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Synthesis and preliminary evaluation of pharmacological properties of some piperazine derivatives of xanthone

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ABSTRACT

A series of 9 piperazine derivatives of xanthone were synthesized and evaluated for cardiovascular activity. The following pharmacological experiments were conducted: the binding affinity for adrenoceptors, the influence on the normal electrocardiogram, the effect on the arterial blood pressure and prophylactic antiarrhythmic activity in adrenaline induced model of arrhythmia (rats, iv). Three compounds revealed nanomolar affinity for α_1-adrenoceptor which was correlated with the strongest cardiovascular (antiarrhythmic and hypotensive) activity in animals' models. The most promising compound was 4-(3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**12**) which revealed antiarrhythmic activity with ED_{50} value of 0.69 mg/kg in adrenaline induced arrhythmia (rats, iv). Other synthesized xanthone derivatives, that is, (*R*,*S*)-4-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**10**) and (*R*,*S*)-4-(2-acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**11**) also acted as potential antiarrhythmics in adrenaline induced model of arrhythmia in rats after intravenous injection (ED_{50} = 0.88 mg/kg and 0.89 mg/kg, respectively). These values were lower than values obtained for reference drugs such as propranolol and urapidil, but not carvedilol.

Results were quite promising and suggested that in the group of xanthone derivatives new potential antiarrhythmics and hypotensives might be found.

1. Introduction

Cardiovascular diseases including coronary heart disease, cerebrovascular disease, hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure are caused by disorders of the heart and blood vessels. Even though there are many modern and effective drugs, cardiovascular diseases still remain the main cause of death, being responsible for 30% of all global deaths.[1]

Arrhythmias account for nearly one quarter of all cardiovascular-related deaths. The majority of them is due to cardiac rhythm disturbances which can be caused by many different conditions. The key role in the pathogenesis of abovementioned cardiac dysfunctions, plays activation of sympathetic nervous system. Therefore, it is not surprising that adrenergic receptors antagonists are effective in the treatment of this disease and are widely used. Sympathetic nervous system is not the only one responsible for cardiac rhythm disturbances. It's worth noticing that the action potential of heart cells also depends on the proper interplay of ion channels, especially sodium, potassium, and calcium.[2] Thus, taking into account the variety of arrhythmias' types, their diverse aetiology and numerous side effects of antiarrhythmic drugs (such as tiredness, changes in mood, sleep disturbances, dry mouth, blurry vision or impotence) there is still a need to search for new agents that can improve heart function with minimal side effects.[3]

Many efforts in this field were made in the group of xanthone derivatives. The first investigation of xanthones date back to 1990. Valenti and co-workers studied a series of xanthone 1,4-dihydropiridine derivatives, xanthone analogues of nifedipine. The tests that had been performed proved negative inotropic and chronotropic properties as well as selectivity on pacemaker activity of these compounds, although precise mechanism of their action was not clearly explained.[4]

Another, serious cardiovascular disorder is hypertension. Most cases of hypertension are so-called essential or primary hypertension, with unknown aetiology. Only 5% of cases, named secondary

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hypertension, have known aetiology and are associated with for example, arteriosclerosis or hyperthyreosis. Nowadays, the number of patients suffering from hypertension is still increasing, partially due to current lifestyle: lack of physical activity, overweight or obesity, using tobacco, too much sodium and too little potassium in diet, too little vitamin D in diet, drinking too much alcohol and stress. Other risk factors are age and family predispositions. Taking into account the global scale of this disorder, searching for new potential hypotensive agents seems to be reasonable.[5]

Xanthone itself was proved to posses vasorelaxating properties in thoracic aorta isolated from rats.[6] These data suggested that xanthone-induced vasorelaxation was endothelium independent and the mechanism might involve an increase in intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) and the blockade of Ca^{2+} channels. Other research on a series of aminoalkanolic xanthone derivatives confirmed hypotensive activity of all tested compounds. It was noticed that, an oxypropanolamine side chain substituted at the C-3 position of the xanthone nucleus significantly enhanced the hypotensive activity. The most potent seemed to be 3-(3*N*-*iso*-propylamine-2-hydroxypropoxy)-9*H*-xanthen-9-one (xanthonolol), which lowered systolic blood pressure by about 32–7% in dependence of dosage (from 5 to 0.1 mg/kg). Its mechanism of action was calcium channel dependant and required the blockade of beta adrenergic receptors.[7] Xanthonolol possess typical, β-blocker moiety (3-amine-2-hydroxypropan-1-yloxy) characteristic for cardiovascular drugs such as propranolol and carvedilol.

The newest communications exhibited interesting properties of xanthone-amine derivatives without typical β-blocker structure and bearing relatively long (4–5 methylene groups) alkane linker connecting pharmacophore structures.[8]

Our Laboratory of Bioorganic Chemistry, Chair of Organic Chemistry (previously Department of Technology and Biotechnology of Drugs) has also documented experience in searching for hypotensive, aminoalkanolic xanthone derivatives.[9,10] The strongest hypotensive effects were observed for compounds containing piperazine moiety. These compounds showed both in vitro (via binding affinity evaluations) and in vivo activity.[10] Piperazine moiety was mentioned as cardiovascular pharmacophore elsewhere.[11]

Basing on literature survey, herein we report in vitro and in vivo cardiovascular activity of some new structures. Structures were designed to combine xanthone and piperazine rings. Some of them possessed typical β-blocker moiety (3-amine-2-hydroxypropan-1-yloxy), others were devoided of hydroxyl group analogously to structures described by Lin et al.[8] and were evaluated to estimate role of hydroxyl group. Piperazine like amines were also diversified. Most of them contained methoxyphenylpiperazine—structural element of urapidil (typical α_1-adrenoceptor antagonist), others contain benzylpiperazine, phenoxyethylpiperazine, cinnamylpiperazine to ascertain pharmacophoric effect of methoxyphenylpiperazine or piperazine itself. We also introduced chlor substituent into xanthone ring to enhance lipophilicity and examine its effect on cardiovascular system.

2. Chemistry

The synthetic route that was used to synthesize of starting materials is outlined in Scheme 1. The detailed description of the method and physico-chemical properties of 4-((oxiran-2-yl)-methoxy)-9*H*-xanthen-9-one, 3-chloro-5-((oxiran-2-yl)methoxy)-9*H*-xanthen-9-one and 4-(3-bromopropoxy)-9*H*-xanthen-9-one were described elsewhere.[12–15] 3-Chloro-5-(3-bromopropoxy)-9*H*-xanthen-9-one was obtained analogously to 4-(3-bromopropoxy)-9*H*-xanthen-9-one from 3-chloro-5-hydroxy-9*H*-xanthen-9-one. The crude products were recrystallized from n-hexane/toluene (1:4). These intermediates are characterised in Experimental protocols.

Compounds **10** and **12–18** were obtained by amination of respective parent compounds with appropriate amines in *n*-propanol (for **10**, **15–18**) or toluene in a presence of K_2CO_3 (for **12–14**), as described previously.[13,16] In addition, compound **10** underwent acetylation to get compound **11**. Acetylation was performed using acetic anhydride, according to well known procedures. All resulted bases were converted into hydrochloride salts using an excess of ethanol saturated with HCl. The crude products were recrystallized from acetone/ethanol (1:3). These procedures are summarized in Scheme 1. Physicochemical properties of compounds **10–18** are summarized in Experimental protocol. In order to optimize synthesis of the tested compounds, compound **10** was obtained also using alternative method including (*R*,*S*)-4-(3-chloro-2-hydroxypropoxy)-9*H*-xanthen-9-one as intermediate (see Scheme 1). This pathway of the synthesis was conducted analogously to the earlier described procedure.[12] Nevertheless, this method seems to be less potent, so other compounds were obtained using oxirane derivatives as intermediates. Structures of the tested compounds are presented in Table 1.

3. Pharmacology

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, Krakow, 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, Krakow.

3.2. Drugs

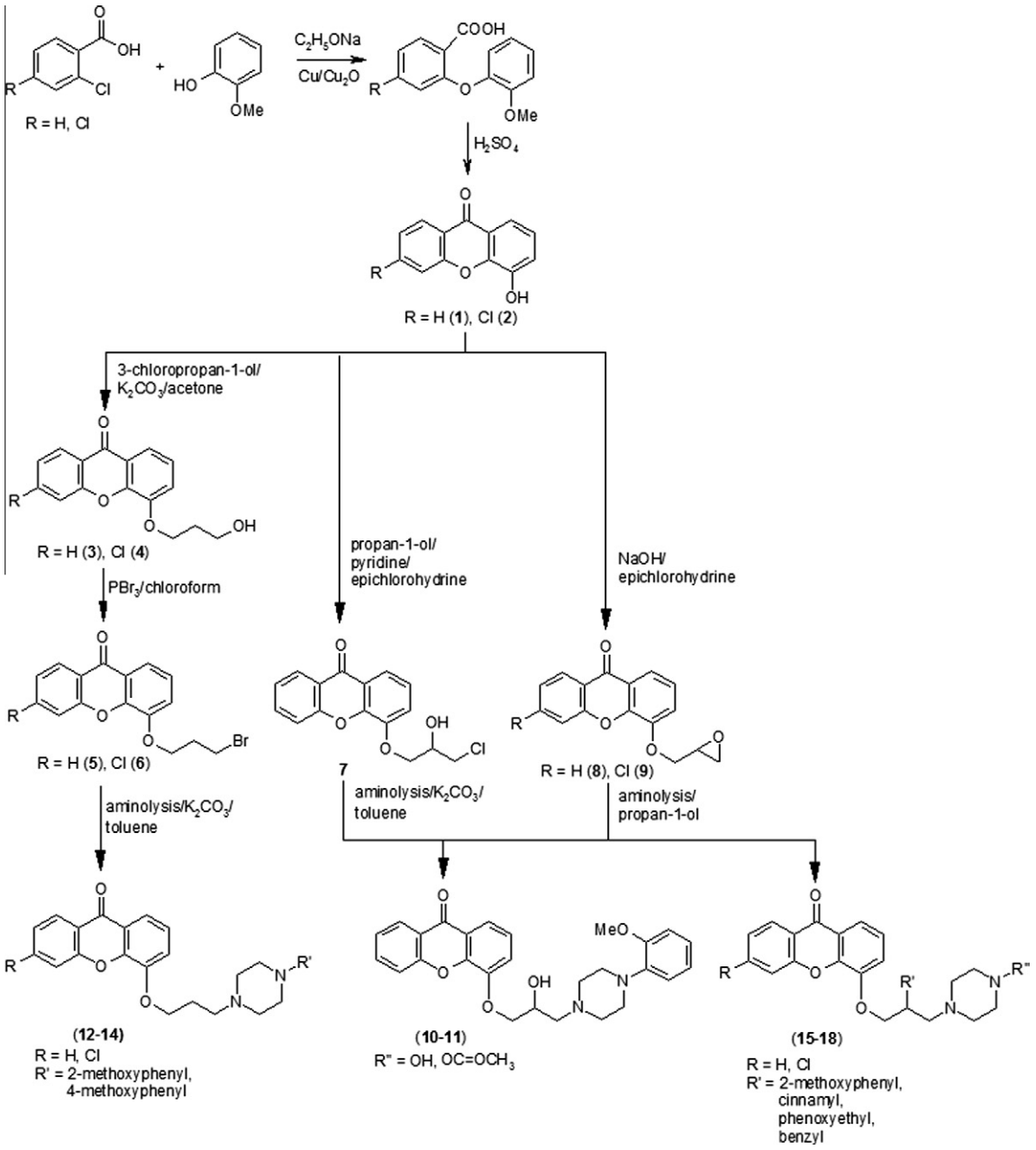
The tested compounds **10–18** were synthesized at the Department of Bioorganic Chemistry UJ CM. Adrenaline (Polfa, Warsaw, Poland), propranolol (Fluka Chemie AG, Seelze, Germany), [^{3}H]CGP-12177 (NEN-Du Pont, Warsaw, Poland), [^{3}H]prazosin (Amersham, Uppsala, Sweden), sodium heparin (Polfa, Warsaw, 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.

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.

3.4. Adrenaline-induced arrhythmia

Prophylactic antiarrhythmic activity was determined according to the method of Szekeres and Papp[17] 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



Scheme 1. General synthetic route of the tested compounds **1**–**18**.

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.[18]

3.5. The effect on the arterial blood pressure

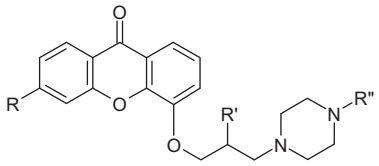
Male Wistar normotensive rats were anesthetized with thiopental (75 mg/kg) by intraperitoneally injection. The right carotid artery was cannulated with polyethylene tub filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments, Columbus OH, USA). 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.

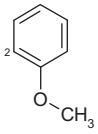
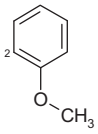
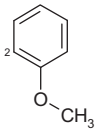
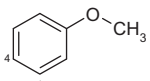
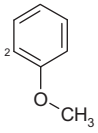
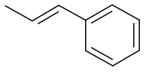
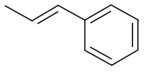
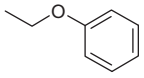
3.6. Adrenoceptor radioligand binding assay

The experiments were conducted on the rat cerebral cortex. [^{3}H]prazosin (19.5 Ci/mmol, α_1-adrenergic receptor), [^{3}H]clonidine (70.5 Ci/mmol, α_2-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[19] 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 20,000×*g* 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), [^{3}H]clonidine (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), 10 μM clonidine (in the case of [^{3}H]clonidine) 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

Table 1
Chemical structures of the tested compounds **10–18**



Compound	R	R′	R″	Form
10	H	OH		HCl
11	H			HCl
12	H	H		HCl
13	H	H		HCl
14	Cl	H		HCl
15	H	OH		2HCl
16	Cl	OH		2HCl
17	Cl	OH		2HCl
18	Cl	OH		2HCl

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.[20]

4. Pharmacological results

4.1. Radioligand binding assay

The pharmacological profile of the new compounds was evaluated by radioligand binding assays. The results obtained are presented in Table 2. Compounds **10–12** and **14** displaced [^{3}H]prazosin from cortical binding sites in low concentration range (K_i = 0.004–0.082 μM). All tested compounds moderately inhibited [^{3}H]clonidine binding and inhibited [^{3}H]CGP12177 binding to cortical β_1-adrenoceptors with μM-range.

4.2. The influence on the normal electrocardiogram

Effect on ECG intervals and the heart rate was determined for a dose of 2.5 mg/kg (compd **10–12**), 5 mg/kg (compd **15**, **16**) or 10 mg/kg (compd **13**, **17**, **18**). Table 3 shows effects of the tested compounds on the heart rate and ECG parameters. Among all studied compounds only **13**, **15** and **17** significantly decreased the number of cardiac beats per minute –**13** by 6%, **15** by 10%, **17** by 16%. Compounds **13** and **15** also significantly prolonged the QT interval and QRS complex.

Table 2
Affinity of compounds **10–18** to α_1-, α_2- and β_1-adrenoceptors

Compound	K_i ± SEM (μM)		
	α_1-Adrenoceptors	α_2-Adrenoceptors	β_1-Adrenoceptors
10	0.018 ± 0.001	4.260 ± 0.100	7.6 ± 3.30
11	0.050 ± 0.009	2.100 ± 0.100	11.2 ± 0.50
12	0.004 ± 0.001	0.695 ± 0.097	3.2 ± 0.30
13	1.100 ± 0.200	–	31.6 ± 2.10
14	0.082 ± 0.006	5.500 ± 0.200	4.5 ± 0.40
15	0.677 ± 0.025	0.634 ± 0.055	5.2 ± 0.10
16	1.100 ± 0.100	3.400 ± 0.300	43.0 ± 2.2
17	0.506 ± 0.020	0.450 ± 0.046	–
18	0.614 ± 0.035	7.700 ± 0.800	27.6 ± 0.20

K_i ± SEM values was obtained from 2 to 3 experiments in duplicates.

4.3. Anti-arrhythmic activity

Prophylactic anti-arrhythmic 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 (100%), which led to the death of approximately 76% of animals of the control group within 15 min of the observation. The tested compounds **10–13**, **15** and **7** injected intravenously 15 min before adrenaline, diminished the occurrence of heart-rhythm disturbances.

Among studied compounds, the strongest prophylactic anti-arrhythmic activity was shown by compounds **10–12**. Their ED_{50} values (a dose producing 50% inhibition of premature ventricular beats) are presented in Table 4. These data show that compound **12** was the most active in this test. This compound as well as compounds **11** and **12** prevented the appearance of other adrenaline-induced arrhythmia symptoms (bradycardia, blocks, bigeminy) and significantly reduced mortality. Tested compounds were more active in this experiment than reference drugs propranolol and urapidil, but not than carvedilol.

4.4. Influence on blood pressure

The hypotensive activity of compounds **10–12** and **15–18** was determined after their iv administration to anaesthetized, normotensive rats. The results are shown in Tables 5 and 6. Four compounds (**10–12** and **17**) significantly decreased systolic and/or diastolic blood pressure in anaesthetized, normotensive rats. Particularly potent hypotensive effect was observed after application of compounds **10** and **12**. Intravenous injections of compound **10** at the dose of 0.31 mg/kg significantly reduced systolic (by 19%) and diastolic (by 17%) blood pressure in normotensive rats and the hypotensive effect lasted throughout the observation. At a lower dose (0.16 mg/kg), compound **10** significantly reduced the blood pressure from the 20th minute of observation (by 14–15%). Compound **11** at the dose of 1.25 mg/kg significantly reduced the systolic blood pressure by 17–15% and diastolic blood pressure by 19–13%. At a lower dose (0.625 mg/kg), the compound **11** significantly reduced systolic blood pressure by 12–10% and diastolic blood pressure by 19–13%. At a dose of 0.31 mg/kg, compound **11** did not have any significant influence on blood pressure (data not shown). Compound **12** revealed hypotensive activity at the

Table 3
The effect of an intravenous injection of the tested compounds on the heart rate and ECG intervals in anaesthetized male Wistar rats

Compound	Dose (mg/kg)	Parameters	Time of observation (min)			
			0	5	10	15
10	2.5	PQ (ms)	43.2 ± 1.2	44.3 ± 0.4	43.3 ± 0.5	42.8 ± 0.8
		QRS (ms)	20.0 ± 0.3	19.6 ± 0.5	19.8 ± 0.5	20.2 ± 0.5
		QT (ms)	68.0 ± 1.1	69.7 ± 1.1	68.2 ± 1.1	68.4 ± 1.2
		Beats per min	330.7 ± 14.2	341.2 ± 23.3	315.2 ± 16.4	303.3 ± 15.3
11	2.5	PQ (ms)	48.0 ± 2.0	48.0 ± 2.0	48.0 ± 2.0	48.0 ± 2.0
		QRS (ms)	19.0 ± 0.6	19.0 ± 0.6	19.0 ± 0.6	19.0 ± 0.6
		QT (ms)	59.3 ± 0.7	61.80 ± 1.3	60.0 ± 0.0	60.00 ± 0.0
		Beats per min	375.0 ± 0.0	375.0 ± 0.0	369.0 ± 5.95	366.3 ± 8.72
12	2.5	PQ (ms)	42.0 ± 0.5	43.0 ± 0.6	43.2 ± 0.7	43.5 ± 0.7
		QRS (ms)	19.9 ± 0.7	20.4 ± 0.4	20.6 ± 0.4	20.3 ± 0.2
		QT (ms)	68.5 ± 1.4	70.0 ± 2.3	69.8 ± 1.9	71.8 ± 1.8
		Beats per min	322.7 ± 12.7	314.4 ± 14.2	306.7 ± 17.4	295.7 ± 12.4
13	10	PQ (ms)	41.6 ± 3.0	43.5 ± 2.8	43.7 ± 2.1	43.7 ± 2.9
		QRS (ms)	20.4 ± 0.4	22.8 ± 0.5*	23.6 ± 0.6**	24.2 ± 0.6**
		QT (ms)	78.2 ± 1.2	79.4 ± 1.5	80.2 ± 1.3*	82.4 ± 0.7*
		Beats per min	333.3 ± 10.5	323.0 ± 12.4	317.4 ± 12.2	311.4 ± 11.1*
15	5	PQ (ms)	39.6 ± 2.0	41.5 ± 2.9	41.7 ± 3.1	41.7 ± 3.1
		QRS (ms)	21.8 ± 0.3	23.4 ± 0.4**	23.5 ± 0.4**	24.0 ± 0.4***
		QT (ms)	73.2 ± 0.7	76.4 ± 0.9*	77.0 ± 0.9**	77.6 ± 0.9**
		Beats per min	341.8 ± 11.7	328.0 ± 8.6	311.3 ± 9.0*	309.0 ± 7.0*
16	5	PQ (ms)	41.7 ± 1.6	45.7 ± 2.5	46.0 ± 2.2	45.2 ± 2.1
		QRS (ms)	20.0 ± 0.0	20.2 ± 0.1	20.2 ± 0.3	20.3 ± 0.2
		QT (ms)	70.4 ± 2.4	73.6 ± 2.9	74.7 ± 2.9	74.6 ± 0.2
		Beats per min	345.8 ± 13.7	317.3 ± 10.5	324.3 ± 8.5	329.3 ± 5.8
17	10	PQ (ms)	29.84 ± 1.72	33.12 ± 1.80	33.60 ± 2.11	32.81 ± 2.42
		QRS (ms)	19.12 ± 1.14	18.80 ± 0.95	20.32 ± 0.54	20.40 ± 0.62
		QT (ms)	50.80 ± 2.12	54.40 ± 1.23	50.48 ± 1.77	53.20 ± 1.25
		Beats per min	338.13 ± 7.72	303.69 ± 10.41	242.43 ± 54.15	283.41 ± 10.33***
18	10	PQ (ms)	39.3 ± 0.4	41.9 ± 0.6	41.1 ± 0.5	40.9 ± 0.6
		QRS (ms)	18.7 ± 0.9	19.2 ± 0.8	19.6 ± 0.9	20.1 ± 0.7
		QT (msms)	78.5 ± 1.9	74.0 ± 1.3	72.8 ± 2.9	78.8 ± 2.5
		Beats per min	323.0 ± 20.1	295.3 ± 20.7	285.0 ± 23.9	268.8 ± 24.8

The data are means of six experiments ± SEM.
Statistical analyses were performed using one-way ANOVA test:
* p <0.05.
** p <0.02.
*** p <0.01.

Table 4
The prophylactic anti-arrhythmic activity in adrenaline induced arrhythmia in anesthetized rats

Compound	ED_{50} (mg/kg)
10	0.88 (0.38–2.00)
11	0.87 (0.41–1.82)
12	0.69 (0.31–1.58)
Propranolol	1.05 (0.64–1.73)
Urapidil[a]	1.26 (0.97–1.64)
Carvedilol[b]	0.25 (0.12–0.53)

Route of administration—iv.
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[18].
[a] Ref. 28.
[b] Ref. 29.

doses of 0.625–0.16 mg/kg and reduced both systolic (17–6%) and diastolic (16–14%) blood pressure. Compound **17** at a dose of 1.25 mg/kg significantly reduced only systolic blood pressure from the 20th minute of observation (by 7–10%). Compounds **15**, **16** and **18** were found to be inactive.

5. Discussion

The aim of this study was to evaluate cardiovascular activity of new compounds-derivatives of xanthone. This study included in particular: design, synthesis, physico-chemical analysis, antiarrhythmic and hypotensive activity estimation and measuring of binding affinity for adrenoceptors.

In the first step the affinity for adrenoceptors was determined using a radioligand binding assay. The highest affinity for α_1-adrenoceptors revealed compounds: **12** > **10** > **11** > **14**, containing 2-methoxyphenylpiperazine moiety in their structure.

The antiarrhythmic effects of novel compounds were examined in rats using the model of adrenaline-induced arrhythmia. The most active were compounds **10**–**12**, administered iv 15 min before arrhythmogen, which prevented or attenuated the symptoms of adrenaline-induced arrhythmia. Data reported in Table 4 suggest that all compounds show more preferable therapeutic indexes than propranolol (a non-selective beta-adrenoceptor blocker) and urapidil (α_1-adrenoceptor antagonist and an 5-HT_{1A} receptor agonist), but not carvedilol (a non-selective beta-adrenoceptor blocker with alpha 1-blocking properties).

Hypotensive activity of investigated compounds was determined after iv administration to normotensive anesthetized rats. The most active were compounds **10**–**12**, which showed a significant hypotensive effect throughout the whole observation period. The hypotensive effect was probably the result of strong α_1-adrenoceptors blockade. Compounds **15**–**17** were less active. Compound **18** slightly increased blood pressure.

Results obtained via adrenoceptor binding assay correlated with in vivo antiarrhythmic and hypotensive evaluation. Compounds **10–12**, which were the most potent substances in hypotensive tests, displaced [^{3}H]prazosine from cortical binding sites

Table 5
Hypotensive activity of compounds **10**, **11** and **12** in anesthetized normotensive rats after iv administration

Compound	Dose mg/kg	Blood pressure (mmHg)	Time of observation (min)					
			0	5	10	20	30	60
10	0.31	Systolic	144.0 ± 3.7	122.8 ± 2.3***	121.8 ± 2.8***	120.6 ± 2.3***	118.6 ± 1.2***	116.0 ± 1.2***
		Diastolic	118.2 ± 3.2	102.8 ± 3.8**	103.0 ± 3.7**	102.0 ± 3.2***	100.6 ± 2.1***	98.4 ± 1.6***
	0.16	Systolic	131.8 ± 3.9	124.0 ± 2.6	124.8 ± 2.9	118.5 ± 3.3**	115.7 ± 3.5***	115.2 ± 2.9***
		Diastolic	115.3 ± 3.9	109.2 ± 2.8	110.2 ± 3.3	103.8 ± 4.0*	101.8 ± 4.1*	101.5 ± 3.3**
11	1.25	Systolic	140.2 ± 3.5	119.0 ± 2.8****	118.4 ± 3.1****	117.0 ± 2.9****	117.6 ± 3.5****	116.6 ± 4.2****
		Diastolic	108.2 ± 5.7	92.8 ± 4.5*	93.6 ± 5.0*	92.2 ± 5.5*	90.8 ± 5.3*	89.6 ± 5.5**
	0.625	Systolic	139.4 ± 8.2	124.8 ± 3.6**	123.8 ± 3.1**	122.2 ± 5.0***	120.6 ± 3.5***	123.2 ± 3.5***
		Diastolic	105.0 ± 4.1	95.6 ± 3.2	96.4 ± 2.7	95.4 ± 3.5*	93.2 ± 2.1**	92.0 ± 3.3***
12	0.625	Systolic	138.5 ± 1.5	122.0 ± 2.9***	125.5 ± 3.2**	129.5 ± 3.6*	129.5 ± 2.5*	129.5 ± 1.5*
		Diastolic	116.2 ± 4.5	101.0 ± 4.1*	106.5 ± 4.4	110.5 ± 4.8	111.7 ± 4.0	112.0 ± 2.3
	0.31	Systolic	135.3 ± 5.8	122.0 ± 3.9*	122.8 ± 4.5	121.0 ± 4.0*	119.5 ± 3.3*	116.0 ± 6.3**
		Diastolic	116.3 ± 5.7	107.2 ± 5.9	107.7 ± 5.7	105.7 ± 5.4	103.3 ± 4.8	97.2 ± 6.7*
	0.16	Systolic	124.2 ± 3.3	109.6 ± 4.1*	114.0 ± 4.5	113.4 ± 5.9*	111.4 ± 4.6*	108.2 ± 5.1*
		Diastolic	110.2 ± 3.8	97.0 ± 4.8*	99.2 ± 4.8	101.0 ± 5.0	100.0 ± 4.3	95.2 ± 5.1*

The data are means of five to six experiments ± SEM.
Statistical analyses were performed using one-way ANOVA test:
* p <0.05.
** p <0.02.
*** p <0.01.
**** p <0.001.

Table 6
Hypotensive activity of compounds **15**–**18** in anesthetized normotensive rats after iv administration

Compound	Dose mg/kg	Blood pressure (mmHg)	Time of observation (min)					
			0	5	10	20	30	60
15	2.5	Systolic	137.6 ± 4.4	144.2 ± 4.1	144.2 ± 4.1	139.8 ± 4.3	132.6 ± 5.0	125.6 ± 4.7
		Diastolic	115.4 ± 5.9	118.4 ± 6.7	117.2 ± 5.4	114.0 ± 5.1	110.8 ± 5.7	105.0 ± 4.8
16	2.5	Systolic	136.20 ± 2.08	125.00 ± 8.55	133.00 ± 2.95	130.80 ± 1.68	129.80 ± 2.03	126.80 ± 1.02
		Diastolic	114.40 ± 1.57	103.20 ± 7.49	110.60 ± 1.69	110.40 ± 1.22	110.80 ± 1.02	108.60 ± 0.93
17	1.25	Systolic	123.80 ± 2.44	117.20 ± 3.53	115.20 ± 7.84	110.80 ± 9.52*	113.20 ± 6.97***	114.80 ± 3.90***
		Diastolic	108.40 ± 5.08	102.60 ± 3.65	101.20 ± 7.10	97.40 ± 8.45	100.60 ± 5.36	103.60 ± 3.81
18	2.5	Systolic	143.0 ± 4.2	147.2 ± 4.6	149.5 ± 4.5	152.5 ± 2.5	152.2 ± 2.4	151.2 ± 0.9
		Diastolic	124.0 ± 4.0	128.7 ± 4.8	132.2 ± 4.9	134.5 ± 2.5	134.2 ± 2.0	134.2 ± 1.3

The data are means of five to six experiments ± SEM.
Statistical analyses were performed using one-way ANOVA test:
* p <0.05.
*** p <0.01.

in low concentration range (K_i = 18, 50 and 4 nM), whereas other compounds moderately inhibited [^{3}H]prazosine binding with μM-range. It is well known that α_1-adrenoceptors play key role in the stimulation of smooth muscle contraction. They stimulate the contraction of veins and arteries and in consequence increase the resistance in circulatory blood flow. In that way, α_1-adrenoceptors are able to regulate blood pressure. As α_1-receptors are important for maintenance of peripheral vascular resistance, α_1-receptor antagonists may be used to relax blood vessels in hypertensive patients and to reduce symptoms of benign prostate hyperplasia. Classic α_1-antagonists, like prazosin, doxazosin and terazosin proved to be a highly effective antihypertensive drugs and remain important options in the treatment regimen available for hypertension.[21]

The above mentioned compounds are also potential antiarrhythmic agents. In human heart, β_1- and β_2-adrenoceptors coexist, whereby β_1-adrenoceptors predominate; in general, the ratio β_1-:β_2-adrenoceptors is about 70%:30% in the atria and 80%:20% in the ventricles. On the other hand 15–20% of adrenoceptors in human heart are different subtypes of α_1-adrenoceptors, especially α_{1A} and α_{1B}.[22] In previously published data α_1-blocking drugs, such as prazosin and phentolamine, have been shown to be effective against ischemia-induced arrhythmias in a variety of animal models. Other studies using transgenic animals have demonstrated that these two α_1-adrenoceptors subtypes expressed in the heart are functionally distinct. To date, the data imply that α_{1B}-adrenoceptors are associated with cardiac growth and α_{1A}-adrenoceptors with contractility and possibly with cardioprotection. It seems that both α_1-adrenoceptors subtypes can provide protection under ischaemia/reperfusion conditions but involve different mechanisms.[23]

Taking into account structure-activity relationship, it can be stated that the most active compounds contained 2-methoxyphenylpiperazine moiety (**10**–**12** and **14**). Compound **13** bearing 4-methoxyphenylpiperazine moiety was less potent in in vivo tests than its 2-substituted analogues and also inhibited [^{3}H]prazosine binding with μM-range in contrast to compounds **10**–**12** that displaced [^{3}H]prazosine from cortical binding sites in nM-range. In vivo tests indicated that compounds **10**–**12** were α_1-receptor antagonists. The binding affinity for α_2-receptors and β_1-adrenoceptors of all of the tested compounds were in μM-range and revealed any structural relationship. Introduction of the chlor substituent in the xanthone ring also diminished binding affinity for α_1-adrenoceptors (compare compounds **12** with **14** and **15** with **16** in Table 2) suggesting that enhancing of the lipophilicity was not desirable. From the synthesized compounds, **12** seemed to be the most potent. This compound was devoided of hydroxyl group and contained 3-amine propan-1-yloxy linker instead of typical β-blocker: 3-amine-2-hydroxypropan-1-yloxy linker. This observation

proved in vitro screenings that evaluated structures acting via α_1-adrenoceptors rather than β_1-adrenoceptors.

6. Conclusion

In conclusion, we have synthesized a series of 9 piperazine like derivatives of xanthone. All compounds were in vitro screened for their affinity for chosen adrenoceptors. Then, in vivo antiarrhythmic and hypotensive activity of the structures was tested. Three compounds revealed nanomolar affinity for α_1-adrenoceptor as well as the highest cardiovascular efficacy in animals' models. The most promising compound was 4-(3-(4-(2-methoxyphenyl) piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**12**) which revealed ED_{50} value of 0.69 mg/kg in adrenaline induced arrhythmia (rats, iv). Other synthesized xanthone derivatives, that is, (*R*,*S*)-4-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**10**) and (*R*,*S*)-4-(2-acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (**11**) were also proved to act as antiarrhythmics and showed ED_{50} values in adrenaline induced arrhythmia (rats, iv) of 0.88 and 0.89 mg/kg, respectively. These values were lower than values obtained for reference drugs such as propranolol and urapidil. The pharmacological results and binding studies suggested that the antiarrhythmic and hypotensive activity of these compounds were related to their adrenolytic properties. More extensive pharmacological studies that are in progress will expand the knowledge of the other possible mechanisms of cardiovascular action of the tested compounds (i.e. antioxidant, vasorelaxant effect via calcium entry blocking activity or the impact on NO pathway).

The results of the tested substances are quite encouraging, therefore further modification of their structures might lead to discovering of new potential drugs. Potential pathways of modifications will include: changes in the longevity of alkane linker, changes in the place of substitution in the xanthone ring and introduction of other substituents in the xanthone ring.

7. Experimental protocols

Reagents: benzylpiperazine, epichlorohydrine, phenoxyethylpiperazine were purchased from Sigma–Aldrich Chemie (Steinheim, Germany), whereas reagents: 2-chlorobenzoic acid, 3-chloro-1-propanol, cinnamylpiperazine, 2,4-dichlorobenzoic acid, 2-methoxyphenol, 2-methoxyphenylpiperazine, 4-methoxyphenylpiperazine, 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 were recorded on a Bruker AMX spectrometer (Brucker, Karlsruhe, Germany) with 500.13 MHz or Varian Mercury-VX 300 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using signal from DMSO in DMSO-d_6 and $CHCl_3$ in $CDCl_3$ 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), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), ddd (double doublet of doublets), qu (quintet), m (multiplet). Elemental analyses were performed on an Elementar Vario EL III (Elementar Analysansysteme, Hanau, Germany). For mass spectrometry analysis samples were prepared in acetonitrile/water (10/90 v/v) mixture. The LC/MS system consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All the analyses were carried out using an Acquity UPLC BEH C18, 1.7 μm, 2.1 × 100 mm column. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 10 min and then 100% B over 2 min was used. Eluent A: water/0.1% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 50 to 1000 Da; eight scans were summed up to produce the final spectrum. 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.

The synthetic rout of the tested compounds and starting materials is outlined in Scheme 1. Synthesis of the compounds mentioned above was multistage. In the first stage there was synthesized xanthone skeleton. This synthesis included Ullmann's condensation of appropriate chlorobenzoic acid and phenol derivative.[24,25] The reaction was performed for few hours, in oil, in the 195–200 °C, in the presence of Cu/Cu_2O catalyst. After condensation, intermediate product underwent cyclization with concomitant demethylation in the presence of concentrated H_2SO_4. It was noticed that, the longer the mixture was heated, the more effective demethylation was achieved. The optimal refluxing time was 2 days. Then reacting mixture was poured into crashed ice, filtered and washed to neutral reaction. Unreacted acid was washed with $NaHCO_3$. The detailed methodology and physicochemical properties of 4-hydroxy-9*H*-xanthen-9-one and 3-chloro-5-hydroxy-9*H*-xanthen-9-one were described in.[14,15] Then synthesis underwent two different pathways to obtain bromopropoxy or oxirane xanthone's derivatives. 4-(3-Bromopropoxy)-9*H*-xanthen-9-one and 3-chloro-5-(3-bromopropoxy)-9*H*-xanthen-9-one were synthesized by the reaction of appropriate hydroxyxanthone with 3-chloropropanol in acetone, in the presence of the big excess of K_2CO_3. After that, solvent was distilled and unreacted hydroxyxanthone was elutriated using NaOH solution. Then obtained intermediate product was taken to bromination using PBr_3 in $CHCl_3$. Subsequently, $CHCl_3$ was distilled and unreacted intermediate product was washed with $NaHCO_3$. Crude product was recrystallized from *n*-hexane/toluene (1:6). The procedure and physicochemical properties of 4-(3-hydroxypropoxy)-9*H*-xanthen-9-one and 4-(3-bromopropoxy)-9*H*-xanthen-9-one were mentioned formerly.[14] 3-Chloro-5-(3-hydroxypropoxy)-9*H*-xanthen-9-one and 3-Chloro-5-(3-bromopropoxy)-9*H*-xanthen-9-one are new and their properties are summarized below. The oxirane derivatives of xanthone were obtained in the reaction of appropriate hydroxyxanthone with epichlorohydrine according to procedures described earlier.[13] Epichlorohydrine was used in double excess. Reacting mixture was stirred during night, at the room temperature. After that, precipitate was filtered and unreacted substrates were washed with 10% NaOH solution. Physicochemical properties of 4-((oxiran-2-yl)-methoxy)-9*H*-xanthen-9-one were described in 14, whereas characteristic of 3-chloro-5-((oxiran-2-yl)methoxy)-9*H*-xanthen-9-one was published in.[15]

Compounds **10** and **12**–**18** were obtained by the amination of respective parent compound with appropriate amines in n-propanol (for **10**, **15**–**18**) or toluene in a presence of K_2CO_3 (for **12–14**), as described previously.[13,16] After aminolysis, solvents were distilled. Resulted amines were dissolved in diluted HCl, cleaned with charcoal and precipitated using NaOH solution. In addition, compound **10** underwent acetylation to get compound **11**. Compound **10** was dissolved in the smallest volume of acetic anhydride and stirred with concomitant heating for 4 h. Then, reacting mixture was pulled into the crushed ice, neutralized with $NaHCO_3$ and precipitated compound **11** was filtered. All resulted bases of compounds **10**–**18** were converted into hydrochloride salts using an excess of

ethanol saturated with HCl. The crude products were recrystallized from acetone/ethanol (1:3). Physicochemical properties of compounds **10**–**18** are summarized below. In order to optimize synthesis of the tested compounds, compound **10** was obtained also using alternative method including (*R*,*S*)-4-(3-chloro-2-hydroxypropoxy)-9*H*-xanthen-9-one as intermediate (see Scheme 1). (*R*,*S*)-4-(3-Chloro-2-hydroxypropoxy)-9*H*-xanthen-9-one was obtained in the reaction of equimolar amounts of 4-hydroxy-9*H*-xanthen-9-one and epichlorohydrine in propan-1-ol in the presence of pyridine. The reaction mixture was stirred for 1 h on water bath, then the unsoluble precipitate was filtered off. From the cooled filtrate the crude product was separated, filtered off and recrystallized from toluene/heptane (5:1) analogously to.[12,26] Nevertheless, this method seems to be less potent, so other compounds were obtained using oxirane derivatives as intermediates. Structures of the tested compounds are presented in Table 1.

7.1. 3-Chloro-5-(3-hydroxypropoxy)-9*H*-xanthen-9-one (compound 4)

3-Chloro-5-(3-hydroxypropoxy)-9*H*-xanthen-9-one (compound **4**) was obtained as white solid (yield 70%), mp 113–115 °C. Anal. calcd for $C_{16}H_{13}O_4Cl$: C, 63.06; H, 4.30. Found: C, 62.99; H, 4.52. IR (KBr) ν (cm^{-1}): 3412, 2935, 1668, 1460, 1271, 1223, 1064, 753. ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.25 (d, J = 8.5, 1H, H-8), 7.86 (dd, J = 7.1, J = 2.4, 1H, H-1), 7.57 (d, J = 2.0, 1H, H-5), 7.34 (dd, J = 8.6, J = 2.0, 1H, H-7), 7.31–7.24 (m, 2H, H-2, H-3), 4.33 (t, J = 5.9, 2H, Ar–O–CH_2–), 3.99 (t, J = 5.4, 2H, –CH_2–OH), 2.20 (qu, J = 5.9, 2H, –CH_2–CH_2–CH_2–). LC–MS: calcd for $[M+H]^+$: $C_{16}H_{14}O_4Cl$ m/z: 305.05, found 305.16. R_F = 0.60 (toluene/acetone (5:3)).

7.2. 3-Chloro-5-(3-bromopropoxy)-9*H*-xanthen-9-one (compound 6)

3-Chloro-5-(3-bromopropoxy)-9*H*-xanthen-9-one (compound **6**) was obtained as white solid (yield 65%), mp 140–142 °C. Anal. calcd for $C_{16}H_{12}O_3ClBr$: C, 52.27; H, 3.29. Found: C, 52.84; H, 3.22. IR (KBr) ν (cm^{-1}): 2933, 1644, 1428, 1273, 1064, 933, 755. ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.25 (d, J = 8.7, 1H, H-8), 7.88 (dd, J = 6.8, J = 2.8, 1H, H-1), 7.60 (d, J = 1.5, 1H, H-5), 7.36–7.24 (m, 3H, H-7, H-2, H-3), 4.29 (t, J = 5.9, 2H, Ar–O–CH_2–), 3.73 (t, J = 6.4, 2H, –CH_2–Br), 2.46 (qu, J = 6.2, 2H, –CH_2–CH_2–CH_2–). LC–MS: calcd for $[M+H]^+$: $C_{16}H_{13}O_3ClBr$ m/z: 366.97, found 367.12. R_F = 0.87 (methanol/ethyl acetate (1:3)).

7.3. (*R*,*S*)-4-(3-Chloro-2-hydroxypropoxy)-9*H*-xanthen-9-one (compound 7)

(*R*,*S*)-4-(3-Chloro-2-hydroxypropoxy)-9*H*-xanthen-9-one (compound **7**) was obtained as white solid (yield 70%), mp 158–160 °C (Lit. 158–159 °C).[27] Anal. calcd for $C_{16}H_{13}O_4Cl$: C, 63.06; H, 4.30. Found: C, 63.00; H, 4.67. IR (KBr) ν (cm^{-1}): 3455, 2930, 1642, 1420, 1271, 1053, 934, 749. ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.33 (dd, J = 8.0, J = 1.5, 1H, H-8), 7.93 (dd, J = 5.6, J = 4.1, 1H, H-1), 7.75–7.22 (m, 5H, H-2, H-3, H-5, H-6, H-7), 4.42–4.32 (m, 1H, =CH–), 4.31–4.24 (m, 2H, Ar–O–CH_2–), 3.95–3.83 (m, 2H, –CH_2–Cl), 2.94 (d, J = 5.9, 1H, –OH). LC–MS: calcd for $[M+H]^+$: $C_{16}H_{14}O_4Cl$ m/z: 305.05, found 305.16. R_F = 0.75 (methanol/ethyl acetate (1:3)).

7.4. (*R*,*S*)-4-(2-Hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound 10)

(*R*,*S*)-4-(2-Hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl) propoxy)-9*H*-xanthen-9-one hydrochloride (compound **10**) was obtained as white solid (yield 80%), mp 218–220 °C. Anal. calcd for $C_{27}H_{29}O_5N_2Cl$: C, 65.25; H, 5.88; N, 5.63. Found: C, 64.94; H, 6.17; N, 5.60. IR (KBr) ν (cm^{-1}): 3361, 2929, 1662, 1462, 1334, 1265, 1245, 1223, 10627, 930, 748. ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 8.21 (dd, J = 8.0, J = 1.5, 1H, H-8), 7.93–7.88 (m, 1H, H-6), 7.78 (dd, J = 7.8, J = 1.4, 1H, H-1), 7.76 (d, J = 7.2, 1H, H-5), 7.60 (dd, J = 8.1, J = 1.4, 1H, H-3), 7.50 (t, J = 8.0, 1H, H-7), 7.40 (t, J = 8.1, 1H, H-2), 7.02–6.90 (m, 4H, H-Ph), 6,12–6.08 (m, 1H, -OH), 4.58–4.48 (m, 1H, CH), 4.24–4.23 (m, 1H, Ar–O–CH_2–), 3.79 (s, 3H, Ar–O–CH_3), 3.67–3.03 (m, 10H, –N–CH_2–). LC–MS: calcd for $[M+H]^+$: $C_{27}H_{29}O_5N_2$ m/z: 461.20, found 461.45. R_F = 0.85 (mathanol/ethyl acetate (1:1)).

7.5. (*R*,*S*)-4-(2-Acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound 11)

(*R*,*S*)-4-(2-Acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound **11**) was obtained as a white solid (yield 70%), mp 214–216 °C. Anal. calcd for $C_{29}H_{31}O_6N_2Cl$: C, 64.62; H, 5.80; N, 5.20. Found: C, 64.83; H, 6.02; N, 5.57. IR (KBr) ν (cm^{-1}): 3430, 2935, 1648, 1594, 1500, 1449, 1275, 1229, 1082, 758. LC–MS: calcd for $[M+H]^+$: $C_{29}H_{31}O_6N_2$ m/z: 503.21, found 503.33. R_F = 0.79 (mathanol/ethyl acetate (1:1)). Base of compound **2**: ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.35 (dd, J = 8.0, J = 1.3, 1H, H-8), 7.94–7.26 (m, 6H, H–Ar), 6.99–6.84 (m, 4H, H-Ph), 5.53–5.50 (m, 1H, CH), 4.45 (d, J = 10.5, J = 3.6, 1H, Ar–O–C*H*H–), 4.32 (d, J = 10.5, J = 5.9, 1H, Ar–O–C*H*H–), 3.86 (s, 3H, Ar–O–CH_3), 3.13–3.00 (m, 4H, –N–CH_2– $(pip)_a$), 2.87–2.74 (m, 6H, –N–CH_2– $(pip)_e$ + –N–CH_2–R), 2.12 (s, 3H, –C–CH_3).

7.6. 4-(3-(4-(2-Methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound 12)

4-(3-(4-(2-Methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound **12**) was obtained as a white solid (yield 80%), mp 210–212 °C. Anal. calcd for $C_{27}H_{29}O_4N_2Cl$: C, 67.42; H, 6.08; N, 5.82. Found: C, 66.86; H, 6.51; N, 5.79. IR (KBr) ν (cm^{-1}): 3421, 3023, 2427, 1665, 1607, 1493, 1467, 1340, 1276, 1065, 746. ^1H NMR (DMSO-d_6, 500.13 MHz) δ ppm: 8.21 (ddd, J = 8.0, J = 1.8, J = 0.5, 1H, H-8), 7.89 (ddd, J = 8.7, J = 7.1, J = 1.8, 1H, H-6), 7.75 (dd, J = 8.0, J = 1.5, 1H, H-1), 7.71 (ddd, J = 8.5, J = 1.1, J = 0.5, 1H, H-5), 7.55 (dd, J = 8.0, J = 1.5, 1H, H-3), 7.50 (ddd, J = 8.0, J = 7.1, J = 1.1, 1H, H-7), 7.41 (dd, J = 8.0, J = 8.0, 1H, H-2), 6.99–6.84 (m, 4H, Ar–H), 4.27 (t, J = 6.4, 2H, Ar–O–CH_2–), 3.77 (s, 3H, Ar–O–CH_3), 3.06–2.92 (m, 4H, –N–CH_2– $(pip)_a$), 2.69–2.53 (m, 6H, –N–CH_2– $(pip)_e$ + –N–CH_2–R), 2.05 (qu, 2H, R–CH_2–R). LC–MS: calcd for $[M+H]^+$: $C_{27}H_{29}O_4N_2$ m/z: 445.20, found 445.36. R_F = 0.70 (methanol/ethyl acetate (1:1)).

7.7. 4-(3-(4-(4-Methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound 13)

4-(3-(4-(4-Methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound **13**) was obtained as a white solid (yield 65%), mp 214–216 °C. Anal. calcd for $C_{27}H_{29}O_4N_2Cl$: C, 67.42; H, 6.08; N, 5.82. Found: C, 67.28; H, 6.22; N, 5.48. LC–MS: calcd for $[M+H]^+$: $C_{27}H_{29}O_4N_2$ m/z: 445.20, found 445.36. R_F = 0.67 (methanol/ethyl acetate (1:1)). Base of compound **4**: IR (KBr) ν (cm^{-1}): 3413, 2919, 2850, 1672, 1607, 1514, 1451, 1261, 1226, 1071, 749. ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 8.35 (dd, J = 8.0, J = 1.5, 1H, H-8), 7.92–7.26 (m, 6H, H–Ar), 6.93–6.83 (m, 4H, H-Ph), 4.26 (t, J = 6.4, 2H, Ar–O–CH_2–), 3.77 (s, 3H, Ar–O–CH_3), 3.31–3.15 (m, 4H, –N–CH_2– $(pip)_a$), 2.72–2.68 (m, 6H, –N–CH_2– $(pip)_e$ + –N–CH_2–R), 2.17 (qu, J = 6.4, 2H, R–CH_2–R).

7.8. 3-Chloro-5-(3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound 14)

3-Chloro-5-(3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride (compound **14**) was obtained as a white solid (yield 70%), mp 270–272 °C. Anal. calcd for $C_{27}H_{28}O_4N_2Cl_2$: C, 62.91; H, 5.48; N, 5.44. Found: C, 62.90; H, 5.66; N, 5.41. LC–MS: calcd for $[M+H]^+$: $C_{27}H_{28}O_4N_2Cl$ *m*/*z*: 479.17, found 479.33. $R_F = 0.72$ (methanol/ethyl acetate (1:1)). Base of compound **5**: IR (KBr) *ν* (cm^{-1}): 3446, 2946, 2817, 1660, 1602, 1499, 1427, 1281, 1242, 1073, 757. ^1H NMR ($CDCl_3$, 300 MHz) *δ* ppm: 8.28 (d, *J* = 8.5, 1H, H-8), 7.88–7.29 (m, 5H, H-Ar), 7.03–6.86 (m, 4H, H-Ph), 4.26 (t, *J* = 6.4, 2H, Ar–O–CH_2–), 3.87 (s, 3H, Ar–O–CH_3), 3.14 (bs, 4H, –N–CH_2– $(pip)_a$), 2.74–2.69 (m, 6H, –N–CH_2– $(pip)_e$ + –N–CH_2–R), 2.17 (qu, *J* = 6.4, 2H, R–CH_2–R).

7.9. (*R*,*S*)-4-(3-(4-Cinnamylpiperazine-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride (compound 15)

(*R*,*S*)-4-(3-(4-Cinnamylpiperazine-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride (compound **15**) was obtained as a white solid (yield 75%), mp 256–258 °C. Anal. calcd for $C_{29}H_{32}O_4N_2Cl_2$: C, 64.09; H, 5.94; N, 5.15. Found: C, 63.73; H, 6.19; N, 5.10. IR (KBr) *ν* (cm^{-1}): 3300, 2977, 2361, 1604, 1572, 1492, 1450, 1340, 1275, 1073, 757. ^1H NMR (DMSO-d_6, 300 MHz) *δ* ppm: 8.21–7.32 (m, 12H, H–Ar), 6.87 (d, *J* = 15.9, 1H, =C<HPh), 6.43–6.33 (m, 1H, RH>C=), 4.49 (bs, 1H, –CH–), 4.22 (d, *J* = 4.6, 2H, Ar–O–CH_2–), 4.08–3.11 (m, 12H, –N–CH_2–, overlap with signal from H_2O in DMSO). LC–MS: calcd for $[M+H]^+$: $C_{29}H_{31}O_4N_2$ *m*/*z*: 471.22, found 471.35. $R_F = 0.53$ (methanol:ethyl acetate (1:1)).

7.10. (*R*,*S*)-3-Chloro-5-(3-(4-cinnamylpiperazine-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride (compound 16)

(*R*,*S*)-3-Chloro-5-(3-(4-cinnamylpiperazine-1-yl)-2-hydroxypropoxy)-9*H*-xanthen-9-one dihydrochloride (compound **16**) was obtained as a white solid (yield 75%), mp 252–254 °C. Anal. calcd for $C_{29}H_{31}O_4N_2Cl_3$: C, 60.27; H, 5.41; N, 4.85. Found: C, 60.57; H, 5.79; N, 4.89. ^1H NMR (DMSO-d_6, 300 MHz) *δ* ppm: 8.18 (d, *J* = 8.5, 1H, H-8), 7.92 (d, *J* = 1.8, 1H, H-5), 7.74 (dd, *J* = 8.0, *J* = 1.3, 1H, H-1), 7.60 (dd, *J* = 8.0, *J* = 1.3, 1H, H-3), 7.54 (dd, *J* = 8.5, *J* = 1.8, 1H, H-7), 7.50–7.21 (m, 6H, H-2, Phenyl), 6.87 (d, *J* = 15.6, 1H, =C<HPh), 6.38 (dt, *J* = 15.6, *J* = 7.4, 1H, RH>C=), 4.48 (bs, 1H, –CH–), 4.21 (d, *J* = 4.6, 2H, Ar–O–CH_2–), 3.92–3.37 (m, 12H, –N–CH_2–, overlap with signal from H_2O in DMSO). LC–MS: calcd for $[M+H]^+$: $C_{29}H_{30}O_4N_2Cl$ *m*/*z*: 505.18, found 505.32. $R_F = 0.46$ (methanol:ethyl acetate (1:1)).

7.11. (*R*,*S*)-3-Chloro-5-(2-hydroxy-3-(4-(2-phenoxyethyl)-piperazine-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride (compound 17)

(*R*,*S*)-3-Chloro-5-(2-hydroxy-3-(4-(2-phenoxyethyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one dihydrochloride (compound **17**) was obtained as a white solid (yield 70%), mp 246–248 °C. Anal. calcd for $C_{28}H_{31}O_5N_2Cl_3$: C, 57.79; H, 5.37; N, 4.81. Found: C, 57.28; H, 5.87; N, 4.78. IR (KBr) *ν* (cm^{-1}): 3426, 2949, 1656, 1603, 1494, 1428, 1277, 1224, 1074, 756. ^1H NMR (DMSO-d_6, 300 MHz) *δ* ppm: 8.19 (d, *J* = 8.7, 1H, H-8), 7.93 (d, *J* = 2.0, 1H, H-5), 7.75 (dd, *J* = 8.2, *J* = 1.3, 1H, H-1), 7.61 (dd, *J* = 8.0, *J* = 1.3, 1H, H-3), 7.54 (dd, *J* = 8.7, *J* = 1.8, 1H, H-7), 7.41 (t, *J* = 8.1, 1H, H-2), 7.34–6.95 (m, 5H, H-Ph), 4.51 (bs, 1H, CH), 4.38 (bs, 2H, –CH_2–O–Ph), 4.22 (d, *J* = 4.4, 2H, Ar–O–CH_2–), 4.04–3.02 (m, 12H, –N–CH_2–, overlap with signal from H_2O in DMSO). LC–MS: calcd for $[M+H]^+$: $C_{28}H_{30}O_5N_2Cl$ *m*/*z*: 509.18, found 509.31. $R_F = 0.55$ (methanol).

7.12. (*R*,*S*)-5-(3-(2-Benzylpiperazine-1-yl)-2-hydroxypropoxy)-3-chloro-9*H*-xanthen-9-one dihydrochloride (compound 18)

(*R*,*S*)-5-(3-(2-Benzylpiperazine-1-yl)-2-hydroxypropoxy)-3-chloro-9*H*-xanthen-9-one dihydrochloride (compound **18**) was obtained as a white solid (yield 65%), mp 286–288 °C. Anal. calcd for $C_{27}H_{29}O_4N_2Cl_3$: C, 58.76; H, 5.30; N, 5.08. Found: C, 58.84; H, 5.53; N, 5.11. IR (KBr) *ν* (cm^{-1}): 3389, 2994, 1657, 1601, 1492, 1428, 1271, 1220, 1074, 756. ^1H NMR (DMSO-d_6, 300 MHz) *δ* ppm: 8.20–7.37 (m, 11H, H–Ar), 4.48 (bs, 1H, –CH–), 4.21 (d, *J* = 4.6, 2H, Ar–O–CH_2–), 4.05–3.12 (m, 12H, –N–CH_2–, overlap with signal from H_2O in DMSO). LC–MS: calcd for $[M+H]^+$: $C_{27}H_{28}O_4N_2Cl$ *m*/*z*: 579.17, found 579.33. $R_F = 0.63$ (methanol:ethyl acetate (1:1)).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.11.014.

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