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Original research article

Biofunctional studies of new 2-methoxyphenylpiperazine xanthone derivatives with α_1-adrenolytic properties

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ABSTRACT

Background: The aim of this study was to assess the selectivity of the studied xanthone derivatives for α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1D}, α_{1L}) in functional experiments in order to verify if they possess any selectivity for a distinct subtype of α_1-adrenoceptor. Moreover, several pharmacological tests were carried out to assess whether they reveal other than α_1-adrenoceptor blocking properties such as: antagonistic for 5-HT_2 receptors, vasorelaxant or spasmolytic.

Methods: The influence on α_{1A}-adrenoceptors was examined in biofunctional studies employing isolated rat vas deferens, on α_{1B}-adrenoceptors in guinea-pig spleen, on α_{1D}-adrenoceptors in rat aorta, and on α_{1L}-adrenoceptors in rabbit spleen. Affinity for 5-HT_2 receptors was measured in radioligand binding assay, whereas antagonistic potency for 5-HT_2 receptors was studied on isolated rat aorta. Vasorelaxant effect of tested compounds was assessed in functional study employing rat aorta, whereas direct spasmolytic activity was investigated using the isolated rabbit small intestine.

Results: The present study provides evidences that the tested 2-methoxyphenylpiperazine xanthone derivatives are non-selective α_1-adrenoceptor blockers. However, at higher concentrations the direct spasmolytic effect could enhance their hypotensive activity. The obtained results indicate that the studied xanthones possessed weak calcium entry blocking activity, as well as antagonistic properties for 5-HT_{2A} receptors.

Conclusions: The results of the present study support the idea that the hypotensive activity of the studied compounds is related to their α_1-adrenolytic properties.

Introduction

Pharmacological research on the new xanthone derivatives with potential biological properties has remained the area of interest for the past several years and has been documented by a number of publications. This kind of compounds have been shown to possess profitable effects on several cardiovascular diseases. It has been reported that xanthone derivatives possess antiarrhythmic, hypotensive, vasorelaxant, antiplatelet, antithrombotic, anti-inflammatory, and antioxidant activities [1–5]. The biological activities of xanthones are associated with their tricyclic scaffold (dibenzo-γ-pyrones), however vary depending on the nature and/or position of substituents [6]. Lin et al. [7] have reported that ω-aminoalkoxylxanthones showed significant antiplatelet effect on the aggregation induced by thrombin, arachidonic acid, collagen or platelet activating factor, as well as vasorelaxant activity in rat thoracic aorta. Some of ω-aminoalkoxylxanthones, as well as xanthonoxypropanolamines have also shown hypotensive activity in normotensive rats [8]. Other research groups have reported the vasodilator action of 1,5-dihydroxy-2,3-dimetoxy-xanthone, which are connected with both endothelium-dependent and endothelium-independent mechanism [9,10]. Furthermore, it has been found that oxygenated and prenylated xanthones scavenge oxygen free radicals and inhibit the production of lipid

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peroxides in myocardial tissues [11]. 3,4,5,6-Tetraxydroxyxanthone possessed protective effect on myocardial ischaemia/reperfusion injury, which could be related to inhibition of TNF-α and reactive oxygen species production [12]. On the other hand, among polysulfated derivatives of xanthone dual anticoagulant/antiplatelet agents have been found [13]. Moreover, our recent findings suggest that among 4-aminoalkanolo- and 2-phenoxyethylpiperazinpropoxy-xanthone derivatives new compounds with antiarrhythmic and/or hypotensive activity may be found [5,14].

In our previous study, we described a series of the new 2-methoxyphenylpiperazine xanthone derivatives with prominent antiarrhythmic and hypotensive activity. These compounds possess high affinity for α_1-adrenoceptors, whereas affinities for α_2- and β_1-adrenoceptors are much weaker [15]. Moreover, we confirmed α_1-adrenolytic activity of the studied agents in the *in vitro* (isolated rat aorta contracted by phenylephrine) and *in vivo* (influence on blood vasopressor response in rats) tests [16]. The obtained results suggest that antiarrhythmic and hypotensive effects could be related to their α_1-adrenoceptors antagonistic properties.

Continuing this line of work, we have now assessed the selectivity of the studied xanthone derivatives for α_1-adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1D}, α_{1L}) in functional experiments in order to verify if they possess any selectivity for a distinct subtype of α_1-adrenoceptor. Moreover, several pharmacological tests were carried out to assess if other than α_1-adrenoceptor blocking properties are involved in the antiarrhythmic and/or hypotensive effect of the selected xanthone derivatives. Hence, we analyzed their affinity for 5-HT_2 receptors in radioligand assay, as well as antagonistic potency for 5-HT_2 receptors in isolated rat aorta. We also tested the interaction of the compounds with voltage-dependent calcium channels in functional study employing rat aorta and we checked if the tested agents possess direct spasmolytic activity using the isolated rabbit small intestine.

Materials and methods

Animals and experimental conditions

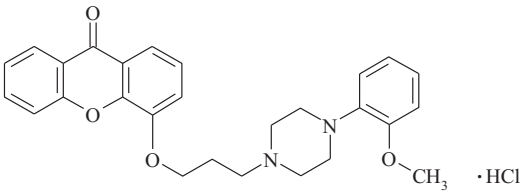
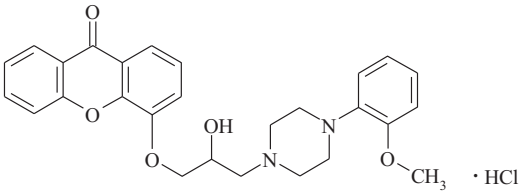
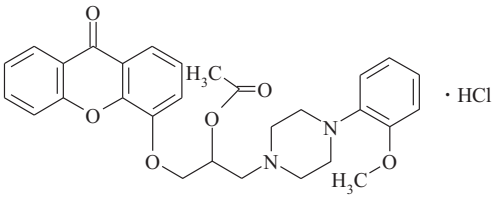
The experiments were carried out on male Wistar rats (180–230 g), Outbred CV guinea-pigs (250–300 g), New Zealand rabbits (1.5–2 kg). Animals were housed in cages in a room at a constant temperature of 20 ± 2 °C. They had free access to standard pellet diet and water. All the procedures were approved by the Local Ethics Committee of the Jagiellonian University in Kraków.

Chemicals

Three compounds, 2-methoxyphenylpiperazine derivatives of xanthone: **MH-94** (4-(3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride), **MH-99** ((*R*,*S*)-4-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride), **MH-105** ((*R*,*S*)-4-(2-acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride), were synthesized at the Department of Bioorganic Chemistry, Chair of Organic Chemistry, Jagiellonian University Medical College (Table 1). The synthesis of the investigated compounds was described earlier [15].

The following drugs were used: (±)-noradrenaline hydrochloride, acetylcholine hydrochloride, nifedipine, phentolamine hydrochloride, serotonin, yohimbine hydrochloride (Sigma–Aldrich, Germany), heparin sodium (Polfa, Poland), and thiopental sodium (Biochemie Gmbh, Austria). Other chemicals used were obtained from POCh (Poland).

Table 1
Schematic structure of the studied 2-methoxyphenylpiperazine derivatives of xanthone.

Compound	Structure
MH-94	
MH-99	
MH-105	

Experimental protocol

Influence on α_{1A}, α_{1B}, α_{1D} and α_{1L}-adrenoceptors

The influence on α_{1A}-adrenoceptors was studied employing isolated rat vas deferens. Rats were sacrificed by cervical dislocation under anaesthesia with thiopental sodium (75 mg/kg, *ip*) and vasa deferentia were carefully isolated and set up in 30 ml chambers containing Tyrode solution (NaCl 130 mM, KCl 2.0 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.9 mM, NaH_2PO_4 0.42 mM, $NaHCO_3$ 25 mM, glucose 5.6 mM) at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1), subjected to 1 g initial optimal tension for the recording of isometric contractile responses (FDT10-A force displacement transducer, BIOPAC Systems, Inc., COMMAT Ltd., Turkey).

The influence on α_{1B}-adrenoceptors was studied employing isolated guinea-pig spleen. Guinea-pigs were anaesthetized with thiopental sodium (100 mg/kg, *ip*) and exsanguinated from the common carotid arteries. The spleen was removed and cut into strips of approximately 20 mm, which were set up in organ bath under a resting tension of 1 g in a Krebs–Henseleit solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 1.9 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 11 mM, EDTA 0.05 mM) at 37 °C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1).

The influence on α_{1D}-adrenoceptors was studied employing isolated rat thoracic aorta. Rats were sacrificed by cervical dislocation under anaesthesia with thiopental sodium (75 mg/kg, *ip*) and the thoracic aorta was carefully dissected, cleaned of surrounding fat tissues, denuded of endothelium and cut into approximately 4 mm long rings. The aorta rings were mounted horizontally under isometric conditions in organ bath filled with a Krebs–Henseleit solution at optimal tension of 2 g and allowed to equilibrate.

The influence on α_{1L}-adrenoceptors was studied employing isolated rabbit spleen. Rabbits were sacrificed by cervical dislocation under anaesthesia with thiopental sodium (150 mg/kg, *ip*). The spleen was removed and cut into strips of approximately 20 mm, which were set up in organ bath under resting tension of 1 g in a Krebs–Henseleit solution. During a 100 min equilibration period, all tissues were stimulated with noradrenaline followed by washout

until the contractile response had become constant. Two cumulative concentration-response curves to noradrenaline were determined on each tissue at a 60 min interval in the absence and presence of antagonist. Tissues were incubated with tested compound for 30 min. The experiments were conducted in the continuous presence of propranolol (1 μM) to minimize the involvement of β-adrenoceptors in the response to noradrenaline [17–22].

Serotonin 5-HT$_2$ receptor radioligand binding assay

The experiments were conducted on the rat cerebral cortex. [^{3}H]ketanserin 60.0 Ci/mmol, was used 5-HT$_{2A}$ receptors. The assay procedure has been carried out according to the methods previously described by Czopek et al. [23]. K_i values were calculated using the Cheng and Prusoff equation [24].

Influence on 5-HT$_2$ receptors

Another series of experiments were performed in order to determine the antagonistic potency of the studied compounds to 5-HT$_2$ receptors located in the rat aorta. Rats were sacrificed by cervical dislocation under anaesthesia with thiopental sodium (75 mg/kg, *ip*) and the thoracic aorta was isolated, denuded of endothelium, cut, mounted and incubated as described above. After the equilibration period, 60 mM KCl was added to test for viability and to normalize individual aortic isometric force response to agonist. After removal of KCl, two cumulative concentration-response curves to serotonin were determined on each arterial ring at 60 min in the absence and presence of tested compounds.

Influence on the contraction induced by KCl

The influence on isolated rat aorta precontracted with KCl in order to examined their calcium entry blocking properties was studied as precisely described earlier [25]. Concentration-relaxation curves were received by cumulative addition of the tested compounds (**MH-94**, **MH-99**, **MH-105**, nifedipine) to the precontracted aortic preparations.

Influence on isolated rabbit ileum

The direct relaxant activity was studied employing isolated rabbit small intestine, as described previously [18]. The influence of every single dose was recorded for 5 min.

Data analysis

Concentration-response curves were analyzed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). Curves were fitted to all the data by non-linear regression to determine Hill slopes for the agonist concentration-response curves and to calculate EC_{50} values. Schild analysis was performed, and where the slope was not significantly different from unity, the pA_2 value was determined [19]. Where a slope appeared to be significantly different from unity, the affinity was estimated with the equation pK_B = log (concentration ratio − 1) − log (molar antagonist concentration) [18].

Concentration-relaxation curves were analyzed using GraphPad Prism 4.0 software. Relaxant responses are expressed as a percentage of inhibition of the maximal tension obtained with the contractile agent. The concentration needed to produce 50% of the maximum relaxation (IC_{50}) was calculated.

Statistically significant differences were calculated using one-way analysis of variance (ANOVA) and the *post hoc* Dunnett's test. The criterion for significance was set at $p < 0.05$.

Results

Functional affinity for α$_1$-adrenoceptor subtypes: α$_{1A}$, α$_{1B}$, α$_{1D}$ and α$_{1L}$

The antagonist activity of test compounds towards α$_{1A}$-adrenoceptors was assessed by inhibition of noradrenaline induced contractions in rat vas deferens. All tested compounds in a concentration-dependent manner produced a shift to right, however at higher concentrations reduced the maximum response (Figs. 1a–3a), which means that they did not show competitive antagonism. The strongest antagonistic activity revealed compound **MH-99** with pK_B value of 7.59 ± 0.02, whereas for compounds **MH-94** and **MH-105** the pK_B values were 7.39 ± 0.06 and 7.35 ± 0.02, respectively.

In contrast, the tested compounds in guinea-pig spleen caused parallel shifts to the right of noradrenaline concentration-response curve, indicating competitive antagonism at splenic α$_{1B}$-adrenoceptors, giving a Schild plot with a slope not significantly different from unity (Figs. 1b–3b). The strongest antagonistic activity

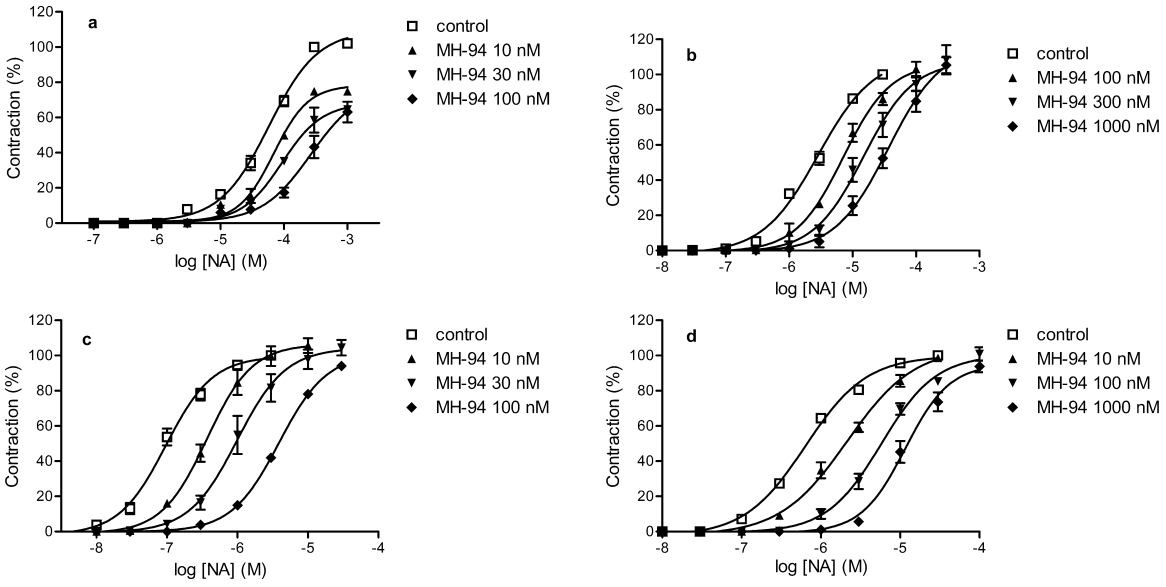


Fig. 1. Effect of compound **MH-94** on α$_1$-adrenoceptors. Concentration-response curves to noradrenaline (NA) in the absence (□) or presence of increasing concentration of **MH-94** (filled symbols). (a) rat vas deferens (α$_{1A}$-adrenoceptors), (b) guinea-pig spleen (α$_{1B}$-adrenoceptors), (c) rat aorta (α$_{1D}$-adrenoceptors), (d) rabbit spleen (α$_{1L}$-adrenoceptors). Values are expressed as percentage of the maximum contractile response induced by NA for each experiment. Each point represents the mean ± SEM (n = 4–8).

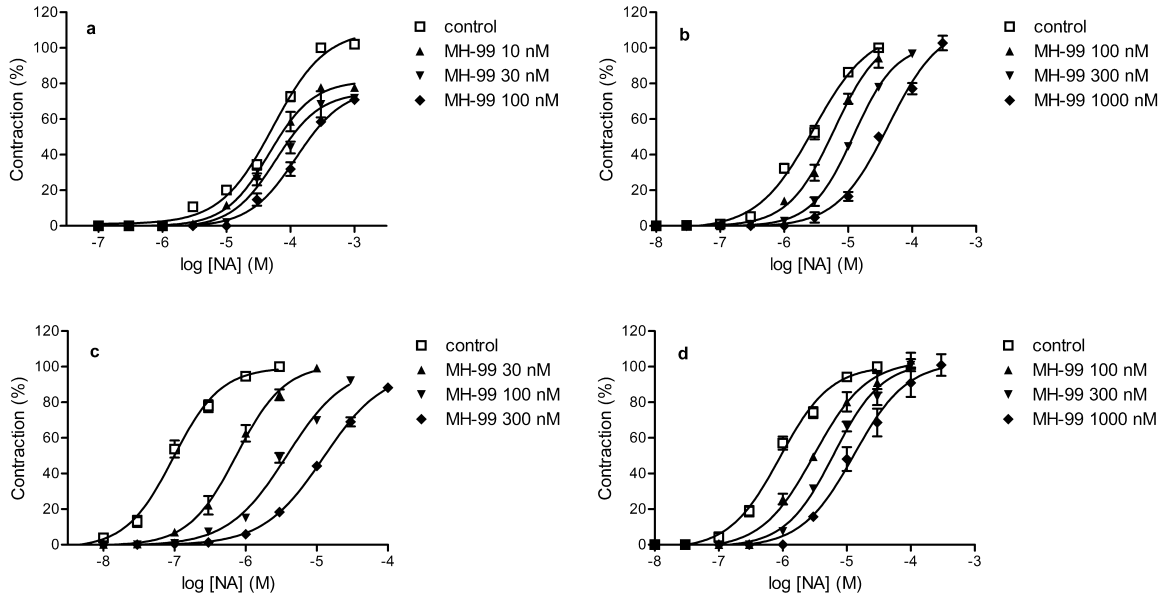


Fig. 2. Effect of compound **MH-99** on α_1-adrenoceptors. Concentration-response curves to noradrenaline (NA) in the absence (□) or presence of increasing concentration of **MH-94** (filled symbols). (a) rat vas deferens (α_{1A}-adrenoceptors), (b) guinea-pig spleen (α_{1B}-adrenoceptors), (c) rat aorta (α_{1D}-adrenoceptors), (d) rabbit spleen (α_{1L}-adrenoceptors). Values are expressed as percentage of the maximum contractile response induced by NA for each experiment. Each point represents the mean ± SEM (n = 4–8).

revealed compound **MH-105** with pA_2 value of 7.47 (s = 0.93 ± 0.01). Weaker antagonistic potency showed compounds **MH-94** and **MH-99** with a pA_2 values of 7.20 (s = 0.90 ± 0.01) and 7.05 (s = 1.07 ± 0.02), respectively.

Similarly, the tested compounds in isolated rat aorta antagonized noradrenaline evoked contraction and shifted the noradrenaline response to the right without affecting the maximum response, indicating competitive antagonism at α_{1D}-adrenoceptors in this tissue (Figs. 1c–3c). The strongest antagonistic activity revealed compound **MH-94** with pA_2 value of 8.39 (s = 1.14 ± 0.02). Weaker antagonistic potency showed compounds **MH-99** and **MH-105** with a pA_2 values of 8.25 (s = 1.16 ± 0.03) and 8.13 (s = 1.0 ± 0.06), respectively.

The tested compounds in rabbit spleen antagonized noradrenaline-evoked contraction and shifted the noradrenaline response to the right without affecting the maximum response, indicating competitive antagonism at α_{1L}-adrenoceptors in this tissue (Figs. 1d–3d). Compounds **MH-94** and **MH-105** revealed similarly antagonistic activity with pA_2 values of 7.69 (s = 0.98 ± 0.01) and 7.68 (s = 0.84 ± 0.03), respectively. Weaker antagonistic potency showed compounds **MH-99** with a pA_2 values of 7.54 (s = 0.84 ± 0.02). Table 2 summarizes the mean pA_2 and pK_B values and slopes determined from Schild plots for the tested compounds.

Binding for 5-HT$_{2A}$ receptors

The pharmacological profile of the new compounds for 5-HT$_{2A}$ receptors was evaluated by radioligand binding assays with [^{3}H]ketanserin as specific ligand. The results indicated that all

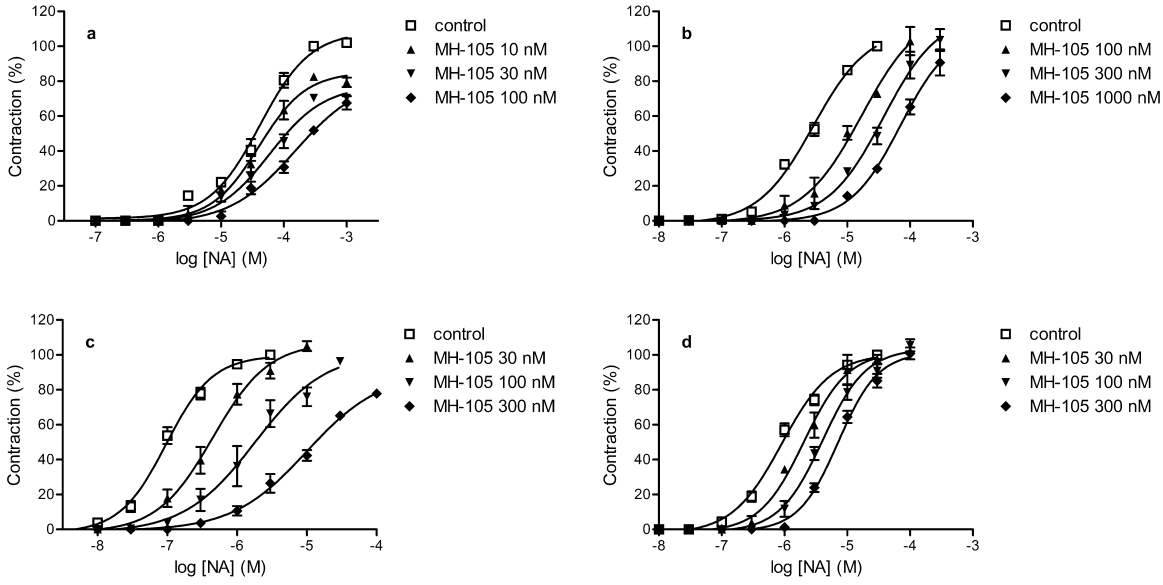


Fig. 3. Effect of compound **MH-105** on α_1-adrenoceptors. Concentration-response curves to noradrenaline (NA) in the absence (□) or presence of increasing concentration of **MH-94** (filled symbols). (a) rat vas deferens (α_{1A}-adrenoceptors), (b) guinea-pig spleen (α_{1B}-adrenoceptors), (c) rat aorta (α_{1D}-adrenoceptors), (d) rabbit spleen (α_{1L}-adrenoceptors). Values are expressed as percentage of the maximum contractile response induced by NA for each experiment. Each point represents the mean ± SEM (n = 4–8).

Table 2
Functional affinities of the tested compounds for α_{1A}-adrenoceptors in rat vas deferens, α_{1B}-adrenoceptors in guinea-pig spleen, α_{1D}-adrenoceptors in rat aorta and α_{1L}-adrenoceptors in rabbit spleen.

Compound	α_{1A}-adrenoceptors $pK_B \pm$ SEM	α_{1B}-adrenoceptors $pA_2 \pm$ SEM (slope $\pm$ SEM)	α_{1D}-adrenoceptors $pA_2 \pm$ SEM (slope $\pm$ SEM)	α_{1L}-adrenoceptors $pA_2 \pm$ SEM (slope $\pm$ SEM)
MH-94	7.39 ± 0.06	7.20 ± 0.03 (0.90 ± 0.01)	8.39 ± 0.09 (1.14 ± 0.02)	7.69 ± 0.02 (0.98 ± 0.01)
MH-99	7.59 ± 0.02	7.05 ± 0.05 (1.07 ± 0.02)	8.25 ± 0.07 (1.16 ± 0.03)	7.54 ± 0.02 (0.84 ± 0.02)
MH-105	7.35 ± 0.02	7.47 ± 0.01 (0.93 ± 0.01)	8.13 ± 0.08 (1.00 ± 0.06)	7.68 ± 0.07 (0.84 ± 0.03)

Antagonist potency of compounds are expressed as $pA_2/pK_B \pm$ SEM of 4–8 experimental results. pA_2 values were obtained from the linear regression of Schild plot. pK_B values were calculated according to relationship pK_B = log(concentration ratio − 1) − log(molar antagonist concentration).

the tested compounds possess weak affinity for 5-HT_{2A} receptors with $K_i > 500$ nM.

Serotonin-induced contraction of isolated rat aortic rings

The antagonistic potency of the studied compounds for 5-HT_2 receptors was studied using isolated rat aorta. Compounds **MH-99** and **MH-105** at the concentration of 1 μM did not influence contraction induced by serotonin. In contrast, compound **MH-94** shifted serotonin concentration-response curve to the right, however it reduced the maximum response, indicating non-competitive antagonism (Fig. 4). The pK_B value was 6.84 ± 0.02.

Influence on the voltage-dependent calcium channels

The studied compounds were tested on isolated rat aorta precontracted with high-K^+ due to investigate their calcium entry blocking properties. Compounds **MH-94** and **MH-105** at the range of concentration 1–300 μM relaxed KCl (60 mM)-precontracted aortic rings in dose-dependent manner with the IC_{50} value 21.2 and 30.8 μM, respectively (Fig. 5). However, none of the tested compounds was able to inhibit the contractile response completely, only by 77.9 and 64.5%, respectively. Compound **MH-99** at that range of concentration was not able to relax aortic rings. The value of maximal relaxation was only 21.4 for that compound. Nifedipine, which is well known voltage-dependent calcium channel blocker, inhibited completely contraction induced by KCl depolariznig solution. The IC_{50} value for nifedipine was 3.75 ± 0.2 nM (n = 4). Under the same experimental condition, urapidil was not able to induce relaxation of aortic rings [20]. The obtained results suggest that two compounds, **MH-94** and **MH-105**, may possess weak voltage-dependent calcium channel blocking properties.

Influence on isolated rabbit ileum

In order to clarify the possible direct relaxant activity of the tested compounds, an experiment on isolated rabbit ileum was performed. The tested compounds **MH-94** and **MH-105** influenced significantly the frequency of contractions of rabbit isolated small intestine only at the highest tested concentration (10 μM), whereas compound **MH-99** influenced the frequency of contraction also at the concentration of 1 μM. Two compounds **MH-94** and **MH-99** diminished significantly amplitude of contractions at concentrations of 1 and 10 μM, whereas **MH-105** only at the highest one. As shown in Figs. 6 and 7, **MH-94** decreased the frequency of contraction by 7.6% (10 μM) and the amplitude by 33.3% (1 μM) and 73.7% (10 μM). Compound **MH-99** decreased the frequency of contraction by 15% (1 μM) and 46.1% (10 μM), whereas amplitude by 31% (1 μM) and 87.6% (10 μM). **MH-105** decreased the frequency of contraction by about 66.1% and the amplitude by about 77.6% (10 μM). Under the same experimental condition, urapidil had no significant influence on of rabbit isolated small intestine [20]. In the control group the frequency was from 10.6 ± 0.5 to 12.5 ± 0.8/min; the amplitude from 2.7 ± 0.3 to 3.4 ± 0.6 g, n = 4–6. These results indicate that the compounds had some direct effects on the smooth muscles at higher concentrations.

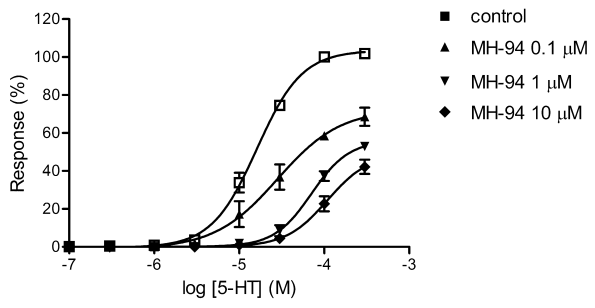


Fig. 4. Effect of compound **MH-94** on $5HT_{2A}$-adrenoceptors in isolated rat aorta. Concentration-response curves to serotonin (5-HT) in the absence (□) or presence of increasing concentration of **MH-94** (filled symbols). Values are expressed as percentage of the maximum contractile response induced by 5-HT for each experiment. Each point represents the mean ± SEM (n = 4).

Discussion

In previously published paper we described a series of xanthone derivatives which showed antiarrhythmic and hypotensive activity, as well as high affinity for α_1-adrenoceptors. The prominent antiarrhythmic and hypotensive activity was found for three compounds, named **MH-94**, **MH-99** and **MH-105**. These compounds possess high affinity for α_1-adrenoceptors (K_i = 4, 18 and 50 nM, respectively), whereas their affinity for α_2-adrenoceptors is much weaker (K_i = 0.69, 4.3 and 2.1 μM, respectively). They reveal also weak affinity for β_1-adrenoceptors (K_i = 3.2, 7.6 and 11.2 μM, respectively). These *in vitro* results reveal that the test compounds are highly selective for α_1-adrenoceptors compared to α_2-adrenoceptors (α_2/α_1 = 173, 42, 236, respectively) [15]. Our extended studies indicated that these compounds demonstrated prominent antiarrhythmic activity in both: prophylactic (given *iv* 15 min before arrhythmogen) and therapeutic (given *iv* at the peak of arrhythmia) model of adrenaline-induced arrhythmia. Moreover, we confirmed their α_1-adrenolytic activity in isolated rat

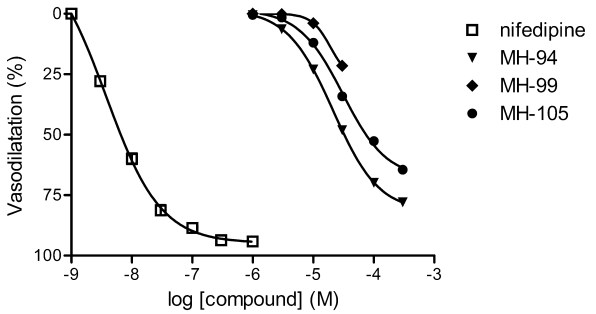


Fig. 5. Effect of the tested compounds and nifedipine on contraction of aortic rings induced by KCl (60 mM). Values are expressed as mean of 4 experiments for each.

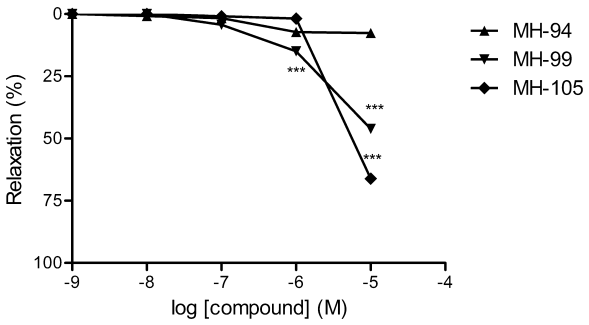


Fig. 6. Effect of the tested compounds on frequency of isolated rabbit ileum contractions. Values are expressed as mean of 4 experiments for each. Statistical analysis: repeated measure ANOVA; *post hoc* Dunnett's test: $^{***}p < 0.001$.

aorta contracted by phenylephrine, where they indicated competitive antagonism for α_1-adrenoceptors. Since all compounds were able to antagonize the vasopressor response elicited by adrenaline, noradrenaline and methoxamine and antagonize contraction induced by phenylephrine in functional studies, we supposed that the antiarrhythmic and hypotensive effects are related to their α_1-adrenoceptors antagonistic properties [16].

In the present study we assessed the selectivity of the studied xanthone derivatives for α_1-adrenoceptor subtypes in functional experiments. It is well established, that the α_1-adrenoceptor subtype predominant in the rat vas deferens has been classified as the α_{1A}-adrenoceptor, whereas contraction to noradrenaline in guinea-pig spleen is mediated predominantly by α_{1B}-adrenoceptors, and in rat aorta by α_{1D}-adrenoceptors [17,21,22]. Whereas, it seems that α_{1L}-adrenoceptor subtype is not new subtype, but represents a functional phenotype of α_{1A}-adrenoceptor subtype [22,26,27].

In human the α_{1A}-adrenoceptor subtype is predominant in prostate and urethra and plays role in mediating the contractile response of the prostate. α_1-adrenoceptor antagonists, such as alfuzosin, doxazosin, tamsulosin and silodosin are effective in reducing benign prostatic hyperplasia and lower urinary tract symptoms. Tamsulosin and silodosin are highly selective α_{1A}-adrenoceptor blockers, therefore they show little cardiovascular adverse events [28]. The group of α_1-adrenoceptor antagonists are well known drugs in the treatment of hypertension. Their action at these receptors prevents the vasoconstrictive effects of catecholamines, resulting in vasodilation and thus a reduction in peripheral vascular resistance. It is remarkable, that α_1-adrenoceptor antagonists, including prazosin, doxazosin and urapidil, have beneficial effects on glucose and lipid metabolism, therefore they may be particularly useful in patients with concomitant hyperlipidemia, type 2 diabetes and/or metabolic syndrome [29]. In the group of α_1-adrenoceptor antagonists, there is also

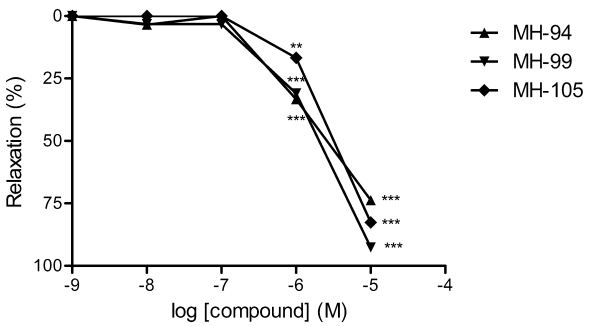


Fig. 7. Effect of the tested compounds on amplitude of isolated rabbit ileum contractions. Values are expressed as mean of 4 experiments for each. Statistical analysis: repeated measure ANOVA; *post hoc* Dunnett's test: $^{**}p < 0.01$, $^{***}p < 0.001$.

urapidil, which is peripheral postsynaptic α_1-adrenoceptor antagonist and in addition, it has central agonist action at serotonin 5-HT_1 receptors. Therefore it decreases the central sympathetic outflow and vagal stimulation, and prevents the reflex tachycardia often observed with vasodilator therapy [30]. Since urapidil in its structure contains the 2-methoxyphenylpiperazine moiety, which is also present in the structure of the tested compounds, it has been used as reference compound.

Functional studies demonstrate that the tested compounds revealed antagonistic properties to all subtypes of α_1-adrenoceptor. The strongest antagonistic activity for α_{1A}-adrenoceptors showed compound **MH-99** with pK_B value of 7.59, whereas for compounds **MH-94** and **MH-105** the pK_B values were 7.39 and 7.35, respectively. All tested compounds indicated competitive antagonism for splenic α_{1B}-adrenoceptors. The strongest antagonistic effect revealed compound **MH-105** with pA_2 value of 7.47. Weaker antagonistic potency showed compounds **MH-94** and **MH-99** with a pA_2 values of 7.20 and 7.05, respectively. Similarly, they indicated competitive antagonism for α_{1D}-adrenoceptors in rat aorta. The pA_2 values for compounds **MH-94**, **MH-99** and **MH-105** are 8.39, 8.25 and 8.13, respectively. Comparing the obtained pA_2 and pK_B values we could state, that the studied agents have similar functional affinities for α_{1A}- and α_{1B}-subtypes, while the affinity for α_{1D}-subtype is approximately 5–16-fold stronger than for α_{1B}-subtype. Moreover, all compounds show also similar affinity for α_{1A}- and α_{1L}-subtypes. In view of the foregoing, the results of functional studies indicate that the tested compounds are non-selective α_1-adrenoceptor blockers, which is in agreement with our earlier studies and support the idea that their prominent hypotensive and antiarrhythmic activities are connected first of all with α_1-adrenolytic properties.

However, as they antagonize α_{1A}- and α_{1D}-subtypes, they may be also beneficial for the treatment of hypertension with benign prostatic hyperplasia or lower urinary tract disorders [31,32]. It has been reported that terazosin (non-selective α_1-adrenoceptor antagonist), tamsulosin (selective $\alpha_{1A} > \alpha_{1D}$-adrenoceptor antagonist) and naftopidil (selective $\alpha_{1D} > \alpha_{1A}$-adrenoceptor antagonist) reduce the nocturia, while silodosin (highly selective α_{1A}-adrenoceptor antagonist) does not decrease the number of nighttime voiding. It suggests that α_{1D}-subtype plays an important role in the facilitation of voiding reflex [31].

The effects of serotonin in the cardiovascular system are complex. On the one hand, serotonin induces release of vasodilator substances such as nitric oxide by stimulating 5-HT_1 receptors on endothelium, on the other hand, vascular smooth muscle contraction induced by serotonin is mediated by 5-HT_{2A} receptors, which activates the rho/rho kinase pathway to regulation of myosin light chain and then vasoconstriction [33]. 5-HT_{2A} receptors are located also in platelets and their stimulation potentiates platelet aggregation [34,35]. Moreover, 5-HT_{2A} receptors are also involved in migration and cell proliferation [36,37]. Therefore, compounds with 5-HT_{2A} antagonistic properties are of significant interest, because of their potential involvement in mediating many cardiovascular diseases. Studies by various group using different models have demonstrated that xanthone possess prominent vasorelaxing and antiplatelet activities [7,13,38]. One of the possible mechanism of this action could be interaction with serotonin receptors located in blood vessels. Wang et al. have reported that 1,5-dihydroxy-2,3-dimetoxy-xanthone and its metabolite were able to antagonize contraction produce by serotonin in concentration-dependent manner, however, in case of parent drug the removal of endothelium significantly affected the vasodilator responses [8,9]. In another study γ-mangostin, which is prenylated xanthone derivative was found as specific 5-HT_{2A} receptor antagonist in vascular smooth muscle cells and platelets [39].

In the present study we used isolated rat aorta to assess antagonistic activity to 5-HT_{2A} receptors. After removing the endothelium, only these receptors are functionally present in rat aorta and mediate potent vasoconstriction [40]. Among tested compounds only **MH-94** revealed antagonistic activity to 5-HT_{2A}. However, its antagonistic potency is much lower ($pK_B = 6.84$) than obtained for ketanserin ($pK_B = 8.67$), which is well known 5-HT_{2A}-antagonist [40]. Compounds **MH-99** and **MH-105** at the concentration of 1 μM did not influence on contraction induced by serotonin. This is in agreement with radioligand binding assay, which shows that tested compounds possess weak affinity for 5-HT_{2A} receptors ($K_i > 500$ nM).

In the present study, the calcium entry blocking properties were examined on isolated rat aorta. The contraction elicited by KCl mainly results from the influx of extracellular Ca^{2+} induced by depolarization of cell membrane and subsequent opening of voltage-dependent calcium channels [41]. It has been reported that unsubstituted xanthone or simple oxygenated xanthones can block L-type Ca^{2+} channels and inhibit calcium influx through voltage-dependent and receptor-operated calcium channels [9,42]. However, all tested herein xanthone derivatives revealed weak Ca^{2+} entry blocking properties. The received EC_{50} values are about 1000 times higher than that of nifedipine, so their inhibitory effects on the contractile response caused by high K^+ are much weaker.

The results obtained from experiment with isolated rabbit ileum revealed that the studied compounds showed significant effect on the spontaneous frequency and amplitude of contractions of isolated rabbit intestine, but only at higher concentrations (1 and 10 μM). These results may indicate that the direct effect on the smooth muscle could enhance hypotensive activity caused by α_1-adrenoceptor blocking properties.

In conclusion, the present study provides evidences that the tested 2-methoxyphenylpiperazine xanthone derivatives are non-selective α_1-adrenoceptor blockers.

These data support the idea that the hypotensive activity of the studied compounds is related to their α_1-adrenolytic properties. However, at higher concentrations the direct spasmolytic effect could enhance their hypotensive activity. The obtained results indicate that the studied xanthones possessed weak calcium entry blocking activity, as well as antagonistic properties for 5-HT_{2A} receptors. Further pharmacological tests are currently being carried out in order to explore their influence on other serotonergic receptors (radioligand binding studies and *in vivo* pharmacological tests).

Conflict of interest

There is no conflict of interests.

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References

[1] Cheng YW, Kang JJ. Mechanism of vasorelaxation of thoracic aorta caused by xanthone. Eur J Pharmacol 1997;336(1):23–8.
[2] Jiang DJ, Dai Z, Li YJ. Pharmacological effects of xanthones as cardiovascular protective agents. Cardiovasc Drug Rev 2004;22(2):91–102.
[3] Librowski T, Czarnecki R, Jastrzębska-Więsek M, Opoka W, Marona H. The influence of some aminoalkanolic xanthone derivatives on central nervous and cardiovascular systems in rodents. Boll Chim Farm 2004;143(7):267–74.
[4] Lin CN, Chung MI, Liou SJ, Lee TH, Wang JP. Synthesis and anti-inflammatory effects of xanthone derivatives. J Pharm Pharmacol 1996;48(5):532–8.
[5] Marona H, Szkaradek N, Rapacz A, Filipek B, Dybała M, Siwek A, et al. Preliminary evaluation of pharmacological properties of some xanthone derivatives. Bioorg Med Chem 2009;17(3):1345–52.
[6] El-Seedi HR, El-Barbary MA, El-Ghorab DM, Bohlin L, Borg-Karlson AK, Göransson U, et al. Recent insights into the biosynthesis and biological activities of natural xanthones. Curr Med Chem 2010;17(9):854–901.
[7] Lin KW, Fang SC, Hung CF, Shieh BJ, Yang SC, Teng CM, et al. Synthesis, antiplatelet and vasorelaxing activities of xanthone derivatives. Arch Pharm (Weinheim) 2009;342(1):19–26.
[8] Wang LW, Kang JJ, Chen IJ, Teng CM, Lin CN. Antihypertensive and vasorelaxing activities of synthetic xanthone derivatives. Bioorg Med Chem 2002;10(3):567–72.
[9] Wang Y, Shi JG, Wang MZ, Che CT, Yeung JH. Mechanisms of the vasorelaxant effect of 1,5-dihydroxy-2,3-dimethoxy-xanthone, an active metabolite of 1-hydroxy-2,3,5-trimethoxy-xanthone isolated from a Tibetan herb, Halenia elliptica, on rat coronary artery. Life Sci 2008;82(1–2):91–8.
[10] Wang Y, Shi JG, Wang MZ, Che CT, Yeung JH. Mechanisms of the vasorelaxant effect of 1-hydroxy-2,3,5-trimethoxy-xanthone, isolated from a Tibetan herb, Halenia elliptica, on rat coronary artery. Life Sci 2007;81(12):1016–23.
[11] Santos CM, Silva AM, Filipe P, Santus R, Patterson LK, Mazière JC, et al. Structure-activity relationships in hydroxy-2,3-diarylxanthone antioxidants. Fast kinetics spectroscopy as a tool to evaluate the potential for antioxidant activity in biological systems. Org Biomol Chem 2011;9(10):3965–74.
[12] Dai Z, Jiang DJ, Hu GY, Du YH, Yu J, Hu CP, et al. 3,4,5,6-Tetrahydroxyxanthone protects against myocardial ischemia-reperfusion injury in rats. Cardiovasc Drugs Ther 2004;18(4):279–88.
[13] Correia-da-Silva M, Sousa E, Duarte B, Marques F, Carvalho F, Cunha-Ribeiro LM, et al. Polysulfated xanthones: multipathway development of a new generation of dual anticoagulant/antiplatelet agents. J Med Chem 2011;54(15):5373–84.
[14] Marona H, Szkaradek N, Kubacka M, Bednarski M, Filipek B, Cegła M, et al. Synthesis and evaluation of some xanthone derivatives for anti-arrhythmic, hypotensive properties and their affinity for adrenergic receptors. Arch Pharm (Weinheim) 2008;341(2):90–8.
[15] Szkaradek N, Rapacz A, Pytka K, Filipek B, Siwek A, Cegła M, et al. Synthesis and preliminary evaluation of pharmacological properties of some piperazine derivatives of xanthone. Bioorg Med Chem 2013;21(2):514–22.
[16] Rapacz A, Pytka K, Sapa J, Kubacka M, Filipek B, Szkaradek N, et al. Antiarrhythmic, hypotensive and α_1-adrenolytic properties of new 2-methoxyphenylpiperazine derivatives of xanthone. Eur J Pharmacol 2014;735:10–6.
[17] Chuliá S, Moreau J, Naline E, Noguera MA, Ivorra MD, D'Ocón MP, et al. The effect of S-(+)-boldine on the alpha 1-adrenoceptor of the guinea-pig aorta. Br J Pharmacol 1996;119(7):1305–12.
[18] Sapa J, Kubacka M. The possible mechanism of hypotensive activity of some pyrrolidin-2-one derivatives with antagonist properties at alpha1-adrenoceptors. Eur J Pharmacol 2011;673:40–8.
[19] Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. 1958. Br J Pharmacol 1997;120(Suppl. 4):151–61.
[20] Kubacka M, Mogilski S, Filipek B, Marona H. The hypotensive activity and alpha1-adrenoceptor antagonistic properties of some aroxyalkyl derivatives of 2-methoxyphenylpiperazine. Eur J Pharmacol 2013;698(1–3):335–44.
[21] Aboud R, Shafii M, Docherty JR. Investigation of the subtypes of alpha 1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. Br J Pharmacol 1993;109(1):80–7.
[22] Eltze M, Grebe T, Michel MC, Czyborra P, Ullrich B. Affinity profile at alpha(1)- and alpha(2)-adrenoceptor subtypes and in vitro cardiovascular actions of (+)-boldine. Eur J Pharmacol 2002;443(1–3):151–68.
[23] Czopek A, Byrtus H, Kołaczkowski M, Pawłowski M, Dybała M, Nowak G, et al. Synthesis and pharmacological evaluation of new 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-dione derivatives. Novel 5-HT1A receptor agonist with potential antidepressant and anxiolytic activity. Eur J Med Chem 2010;45(4):1295–303.
[24] Cheng YC, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 1973;22(23):3099–108.
[25] Rapacz A, Sapa J, Bednarski M, Filipek B, Szkaradek N, Marona H. Antiarrhythmic activity of some xanthone derivatives with β1-adrenoceptor affinities in rats. Eur J Pharmacol 2014;738:14–21.
[26] Hieble JP. Adrenoceptor subclassification: an approach to improved cardiovascular therapeutics. Pharm Acta Helv 2000;74(2–3):163–71.
[27] Jähnichen S, Eltze M, Pertz HH. Evidence that alpha(1B)-adrenoceptors are involved in noradrenaline-induced contractions of rat tail artery. Eur J Pharmacol 2004;488(1–3):157–67.
[28] Cui Y, Zong H, Zhang Y. The efficacy and safety of silodosin in treating BPH: a systematic review and meta-analysis. Int Urol Nephrol 2012;44(6):1601–9.
[29] Buch J. Urapidil, a dual-acting antihypertensive agent: current usage considerations. Adv Ther 2010;27(7):426–43.
[30] Dooley M, Urapidil Goa KL. A reappraisal of its use in the management of hypertension. Drugs 1998;56(5):929–55.
[31] Ishizuka O, Imamura T, Kurizaki Y, Nishizawa O, Andersson KE. Male lower urinary tract symptoms and α1D-adrenoceptors. Int Urol Nephrol 2013;20(1):73–8.
[32] Yamaguchi O. Latest treatment for lower urinary tract dysfunction: therapeutic agents and mechanism of action. Int Urol Nephrol 2013;20(1):28–39.
[33] Nelson PM, Harrod JS, Lamping KG. 5HT(2A) and 5HT(2B) receptors contribute to serotonin-induced vascular dysfunction in diabetes. Exp Diabetes Res 2012;2012:398406.
[34] Nagatomo T, Rashid M, Abul Muntasir H, Komiyama T. Functions of 5-HT2A receptor and its antagonists in the cardiovascular system. Pharmacol Ther 2004;104(1):59–81.

[35] Ogawa T, Sugidachi A, Tanaka N, Fujimoto K, Asai F. Pharmacological profiles of R-96544, the active form of a novel 5-HT2A receptor antagonist R-102444. Eur J Pharmacol 2002;457(2–3):107–14.
[36] Sharma SK, Zahradka P, Chapman D, Kumamoto H, Takeda N, Dhalla NS. Inhibition of serotonin-induced vascular smooth muscle cell proliferation by sarpogrelate. J Pharmacol Exp Ther 1999;290(3):1475–81.
[37] Tamura K, Kanzaki T, Saito Y, Otabe M, Saito Y, Morisaki N. Serotonin (5-hydroxytryptamine, 5-HT) enhances migration of rat aortic smooth muscle cells through 5-HT2 receptors. Atherosclerosis 1997;132(2):139–43.
[38] Pinto MM, Sousa ME, Nascimento MS. Xanthone derivatives: new insights in biological activities. Curr Med Chem 2005;12(21):2517–38.
[39] Chairungsrilerd N, Furukawa KI, Ohta T, Nozoe S, Ohizumi Y. Gamma-mangostin, a novel type of 5-hydroxytryptamine 2A receptor antagonist. Naunyn Schmiedebergs Arch Pharmacol 1998;357(1):25–31.
[40] Villazón M, Padín JF, Cadavid MI, Enguix MJ, Tristán H, Orallo F, et al. Functional characterization of serotonin receptors in rat isolated aorta. Biol Pharm Bull 2002;25(5):584–90.
[41] Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995;268(4 Pt 1):C799–822.
[42] Fang LH, Mu YM, Lin LL, Xiao PG, Du GH. Vasorelaxant effect of euxanthone in the rat thoracic aorta. Vasc Pharmacol 2006;45(2):96–101.