3	7.3 ± 0.5	0.084 ± 0.010	—
4	49.8 ± 5.5	0.004 ± 0.0007	1.753 (0.77–4.01)
5	4.6 ± 0.5	7.7 ± 0.3	—
6	8.7 ± 0.2	2.6 ± 0.3	—
7	5.6 ± 0.6	0.011 ± 0.003	2.46 (1.05–5.73)
8	20.6 ± 2.1	0.047 ± 0.004	—
Propranolol	—	9.7 ± 0.8	1.05 (0.64–1.73)

[a,b] $K_i \pm$ SEM values were derived from 2 to 3 experiments in duplicates.
[b] 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.

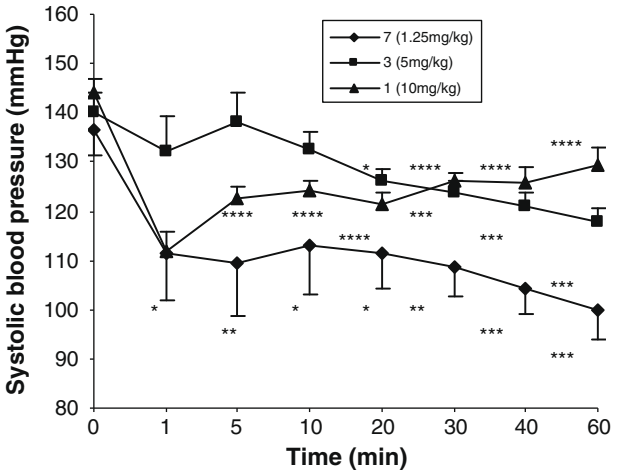


Figure 4. The influence of compounds **1**, **3** and **7** on the systolic blood pressure in anaesthetized rats after iv administration. Statistical analyses were performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

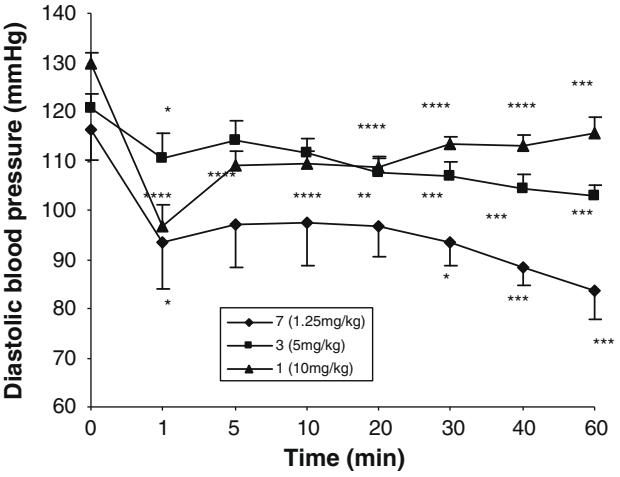


Figure 5. The influence of compounds **1**, **3** and **7** on the diastolic blood pressure in anaesthetized rats after iv administration. Statistical analyses were performed using a one-way ANOVA test: $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{****}p < 0.001$.

as in the dose of 100 mg/kg (compounds **1**, **2**, **4**, **6**, **8**). Except **8**, all of the evaluated compounds revealed diverse (from 25% to 100%)

range of neurotoxicity after 0.5 h of administration in the dose of 300 mg/kg.

3. Discussion

The aim of this study was to evaluate cardiovascular activity of eight new xanthone derivatives. This work includes synthesis and pharmacological studies of these compounds. It examines both cardiovascular activity: anti-arrhythmic, hypotensive, α_1- and β_1-adrenergic blocking activities, effect on the normal electrocardiogram and influence on central nervous system.

Searching for new xanthone derivatives with potential biological properties has remained in the area of our interest for the last several years and has been documented by a number of publications. Among the evaluated compounds there were structures revealing for example anticonvulsant[13–15], antitumour,[16] antituberculotic,[17] aniarrhythmic and/or hypotensive[9–11] activity. Thus our own experience and former articles which treated xanthone derivatives possessing cardiovascular activity[6–8] prompted us to synthesize a novel series of xanthone derivatives with aminoalcanolic moieties—well known structural element of β-blockers. Additionally we synthesized and evaluated their two aminic analogues. Results obtained via antiarrhythmic, hypotensive and binding affinity assays confirmed that aminoalcanolic moiety play an important role in the structure of cardiovascular drugs. For that reason, compounds **5** and **6** were less active than their alcanolic derivatives. Presence of one hydroxylic group seems to be essential also in the amine molecule. Fact that many of β-blockers possess *iso*-propylamine or *tert*-butylamine moiety in their structure encouraged us to synthesize their xanthonic analogues and xanthonic analogues with additional hydroxylic groups. Evaluation of the obtained substances towards cardiovascular properties indicated that compounds with no hydroxylic groups were inactive neither in reducing blood pressure nor in cardioprotection in the adrenaline model of arrhythmia (data not published). On the other hand, the most potent in the adrenaline model of arrhythmia were compounds **4** and **7**, with the ED_{50} value 1.75 mg/kg for compound **4** and 2.46 mg/kg for compound **7**. Propranolol, which was used in this test as a reference compound had ED_{50} value 1.05 mg/kg. From the radioligand-binding assay it appeared that compounds **4** and **7** had high affinity to β_1-adrenergic receptors in rat cerebral cortex (4 and 11 nM, respectively, see Table 3). Based on our results, we suggest that the antiarrhythmic effects of the tested compounds are related to the β_1-adrenoceptor blockade in heart tissue.

However, compound **8** in comparison with **7** was less active. Analogously compound **4** was less active than its unhydroxylated analogue: 4-(3*N*-(2-amino-2-methyl-1-hydroxypropyl)-2-hydroxypropoxy)-9*H*-xanthen-9-one hydrochloride revealing β_1-affinity amounted to 2.6 nM.[18] These two facts suggested that presence of one hydroxylic group is favourable to potential antiarrhythmic properties of the tested compound. Nevertheless, presence of two hydroxylic group and in consequence lower lipophilicity caused decreasing of efficacy.

Concerning influence on blood pressure, the most potent seem to be compounds **3** and **7**, containing allylamine moiety, new, potential hypotensive farmacophore. Compound **7** significantly reduced the systolic and diastolic blood pressure at the dose 1.25 mg/kg. Compound **3** decreased blood pressure all the time during the observation but at higher doses. In binding studies compounds **3** and **7** had additionally moderately high affinity for α_1 adrenoceptors in binding studies. The hypotensive effect is probably the result of β_1-adrenoceptor blockade in heart and α_1-adrenoceptor blockade in arteries.

Among all of the tested compounds with allylamine group, place of substitution played a key role. Curiously enough, the most required was position 4 in the xanthone rings, while according to the former publication[19] treating xanthonolol as a potential hypotensive agent, the most suitable was substitution in the 3 position.

Table 3
Anticonvulsant screening project (ASP): phase I-test results in mice (*i.p.* administration)

Compound	Dose (mg/kg)	Activity MES[a] time (h)		TOX[b] time (h)	
		0.5	4	0.5	4
1	30	0/1	0/1	0/4	0/2
	100	2/3	0/3	0/8	0/4
	300	1/1	1/1	4/4	1/2
2	30	0/1	0/1	0/4	0/2
	100	1/3	0/3	0/8	0/4
	300	1/1	1/1	4/4	0/2
3	30	0/1	0/1	1/4	0/2
	100	0/3	0/3	1/8	0/4
	300	0/1	0/1	2/4	0/2
4	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/8	0/4
	300	0/1	0/1	1/4	0/2
5	30	0/1	0/1	0/4	0/2
	100	0/2	0/2	5/8	0/3
	300	—	—	4/4	—
6	30	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/8	0/4
	300	0/1	0/1	1/4	0/2
7	30	0/1	0/1	1/4	0/2
	100	0/3	0/3	5/8	1/4
	300	0/1	0/1	4/4	2/2
8	30	0/1	0/1	0/4	—
	100	0/3	0/3	0/8	0/5
	300	0/1	0/1	0/4	—

[a] Maximal electroshock test (number of animals protected/number of animals tested).
[b] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested).

4. Conclusion

The results confirmed that among the investigated compounds, some of them exhibited significant β_1-adrenolitic, antiarrhythmic and/or hypotensive activities. The strongest anti-arrhythmic activity in the adrenaline-induced model of arrhythmia revealed compounds **4** and **7**. The most potent hypotensive activity in our research has the compound **7**. The results obtained from radioligand binding assays showed that tested compounds had affinity to adrenoceptors. For compounds **3**, **4**, **7** and **8** the K_i value was in nM range towards β_1 adrenoceptors. The obtained data concluded, that binding studies are in agreement with our pharmacological results and we put forward a hypothesis that the antiarrhythmic and hypotensive activity are related to adrenolitic properities of examined compounds.

5. Experimental

5.1. Chemistry

Reagents: epichlorohydrin, allylamine, 2-amino-2-methyl-1-propanol, 2-amino-2-methyl-1,3-propanediol were purchased from Sigma–Aldrich Chemie (Steinheim, Germany), wherease reagents: 3-chloro-1-propanol, phosphorus tribromide were provided by Lancaster Synthesis (Frankfurt am Main, Germany). Solvents were commercially available materials of reagent grade. Melting points (mp) are uncorrected and were determined using a Büchi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland). The infrared spectra

were recorded on potassium bromide pellets using a Jasco FT/IR 410 spectrometer (Jasco Inc., Easton, MD, USA). The ^{1}H NMR for compounds **3**, **5**, **6**, **7** were recorded on a Bruker AMX spectrometer (Brucker, Karlsruhe, Germany) with 500.13 MHz, using signal from DMSO in DMSO-d_6 and TMS in $CDCl_3$ as an internal standard, whereas ^{1}H NMR spectra for the other compounds were obtained in $CDCl_3$ or DMSO-d_6 with a Varian Mercury-VX 300 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) with TMS or DMSO, respectively, as an internal standard. The results are presented in the following format: chemical shift δ (ppm), multiplicity, J values in Hertz (Hz), number of protons, proton's position. Multiplicities were shown as the abbreviations: s (singlet), br s (broad singlet), br b (broad bond), d (doublet), dd (doublet of doublets), td (triplet of doublets), t (triplet), dt (doublet of triplets), tt (triplet of triplets), qu (quintet), m (multiplet). Elemental analyses were performed on a Elementar Vario EL III (Elementar Analysansysteme, Hanau, Germany). The purity of obtained compounds was confirmed by the thin-layer chromatography (TLC), carried out on precoated plates (Silica Gel, 60 F-254, Merck, Darmstadt, Germany) using the solvent systems mentioned below. The obtained corresponding spots were visualized under UV light.

5.2. Chemical syntheses

5.2.1. Synthesis of the starting materials

The synthetic route used to synthesize starting materials is outlined in Scheme 1. The detailed description of the method and physico-chemical properties of 2-((Oxiran-2-yl)methoxy)-9*H*-xanthen-9-one, 3-((Oxiran-2-yl)methoxy)-9*H*-xanthen-9-one and 4-((Oxiran-2-yl)methoxy)-9*H*-xanthen-9-one were described elsewhere.[9–11] 3-Chloro-5-((Oxiran-2-yl)methoxy)-9*H*-xanthen-9-one was obtained analogously from 3-chloro-5-hydroxy-9*H*-xanthen-9-one. The crude products were recrystallized from ethanol. 4-(3-bromopropoxy)-9*H*-xanthen-9-one was prepared from 4-hydroxy-9*H*-xanthen-9-one according to the previously described procedure[11] using 3-chloro-1-propanol instead of 2-chloroethanol (see Scheme 1). The crude products were recrystallized from *n*-hexane/toluene (1:4).

5.2.1.1. *3-Chloro-5-hydroxy-9H-xanthen-9-one* was obtained as white solid (yield 70%), mp 174–176 °C. Anal. Calcd for $C_{13}H_7O_3Cl$: C, 63.30; H, 2.86. Found: C, 63.34; H, 3.03. IR (KBr) υ (cm^{-1}): 3229, 1647, 1600, 1496, 1432, 1252, 1202, 1071, 934, 831. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.76 (s, 1H, –OH), 7.24–8.32 (m, 6H, Ar–H). R_f = 0.91 (methanol/ethyl acetate (1:1)).

5.2.1.2. *3-Chloro-5-((oxiran-2-yl)methoxy)-9H-xanthen-9-one* was obtained as white solid (yield 65%), mp 183–185 °C. Anal. Calcd for $C_{16}H_{11}O_4Cl$: C, 63.48; H, 3.66. Found: C, 63.05; H, 4,01. IR (KBr) υ (cm^{-1}): 3437, 3069, 1602, 1492, 1332, 1274, 1220, 1070, 928, 753. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.87 (dd, J = 2.7 Hz, J = 8.7 Hz, 1H, –C*H*H–), 2.99 (dd, J = 4.1 Hz, J = 4.9 Hz, 1H, –CH*H*–), 3.47–3.52 (m, 1H, –CH=), 4.13 (dd, J = 5.9 Hz, J = 11.3 Hz, 1H, Ar–O–C*H*H–CH=), 4.47 (dd, J = 2.8 Hz, J = 11.3 Hz, 1H, Ar–O–CH*H*–CH=), 7.26–8.28 (m, 6H, Ar–H). R_F = 0.88 (methanol/ethyl acetate (1:1)).

5.2.1.3. *4-(3-Hydroxypropoxy)-9H-xanthen-9-one* was obtained as white solid (yield 65%), mp 136–137 °C. Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.02; H, 5.23. IR (KBr) υ (cm^{-1}): 3365, 2935, 1668, 1467, 1337, 1274, 1256, 1226, 1067, 940, 751. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.17–2.24 (m, 2H, –CH_2–C*H*$_2$–CH_2–), 2.55 (t, J = 5.6, 1H, –OH), 3.97–4.03 (m, 2H, –C*H*$_2$–OH), 4.34 (t, J = 5.9 Hz, 2H, –O–CH_2-), 7.21–8.36 (m, 7H, Ar–H). R_f = 0.53 (toluene/acetone (5:3)).

5.2.1.4. *4-(3-Bromopropoxy)-9H-xanthen-9-one* was obtained as white solid (yield 50%), mp 124–126 °C. Anal. Calcd for $C_{16}H_{13}O_3Br$: C, 57.68; H, 3.93. Found: C, 57.73; H, 4.11. IR (KBr) υ (cm^{-1}): 1662, 1606, 1492, 1468, 1327, 1270, 1228, 1062, 926, 749. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.48 (m, 2H, –CH_2–C*H*$_2$–CH_2–), 3.75 (t, J = 6.4, 2H, –CH_2–Br), 4.32 (t, J = 5.9 Hz, 2H, –O–CH_2–), 7.21–8.37 (m, 7H, Ar–H). R_f = 0.93 (toluene/acetone (5:3)).

5.2.2. Synthesis of the tested compounds

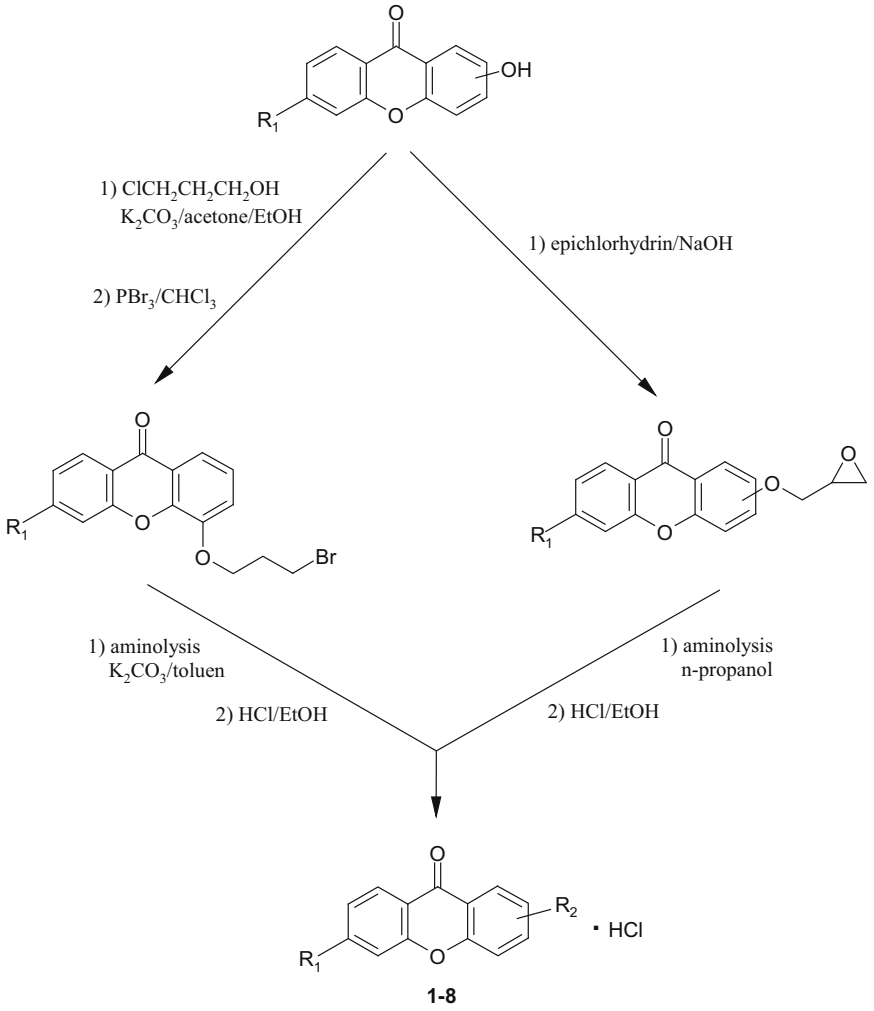
Racemic compounds **1–8** (see Table 1) were obtained as presented in Scheme 1 by amination of respective parent compound with appropriate amines in n-propanol (for **1–4**, **7**, **8**) or toluene in a presence of K_2CO_3 (for **5**, 6), according to the earlier published procedures.[9,13] Resulted bases were converted into hydrochloride salts in propanol/acetone (4:1) with an excess of ethanol saturated with HCl. The crude products were recrystallized from acetone/ethanol (1:3).

5.2.2.1. *2-(3-(Allylamino)-2-hydroxypropoxy)-9H-xanthen-9-one hydrochloride* (**1**) was obtained as white solid (yield 70%), mp 206–207 °C. Anal. Calcd for $C_{19}H_{20}NO_4Cl$: C, 63.07; H, 5.57; N, 3.87. Found: C, 63.06; H, 5.72; N, 3.75. IR (KBr) υ (cm^{-1}): 3384, 2932, 2788, 1619, 1467, 1215, 1037, 764. Base of compound **1**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.72 (br s, 1H, –NH–), 2.79–2.93 (m, 2H, –CH(OH)–C*H*$_2$–NH–), 3.26–3.38 (m, 2H, –NH–C*H*$_2$–CH=CH_2), 4.05–4.17 (m, 4H, Ar–O–CH_2–CH(OH)–), 5.11–5.25 (m, 2H, –CH=C*H*$_2$), 5.85 (m, 1H, –C*H*=CH_2), 7.35–8.36 (m, 7H, Ar–H). R_f = 0.28 (methanol/ethyl acetate (1:1)).

5.2.2.2. *3-(3-(Allylamino)-2-hydroxypropoxy)-9H-xanthen-9-one hydrochloride* (**2**) was obtained as white solid (yield 64%), mp 184–186 °C. Anal. Calcd for $C_{19}H_{20}NO_4Cl$: C, 63.07; H, 5.57; N, 3.87. Found: C, 63.04; H, 5.53; N, 3.75. IR (KBr) υ (cm^{-1}): 3266, 2929, 1622, 1467, 1283, 1166, 1106, 759. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.94–3.15 (m, 2H, –CH(OH)–C*H*$_2$–NH_2^+–), 3.61 (d, J = 6.4 Hz, 2H, –NH_2^+–C*H*$_2$–CH=CH_2), 4.13–4.27 (m, 3H, Ar–O–C*H*$_2$–C*H*(OH)-), 5.38–5.51 (m, 2H, –CH=C*H*$_2$), 5.86–5.98 (m, 2H, –C*H*=CH_2, –OH), 7.05–8.18 (m, 7H, Ar–H), 9.02 (br s, 1H, –NH*H*$^+$–), 9.19 (br s, 1H, –N*H*H$^+$–). R_f = 0.28 (methanol/ethyl acetate (1:1)).

5.2.2.3. *4-(3-(Allylamino)-2-hydroxypropoxy)-9H-xanthen-9-one hydrochloride* (**3**) was obtained as white solid (yield 65%), mp 163–165 °C. Anal. Calcd for $C_{19}H_{20}NO_4Cl$: C, 63.07; H, 5.57; N, 3.87. Found: C, 62.58; H, 5.68; N, 3.80. IR (KBr) υ (cm^{-1}): 3357, 2942, 1651, 1594, 1467, 1278, 1082, 756. ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 3.07 (dd, J = 8.7 Hz, J = 12.5 Hz, 1H, –CH–CH*H*–NH_2^+–), 3.22 (dd, J = 3.5 Hz, J = 12.5 Hz, 1H, –CH–C*H*H–NH_2^+–), 3.64 (ddd, J = 0.9 Hz, J = 1.2 Hz, J = 6.6 Hz, 2H, –NH_2^+–C*H*$_2$-), 4.22 (dd, J = 5.6 Hz, J = 10.2 Hz, 1H, Ar–O–CH*H*–), 4.26 (dd, J = 4.9 Hz, J = 10.2 Hz, 1H, Ar–O–C*H*H–), 4.30–4.41 (m, 1H, –CH–), 5.40 (ddd, J = 0.9 Hz, J = 1.5 Hz, J = 10.2 Hz, 1H, =CH*H*), 5.49 (ddt, J = 1.2 Hz, J = 1.5 Hz, J = 17.2 Hz, 1H, =C*H*H), 5.97 (ddt, J = 6.6 Hz, J = 10.2 Hz, J = 17.2 Hz, 1H, –CH_2C*H*=CH_2), 6.03 (br s, 1H, –OH), 7.40 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H, 2Ar–H), 7.51 (ddd, J = 1.1 Hz, J = 7.1 Hz, J = 8.0 Hz, 1H, 7Ar–H), 7.59 (dd, J = 1.5 Hz, J = 8.0 Hz, 1H, 3Ar–H), 7.71 (dd, J = 1.1 Hz, J = 8.7 Hz, 1H, 5Ar–H), 7.77 (dd, J = 1.5 Hz, J = 8.0 Hz, 1H, 1Ar–H), 7.92 (ddd, J = 1.8 Hz, J = 7.1 Hz, J = 8.7 Hz, 1H, 6Ar–H), 8.21 (dd, J = 1.8 Hz, J = 8.0 Hz, 1H, 8Ar–H), 8.89 (br s, 2H, –NH_2^+–). ^{13}C NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 48.81 (R–CH_2–NH–), 49.31 (–NH–CH_2–CH=), 64.92 (R–CHOH–R), 71.34 (Ar–O–CH_2-), 117.26 (C-1), 118.30 (C-3), 118.49 (C-5), 121.03 (C-8a), 122.16 (C-8b), 122.89 (=CH_2), 124.11 (C-2), 124.69 (C-7), 126.09 (C-8), 129.27 (=CH–), 135.69 (C-6), 146.17 (C-4a), 147.55 (C-4), 155.41 (C-4ba), 176.10 (C=O). R_f = 0.29 (methanol/ethyl acetate (1:1)).

5.2.2.4. *4-(3N-(2-Amino-2-methyl-1,3-dihydroxypropyl)-2-hydroxypropoxy)-9H-xanthen-9-one hydrochloride* (**4**) was obtained as white solid (yield 75%), mp 242–244 °C. IR (KBr) υ (cm^{-1}): 3410, 2921, 1657, 1608, 1469, 1278, 1079, 751. R_f = 0.12 (methanol/ethyl acetate (1:1)). Base of compound **4**: Anal. Calcd for $C_{20}H_{23}NO_6$: C, 64.33; H, 6.21; N, 3.75. Found: C, 64.18; H, 6.17; N, 3.47. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 0.87 (s, 3H, –CH_3), 1.75 (s, 1H, –NH–), 2.61–2.77



Scheme 1. Synthesis of the tested compounds (**1**–**8**).

(m, 1H, –NH–C*H*$_2$-), 3.23 (t, *J* = 4.5 Hz, 4H, (–C*H*$_2$–OH)$_2$), 3.90–3.92 (m, 1H, –C*H*–OH), 4.08–4.21 (m, 2H, Ar–O–C*H*$_2$-), 4.33 (br s, 2H, (–CH$_2$–O*H*)$_2$), 5.03 (d, *J* = 4.6 Hz, 1H, –CH–O*H*), 7.33–7.91 (m, 6H, Ar–H), 8.18 (d, *J* = 6.7 Hz, 1H, 8Ar–H).

5.2.2.5. *4-(3-(Allylamino)propoxy)-9H-xanthen-9-one hydrochloride* (**5**) was obtained as white solid (yield 72%), mp 185–187 °C. Anal. Calcd for $C_{19}H_{20}NO_3Cl$: C, 65.99; H, 5.83; N, 4.05. Found: C, 65.98; H, 6.14; N, 3.82. IR (KBr) υ (cm^{-1}): 3434, 2956, 2759, 1656, 1606, 1469, 1343, 1277, 1075, 966, 756. ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 2.25 (qu, *J* = 6.6 Hz, 2H, -R–CH$_2$-R-), 3.18 (t, *J* = 6.5 Hz, 2H, –C*H*$_2$–NH–), 3.67 (ddd, *J* = 0.9 Hz, *J* = 1.2 Hz, *J* = 6.5 Hz, 2H, –NH–C*H*$_2$–CH=), 4.33 (t, *J* = 6.5 Hz, 2H, Ar–O–CH$_2$–), 5.40 (ddt, *J* = 0.9 Hz, *J* = 1.5 Hz, *J* = 10.3 Hz, 1H, =CH*H*), 5.49 (ddt, *J* = 1.2 Hz, *J* = 1.5 Hz, *J* = 17.2 Hz, 1H, =CH*H*), 5.96 (ddt, *J* = 6.6 Hz, *J* = 10.3 Hz, *J* = 17.2 Hz, 1H, –CH=), 7.41 (dd, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H, 2Ar–H), 7.52 (ddd, *J* = 1.1 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, 7Ar–H), 7.56 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H, 3Ar–H), 7.71 (ddd, *J* = 0.6 Hz, *J* = 1.1 Hz, *J* = 8.5 Hz, 1H, 5Ar–H), 7.77 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H, 1Ar–H), 7.92 (ddd, *J* = 1.8 Hz, *J* = 7.1 Hz, *J* = 8.5 Hz, 1H, 6Ar–H), 8.21 (ddd, *J* = 0.6 Hz. *J* = 1.8 Hz, *J* = 8.0 Hz, 1H, 8Ar–H), 9.00 (br s, 2H, –NH$_2^+$–). ^{13}C NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 26.48 (R–CH$_2$-R), 44.71 (R–CH$_2$–NH–), 49.92 (–NH–CH$_2$–CH=), 67.58 (Ar–O–CH$_2$-), 118.02 (C-1), 118.98 (C-3), 119.50 (C-5), 122.02 (C-8a), 123.11 (C-8b), 123.59 (=CH$_2$), 125.11 (C-2), 125.67 (C-7), 127.07 (C-8), 130.27 (=CH–), 136.66 (C-6), 147.13 (C-4a), 148.47 (C-4), 156.41 (C-4ba), 177.09 (C=O). R_f = 0.14 (methanol/ethyl acetate (1:1)).

5.2.2.6. *4-(3N-(2-Amino-2-methyl-1-hydroxypropyl)propoxy)-9H-xanthen-9-one hydrochloride* (**6**) was obtained as white solid (yield 67%), mp 226–228 °C. Anal. Calcd. for $C_{20}H_{24}NO_4Cl$: C, 63.57; H, 6.40; N, 3.71. Found: C, 63.51; H, 6.72; N, 3.79. IR (KBr) υ (cm^{-1}): 3389, 2962, 2795, 1657, 1604, 1467, 1272, 1227, 1073, 753. ^{1}H NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 0.96 (s, 6H, C*H*$_3$–CH–C*H*$_3$), 1.94 (tt, *J* = 6.5 Hz, *J* = 6.7 Hz, 2H, –R–CH$_2$–R–), 2.71 (t, *J* = 6.7 Hz, 2H, –C*H*$_2$–NH–), 3.20 (s, 2H, –C*H*$_2$–OH), 3.36 (br s, 2H, –NH–, –OH), 4.27 (t, *J* = 6.5 Hz, 2H, Ar–O–CH$_2$-), 7.38 (dd, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H, 2Ar–H), 7.50 (ddd, *J* = 1.0 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, 7Ar–H), 7.52 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H, 3Ar–H), 7.68 (ddd, *J* = 0.5 Hz, *J* = 1.0 Hz, *J* = 8.5 Hz, 1H, 5Ar–H), 7.73 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H, 1Ar–H), 7.89 (ddd, *J* = 1.8 Hz, *J* = 7.1 Hz, *J* = 8.5 Hz, 1H, 6Ar–H), 8.20 (ddd, *J* = 0.6 Hz. *J* = 1.8 Hz, *J* = 8.0 Hz, 1H, 8Ar–H). ^{13}C NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 23.67 ((–CH$_3$)$_2$, 30.12 (R–CH$_2$–R), 38.11 (R–CH$_2$–NH–), 53.35 (–NH–C), 67.70 (Ar–O–CH$_2$–), 68.05 (–CH$_2$–OH), 116.43 (C-1), 117.65 (C-3), 118.31 (C-5), 122.92 (C-8a), 121.98 (C-8b), 123.97 (C-2), 124.42 (C-7), 125.92 (C-8), 135.46 (C-6), 146.03 (C-4a), 147.83 (C-4), 155.34 (C-4ba), 176.02 (C=O). R_f = 0.06 (methanol/ethyl acetate (1:1)).

5.2.2.7. *5-(3N-(2-Amino-2-methyl-1-hydroxypropyl)-2-hydroxypropoxy)-3-chloro-9H-xanthen-9-one hydrochloride* (**7**) was obtained as white solid (yield 65%), mp 236–238 °C. Anal. Calcd. for

$C_{20}H_{23}NO_5Cl_2$: C, 56.08; H, 5.41; N, 3.27. Found: C, 56.05; H, 5.59; N, 3.20. IR (KBr) ν (cm^{-1}): 3342, 1661, 1604, 1428, 1276, 1222, 1075, 929, 754. ^1H NMR ($CDCl_3$, 500.13 MHz) δ (ppm): 1.29 (d, J = 3.4 Hz, 6H, $(-CH_3)_2$), 3.05–3.15 (m, 1H, –CH*H*–NH–), 3.20–3.30 (m, 1H, –C*H*H–NH–), 3.52 (d, J = 4.2 Hz, 2H, $-CH_2$–OH), 4.22–4.32 (m, 2H, $-CH_2$–O–Ar), 4.32–4.39 (m, 1H, CH), 5.60 (t, J = 4.2 Hz, 1H, $-CH_2$–O*H*), 5.95 (d, J = 4.7 Hz, 1H, –CH–O*H*), 7.42 (dd, J = 8.0 Hz, J = 8.0 Hz, 1H, 2Ar–H), 7.55 (dd, J = 2.0 Hz, J = 8.5 Hz, 1H, 7Ar–H), 7.61 (dd, J = 1.4 Hz, J = 8.0 Hz, 1H, 3Ar–H), 7.76 (dd, J = 1.4 Hz, J = 8.6 Hz, 1H, 1Ar–H), 7.84 (d, J = 2.0 Hz, 1H, 5Ar–H), 8.20 (d, J = 8.5 Hz, 1H, 8Ar–H), 8.38 (t, J = 8.7 Hz, 1H, –N*H*H$^+$–), 8.82 (t, J = 8.7 Hz, 1H, –NH*H*$^+$–). ^{13}C NMR (DMSO-d_6, 500.13 MHz) δ (ppm): 20.41 ($-CH_3$), 20.60 ($-CH_3$), 44.28 (R–CH_2–NH–), 59.85 (–NH–C), 64.68 ($-CH_2$–OH), 65.42 (–CH–OH), 71.41 (Ar–O–CH_2–), 117.27 (C-1), 118.25 (C-5), 118.85 (C-3), 119.94 (C-8a), 122.15 (C-8b), 124.54 (C-2), 125.20 (C-7), 128.03 (C-8), 139.88 (C-6), 146.16 (C-4a), 147.52 (C-4), 155.69 (C-4ba), 175.46 (C=O). R_f = 0.06 (methanol/ethyl acetate (1:1)).

5.2.2.8. *5-(3N-(2-Amino-2-methyl-1,3-dihydroxypropyl)-2-hydroxypropoxy)-3-chloro-9H-xanthen-9-one hydrochloride* (**8**) was obtained as white solid (yield 70%), mp 218–220 °C. Anal. Calcd. for $C_{20}H_{23}NO_6Cl_2$: C, 54.06; H, 5.22; N, 3.15. Found: C, 54.06; H, 5.50; N, 3.05. IR (KBr) ν (cm^{-1}): 3352, 1599, 1426, 1276, 1071, 928, 756. ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.20 (br s, 3H, $-CH_3$), 3.15–3.21 (m, 1H, –NH–CH*H*–), 3.36–3.40 (m, 1H, –NH–C*H*H–), 3.50–3.64 (m, 4H, $(-CH_2$–OH$)_2$), 4.19–4.32 (m, 3H, Ar–O–CH_2–C*H*(OH)), 5.47 (br s, 2H, $(-CH_2$–O*H*$)_2$), 5.89 (d, J = 4.9 Hz, 1H, –CH–O*H*), 7.38–7.84 (m, 5H, Ar–H), 8.09 (br s, 1H, –NH*H*$^+$–), 8.19 (d, J = 8.5 Hz, 1H, 8Ar–H), 8.62 (br s, 1H, –N*H*H$^+$–). R_f = 0.11 (methanol/ethyl acetate (1:1)).

5.3. Pharmacology

5.3.1. Animals and experimental conditions

The studies were carried out on normotensive male Wistar rats weighing 170–350 g (Source: Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Cracow, Poland, stocks name: KRF: WI(WU). The animals were kept in plastic cages in a room at a temperature of 20 ± 4 °C, under 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.). They were fed with granulated feed (standard laboratory pellets) and had free access to water. The control and study groups consisted of six animals each. All procedures were conducted according to the Animal Care and Use Committee guidelines, and approved by the Ethical Committee of Jagiellonian University, Cracow.

5.3.2. Drugs

The tested compounds **1**–**8** were synthesized at the Department of Chemical Technology and Biotechnology of Drugs UJ CM. Adrenaline (Polfa, Warsaw, Poland), propranolol (Fluka Chemie AG, Seelze, Germany), [^{3}H]CGP-12177 (NEN-Du Pont, Warsaw, Poland), [^{3}H]prazosin (Amersham, Uppsala, Sweden), sodium heparin (Polfa, Warsaw, Poland), thiopental sodium (Biochemie Gmbh, Vienna, Austria) were dissolved in saline. The tested compounds were administered intravenously (iv.) at a constant volume of 1 mL/kg and were investigated at the wide range of doses starting at a dose of 10 mg/kg.

5.3.3. The effect on the normal electrocardiogram

Electrocardiographic investigations were carried out using ASPEL ASCARD B5 apparatus, (Aspel SA, Zabierzów, Poland) standard lead II and paper speed of 50 mm/s. The ECG was recorded just prior to and also 1, 5, and 15 min following the administration of the compounds.

5.3.4. Adrenaline-induced arrhythmia

Prophylactic antiarrhythmic activity was determined according to the method of Szekeres and Papp.[20] The arrhythmia was evoked in rats under anesthesia with thiopental (75 mg/kg, ip.) by iv. injection of adrenaline (20 μg/kg). The studied compounds were administered via iv. route 15 min before adrenaline administration. The criteria of antiarrhythmic activity were the lack of premature beats and inhibition of cardiac arrhythmia in comparison to the control group. The ED_{50} value was calculated according to the method of Litchfield and Wilcoxon.[21]

5.3.5. The effect on the arterial blood pressure

Male Wistar normotensive rats were anesthetized with thiopental (75 mg/kg) 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, Columbus OH, USA). The studied compounds were injected into the caudal veins of rats after a 5 min stabilization period, at a constant volume of 1 mL/kg.

5.3.6. Adrenoceptor radioligand-binding assay

The experiments were conducted on the rat cerebral cortex. [^{3}H]prazosin (19.5 Ci/mmol, α_1-adrenergic receptor) and [^{3}H]CGP-12177 (48 Ci/mmol, β_1-adrenergic receptor) were used. The membrane preparation and the assay procedure were carried out according to the published procedure[22] with slight modifications. The brains of the rats were homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.6) and centrifuged at 20,000g for 20 min (0–4 °C). The cell pellet was resuspended in Tris–HCl buffer and centrifuged again.

The final incubation mixture (final volume 300 μL) consisted of 240 μL membrane suspension, 30 μL of a [^{3}H]prazosin (0.2 nM) or [^{3}H]CGP-12177 (0.2 nM) solution and 30 μL buffer containing from seven to eight concentrations (10^{-11}–10^{-4} M) of investigated compounds. For measuring unspecific binding, phentolamine—10 μM (in the case of [^{3}H]prazosin), and propranolol—1 μM (in the case of [^{3}H]CGP-12177), were applied. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter (Perkin Elmer, USA). All assays were done in duplicates. Radioligand binding data were analyzed using iterative curve fitting routines with GraphPAD/Prism, Version 3.0 (GraphPad ware, San Diego, CA, USA). K_i values were calculated using the Cheng and Prusoff equation.[23]

5.3.7. Anticonvulsant and neurotoxicity assay

Initial evaluations for anticonvulsant activity were performed due to the ADD (anticonvulsant drug development) program Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, Rockville, USA. The evaluations of anticonvulsant activity included phase I test procedure. The screens were performed in male Carworth Farms no. 1 (CF 1) mice (18–25 g). In the phase I studies which deal with qualitative assay, all the compounds were tested for activity in the MES test as well as in the rotorod screen for TOX. The examined compounds were suspended in 0.5% aq methylcellulose and then administered at three dosage levels (30, 100 and 300 mg/kg) with anticonvulsant activity observed 0.5 and 4 h after *i.p.* administration in mice. The details of these procedures were published formerly.[24]

5.4. Statistical analysis

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

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References and notes

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