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Antihypertensive and Vasorelaxing Activities of Synthetic Xanthone Derivatives

Li-Wen Wang,[a] Jaw-Jou Kang,[b] Ing-Jun Chen,[c] Che-Ming Teng[d] and Chun-Nan Lin[a,*]

[a]*School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, ROC*
[b]*Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan 100, ROC*
[c]*Department of Pharmacology, Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan 807, ROC*
[d]*Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan 100, ROC*

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Abstract—A series of xanthones and xanthonoxypropanolamines have been synthesized. The activity of compounds on cardiovascular system was evaluated. All the compounds tested exhibited effective hypotensive activity in anesthetized rats. An oxypropanolamine side chain substituted at the C-3 position of the xanthone nucleus significantly enhanced the hypotensive activity. In rat thoracic aorta, all the compounds tested significantly depressed the contractions induced by Ca^{2+} (1.9 mM) in high K^+ (80 mM) medium and the phasic and tonic contractions caused by norepinephrine (3 μM). In the rat thoracic aorta, the phenylephrine- and high K^+-induced $^{45}Ca^{2+}$ influx were both inhibited by a selective xanthone derivative, **13**. In addition to the previously reported result of **13**, evaluated as beta adrenoceptor blocker, the depressor and bradycardia effects of **9** are independent of the parasympathetic passway. These results suggest that **13** showed inhibitory effects on the contractile response caused by high K^+ and norepinephrine in rat thoracic aorta are mainly due to inhibition of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. The vasodilating properties of **13** is due to its calcium channel and beta adrenergic blocking effects.

Introduction

A series of xanthones, xanthonoxypropanolamines, and ω-aminoalkoxyxanthones have been synthesized.[1–5] Most of the synthetic compounds showed antiplatelet effect in washed rabbit platelets and human platelet-rich plasma (PRP) and several compounds caused vasorelaxing action in rat thoracic aorta.[1–5] Two synthetic compounds, named xanthonolol (3-[3-(isopropylamino)-2-hydroxypropoxy]xanthone (**13**) and 3-hydroxyxanthone (**1**) reduced the blood pressure, heart rate, and L-isoproterenol-induced tachycardia in rats.[6] For evaluating compounds as a antithrombotic antihypertensive agent, we continually studied the cardiovascular, the structure–activity relationships of antihypertensive and vasorelaxing effects, of various xanthones, xanthonoxypropanolamines, and ω-aminoalkoxyxanthones and reported in the present paper.

*Corresponding author. Tel.: +886-7-3121101; fax: +886-7-5562365; e-mail: lincna@cc.kmu.edu.tw

Result and Discussion

The antihypertensve activity of these compounds was evaluated in male normotensive anaesthetized Wistar rats by intravenous administration at doses of 1, 5, or 10 mg/kg, respectively. All the compounds (Tables 1 and 2) were assayed for their ability to lower blood pressure in normotensive Wistar rat. As shown in Tables 1 and 2, all the compounds showed significant hypotensive activity. Of all oxygenated xanthones tested, **7** was the most effective one. A hydroxyl groups substituted at C-1,-5, or,-6 of **1** enhanced the hypotensive activity while it substituted at C-2 or -4 of **1** did not enhance the activity. Compounds **9** and **10**, did not show good hypotensive activity. Based on the above results, it clearly indicated that a xanthone molecule with orthodiphenolic moiety did not show potent hypotensive activity in normotensive rats. As shown in Table 2, an oxypropanolamine side chain substituted at C-3 of **1** significantly enhanced the hypotensive activity in normotensive rats. The greater activity of the branched *N*-alkyl compound (**13**) relative to the straight *N*-alkyl compound (**12**) reveals the steric preference for a branched radical substituted in the nitrogen atom. The

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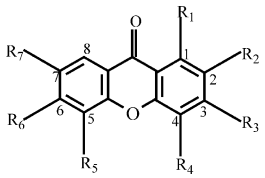
Table 1. Antihypertensive properties of syntetic xanthone derivatives

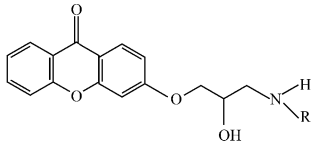


Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Blood pressure (mm Hg)[a]			
								Dose (mg/kg) iv	Before	After	Change (%)
1	H	H	OH	H	H	H	H	1	105.000±10.000	96.500±11.500	−8.1
								5	103.667±5.364	89.833±5.256	−13.3
								10	123.000±3.578	102.000±5.367*	−17.1
2	OH	H	OH	H	H	H	H	1	121.750±1.765	110.500±2.082*	−9.2
								5	137.000±1.371	109.250±1.803**	−20.3
3	H	OH	OH	H	H	H	H	1	109.600±3.415	101.000±3.795	−7.8
								5	110.600±2.064	99.200±2.596**	−10.3
								10	112.600±3.957	81.600±3.803**	−27.3
4	H	H	OH	OH	H	H	H	1	110.000±5.000	17.667±5.364	−11.2
								5	111.250±4.270	98.250±3.119*	−11.7
								10	116.250±6.787	87.500±5.852*	−24.7
5	OH	H	H	H	H	OH	H	1	117.500±2.887	108.500±3.152	−7.7
								5	101.667±1.126	87.000±3.550*	−19.2
								10	129.667±2.017	92.333±2.978**	−28.8
6	H	OH	H	H	H	OH	H	1	107.200±2.083	102.400±1.536	−4.5
								5	110.000±3.851	81.000±3.894*	−26.4
7	H	H	OH	H	OH	H	H	1	118.600±4.632	107.000±4.889	−9.8
								5	114.000±3.531	79.333±7.775**	−30.9
8	H	H	OH	H	H	OH	H	1	107.500±7.500	98.500±8.684	−8.4
								5	105.000±5.244	83.000±4.754*	−21.0
								10	115.000±1.401	81.710±1.752**	−29.0
9	OH	H	OH	H	H	OH	OH	1	141.000±4.546	136.667±4.907	−3.1
								5	128.667±2.373	122.567±3.569*	−4.7
								10	124.667±2.722	110.000±7.360*	−11.8
10	H	OH	OH	H	H	OH	OH	1	125.333±2.126	117.000±3.859*	−6.6
								5	147.667±3.538	138.333±5.932*	−6.3
								10	146.667±3.600	134.000±3.742*	−8.6
11	H	H	OH	OH	H	OH	OH	1	134.333±1.905	126.667±1.361*	−5.7
								5	130.000±16.330	113.333±15.694	−12.8
								10	131.567±5.443	85.667±13.007*	−34.9

[a]Each value represents the mean±SEM ($n=5$); (*$p<0.05$; **$p<0.01$).

above results suggest that, in addition to hydrogen bonding with the NH group, optimal lipophilicity may be another important factor in the recognition site in eliciting antihypertensive activity. A series of analogue of **12** or **13**, flavonoxypropanolamines have been shown to inhibit antihypertensive activity in spontaneously hypertensive rats.[7] An optimal chain length of three carbon atoms in substituents of the secondary amine has obtained from the study of structure–activity relationships.[7] Of these analogues of xanthonoxypropanolamines, **13** may be the most effective compound in eliciting hypotensive activity. It needs more experiments to clarify the above structure–activity relationships obtained from Tables 1 and 2, and the above suggestion. In the rat thoracic aorta, all the compounds (Table 3) depressed markedly the contractions induced by Ca^{2+} (1.9 mM) in high K^+ (80 mM) medium and by norepinephrine (3 μM)-induced phasic and tonic contractions. As shown in Table 3, acetylation of oxygenated xanthone did not enhance the inhibitory effects on high K^+- and Ca^{2+}-induced and norepinephrine induced contraction of rat thoracic aorta. A hydroxyl group substituted at C-1 or C-4 or C-6 of **1** siginificantly enhanced the inhibitory effects on high K^+- and Ca^{2+}-induced and norepinephrine induced contractions of rat thoracic aorta. As shown in Table 3, an oxypropanolamine side chain substituted at C-3 of **1** significantly enhanced the inhibitory effects on high K^+- and Ca^{2+}-induced and norepinephrine-induced contractions of rat thoracic aorta. A series of synthetic ω-aminoalkoxyxanthones have been shown to inhibit the contractions induced by Ca^{2+} in high-K^+ medium and by norepinephrine.[4] An increase of the length of the *N*-substituted groups from three carbons did not enhance the inhibitory effects on high K^+- and Ca^{2+}-induced and norepinephrine-induced contractions of rat thoracic aorta. Of these analogues of xanthonoxypropanolamines, **13** may be also the most potent compound in eliciting the inhibitory effects on high K^+- and Ca^{2+}-induced and norepinephrine-induced contractions of rat thoracic aorta. It needs more experiments to clarify the above structure–activity relationships. Compound **13** with an oxypropanolamine side chain, a side chain traditionally associated with β-antagonist activity, is suggested to have a calcium channel and beta adrenergic blocking effects with vasodilating activity in one

Table 2. Antihypertensive properties of synthetic xanthone derivatives



Compound	R	Blood pressure (mm Hg)[a]			
		Dose (mg/kg) iv	Before	After	Change (%)
12	$-CH_2CH_2CH_3$	0.1	130.0±8.0	128.0±5.0	−1.6±0.0*
		0.5	119.5±1.8	114.0±2.3	−4.7±0.6*
		1.0	124.8±4.5	101.8±3.7	−18.4±1.8*
		5.0	105.4±3.9	80.3±2.9	−23.71±1.6*
13	$-CH-(CH_3)_2$	0.1	120.0±7.9	111.0±6.7	−7.4±1.1*
		0.5	127.56±6.5	115.7±6.9	−9.3±0.7*
		1.0	127.0±2.6	108.4±3.0	−14.6±1.4*
		5.0	123.6±3.9	84.5±5.5	−32.1±2.8*

[a]Value represents the mean±SEM ($n=5$) (*$p<0.05$, **$p<0.01$).

molecule.[6] The tonic contraction in response to norepinephrine results primarily from Ca^{2+} entry through receptor-activated Ca^{2+} channels with little requirement for Ca^{2+} entry through voltage-dependent Ca^{2+} channels.[8–10] The norepinephrine-induced tonic contraction and phenylephrine-induced $^{45}Ca^{2+}$ influx were both inhibited by **13** (Table 3 and Fig. 1), indicating that **13** is a blocker of receptor-operated Ca^{2+} channels. It has been reported that high K^+-induced contraction in vascular smooth muscle is mediated by an increase in Ca^{2+} influx through voltage-dependent Ca^{2+} channels.[11] Since **13** inhibited the Ca^{2+}-dependent contraction and $^{45}Ca^{2+}$ influx in high K^+ medium, it may be a blocker of voltage-dpendent Ca^{2+} channels (Table 3 and Fig. 1). As shown in Figure 1, high K^+-induced $^{45}Ca^{2+}$ influx was more easily inhibited by verapamil, a relatively selective inhibitor of the voltage-dependent Ca^{2+} channels.[12] The results from this study indicate that **13** at high concentration is a relatively selective inhibitor of the receptor-operated Ca^{2+} channels and its inhibitory effect on the contractile response caused by high K^+ and norepinephrine in rat thoracic aorta is mainly due to the inhibition Ca^{2+} influx both in voltage-dependent and receptor-operated Ca^{2+} channels.

As shown in Table 1, intravenous injections of **9** at doses 1, 5, or 10 mg/kg reduces the blood pressure and heart rate in normotensive Wistar rats (Figure, not shown). As shown in Figure 2, in measuring the blood pressure and heart rate of rats, pretreatment with a bilateral vagotomy and atropine could not abolish or diminish the depressor response and bradycardia effect of **9**. Thus, the depressor and bradycardia effects are independent of the parasympathetic passway. Further experiments need to elucidate the exact mechanism of action. Compound **9**, a tetrahydroxy-xanthone, inhibited angiotensin-I-converting-enzyme activity.[13] The hypotensive activity of **9** is due to an inhibitory effect

Table 3. Effect of synthetic xanthone derivatives on high K^+- and Ca^{2+}-induced and norepinephrine-induced contraction of rat thoracic aorta[a]

Compound (μg/mL)	K^+ (80 mM) + Ca^{2+} (1.9 m)	Norepinephrine	
		Phasic	Tonic
Control	100.0±4.5	100.0±4.4	100.0±1.2
1 (100)	19.4±4.5***	30.6±0.4**	11.0±0.5***
2 (100)	5.2±1.3***	6.3±4.4**	2.9±2.1***
3 (100)	53.4±2.4**	64.9±8.2*	15.3±0.2***
4 (100)	9.0±1.4***	15.6±11.0***	0.0±0.0***
5 (100)	3.3±2.4***	0.0±0.0***	0.0±0.0***
5 diacetate (100)	0.0±0.0***	2.1±1.5***	0.0±0.0***
6 (100)	40.5±7.3**	79.5±4.4*	57.3±6.1**
6 diacetate (25)	56.7±1.5*	78.6±11.3*	74.9±9.1*
7 (25)	41.4±1.0**	70.8±14.7*	65.6±5.9*
7 diacetate (100)	43.9±9.4**	62.4±1.7*	50.7±4.6**
8 (100)	4.0±2.8***	7.1±5.1***	0.9±0.7***
12 (100)	38.4±2.5**	9.9±3.1***	8.5±4.4***
13 (100)	3.3±2.4***	21.6±2.4***	15.4±0.5**

[a]Rat aorta was preincubated with various xanthone derivatives or DMSO (0.1%, control) at 37 °C for 15 min, then high K^+ (80 mM) and Ca^{2+} (1.9 mM) or norepinephrine (3 μM) was added. Percentages of the contraction were calculated and presented as mean±SEM ($n=3$); *$p<0.05$, **$p<0.01$, ***$p<0.001$, as compared with the respective control values.

on angiotensin-I-converting-enzyme and a calcium channel and an independent of the parasympathetic blocking effect with vasodilating properties.

Conclusions

The present screening of hypotensive and vasorelaxing activities of synthetic xanthone derivatives has led to the identification of several potent cardiovascular agents. Previously, **13** is suggestd to have a calcium channel and beta adrengeric blocking effect with vasodilating activities in one molecule.[6] The results from this study indicate that **13** at high concentration is a relatively selective inhibitor of receptor-operated Ca^{2+} channels and its inhibitory effect on the contractile response caused by high K^+ and norepinephrine in rat thoracic aorta is mainly due to the inhibition of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. A selective oxygenated xanthone, **9**, inhibited angiotensin-I-converting-enzyme activity.[13] Its hypotensive activity is due to an inhibitory effect on angiotensin-I-converting-enzyme, and a calcium channel and an independent of parasympathetic blocking effects with vasodilating properties.

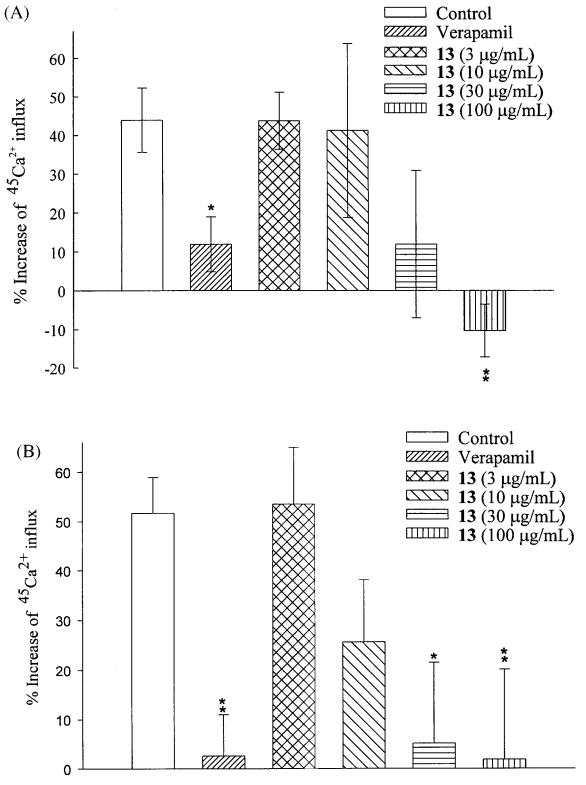


Figure 1. Effect of **13** on the $^{45}Ca^{2+}$ influx induced by phenylephrine and KCl. Aortic rings were placed in test tubes containing Krebs solution with $^{45}Ca^{2+}$ ($1\,\mu Ci\,mL^{-1}$) and incubated for 20 min with DMSO (0.1%) or verapamil (2 μM) or various concentrations of **13**, then phenylephrine (3 μM, panel A), KCl (60 mM, panel B) or saline (resting) was added and incubated for another 15 min. $^{45}Ca^{2+}$ influx into the muscle was measured. Data are expressed as percent increase of $^{45}Ca^{2+}$ uptake over the resting value. Values are expressed as the means ± SEM ($n = 3$–4); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ as compared with the respective control (phenylephrine or K^+ alone).

Experimental

Xanthones (**1**–**13**) were synthesized by a previously described method.[1–3] Elemental analyses were within ±0.4% of the theoretical values, unless otherwise noted. Atropine sulfate, L-phenylephrine HCl, EGTA, perchloric acid, and verapamil HCl were all purchased from Sigma Chemical Co. (USA). Xanthone derivatives were dissolved in distilled water (10% EtOH and 10% tween 80) and then diluted with normal saline for injection. Atropine sulfate and L-phenylephrine HCl were all diluted with normal saline. Pentobarbital sodium was purchased from Tokyo Chemical Co. (TCI, Japan). Heparin was obtained from the Green Cross Co. (Japan).

Measurement of blood pressure and heart rate

Male Wistar rats, weighing 250–300 g (provided by the Experimental Animal Center, Medical College, National Cheng-Kung University, Taiwan) were anethesized with sodium pentobarbital (40 mg/kg, ip). Following tracheal cannulation, systemic arterial blood pressure and heart rates were recorded from the femoral artery with a pressure transducer (Gould, Model P50). Body temperature were maintained at 37 °C. A femoral vein of rat was cannulated and then heparinized (heparin, 20 units/mL) for iv injections. All drug solutions were administered in a volume of 0.4 mL/kg. Equivolumetric injections of the vehicle were administrated as a control. The magnitudes of the effects elicited after injections were evaluated by measuring the changes in arterial pressure and heart rate between the responses and basal blood pressure or heart rate.

Pretreatments

In order to examine whether the effect of **9** resulted from the change of autonomic function, some pretreatments were performed on the rats before the experiments. Bilateral cervical vagotomies were performed 1 h prior to the experiments and atropine pretreatments (1.0 mg/kg, ip) were performed 15 min prior to the experiments.

Aortic contraction

Wistar rats of either sex, 250–300 g, were killed by a blow to the head. The thoracic aorta was isolated, removed excess fat and connective tissue and placed in organ bath containing 5 mL Krebs solution, maintained at 37 °C, and bubbled with a 95% O_2–5% CO_2 mixture. Two stainless-steel hooks were inserted into the aortic lumen, one was fixed while the other was connected to a transducer. Aorta were equilibrated in the medium for 90 min with three changes of Krebs solution and maintained under an optimal tension of 1 g before specific experimental protocols were initiated. Contractions were recorded isometrically via a force displacement transducer connected to a Gould polygraph (Model 2400). The final concentration of DMSO was fixed at 0.1%.

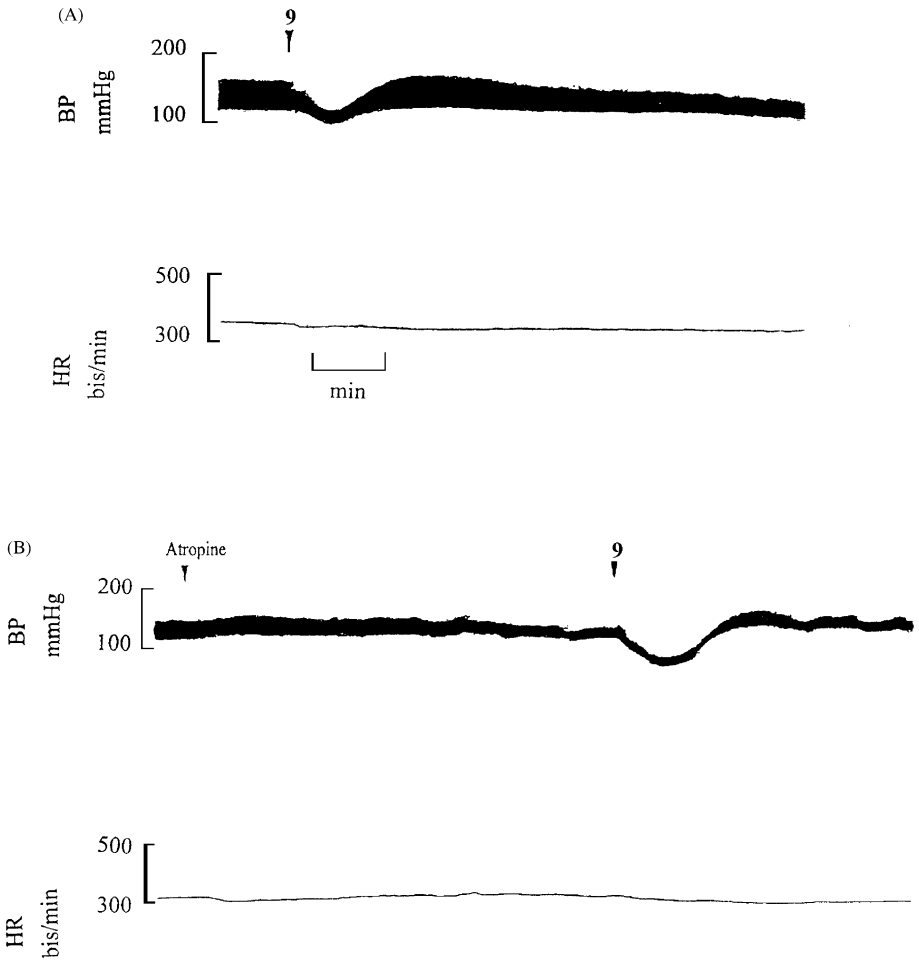


Figure 2. Effects of intravenous injection of **9** (10 mg /kg) on pressure and heart rate after bilateral cervical vagotomy (A) or treatment with atropine (1.0 mg/kg, ip) (B) with typical recordings.

$^{45}Ca^{2+}$ influx

$^{45}Ca^{2+}$ influx was measured in a manner similar to that described by Kaushik et al.[14] Aortic rings were placed in test tubes containing Krebs solution with 1 μ Ci mL^{-1} $^{45}Ca^{2+}$ in the presence of DMSO (0.1%) or various concentrations of tested compounds and incubated for 20 min. Phenylephrine (3 μM) or K^+ (60 mM) was then added and incubated for another 15 min. After the incubation period, the tissues were transferred into test tubes containing 2 mL of ice-cold Ca^{2+}-free Krebs solution with 2 mM EGTA for 40 min in order to remove extracellular $^{45}Ca^{2+}$. The tissues were then removed, lightly blotted with No. 5 Whatman filter paper, weighed, and dissolved in 37% perchloric acid (0.1 mL) at 75 °C. The radioactivity was counted in a liquid scintillation counter (Packard Model 2200 CA).

Data analysis

Data are presented as the means ± SEM. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if there was significant variation between treatment group, then the inhibitor-treated groups were compared with the control group by Student's *t*-test, and *p* values of less than 0.05 were considered to be statistically significant.

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