J. Pharm. Pharmacol. 1996, 48: 887–890
Received May 15, 1995
Accepted March, 22 1996

Synthesis and Antithrombotic Effect of Xanthone Derivatives

CHUN-NAN LIN, HSIN-KAW HSIEH*, SHIOU-JYH LIOU, HORNG-HUEY KO, HSIEN-CHENG LIN, MEI-ING CHUNG, FENG-NIEN KO, HONG-WEN LIU** AND CHE-MING TENG

*School of Pharmacy, School of Technology for Medical Sciences, **Department of Internal Medicine, Kaohsiung Medical College, Kaohsiung, Taiwan 807, *China Junior College of Medical Technology, Tainan Hsien, Taiwan 717 and Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan 100, R.O.C.*

Abstract

A series of xanthone derivatives was synthesized and tested in-vitro for their ability to inhibit aggregation of rabbit washed platelets and human platelet-rich plasma (PRP) induced by various inducers.

2-Prenyloxyxanthone showed the most potent inhibition of rabbit washed platelet aggregation induced by arachidonic acid (IC50 = 10.2 μM). Of the compounds tested in human PRP, 2-[3 (propylamino)-2-hydroxypropoxy]xanthone (**4**) hydrochloride salt exhibited the most potent inhibition of platelet aggregation induced by adrenaline (IC50 = 4.4 μM), whereas in evaluation of mouse antithrombotic activity, compound **4** exhibited the most potent protection of mice from thrombotic challenge. Compound **4**, 2-[3-(isopropylamino)-2-hydroxypropoxylxanthone hydrochloride salt and 2,5 dihydroxyxanthone suppressed the secondary aggregation induced by adrenaline in human PRP.

We conclude that the antiplatelet effects of these compounds are mainly due to an inhibitory effect on thromboxane formation.

We have previously reported that synthetic antiplatelet agents also had a vasorelaxation effect in rat thoracic aorta (Lin et al 1995), reduced the blood pressure and heart rate, and attenuated isoprenaline-induced tachycardia in rats (Chen et al 1993). For the study of structure-activity relationships of various xanthone derivatives and their design as antithrombotic or antihypertensive agents, we have now synthesized a series of xanthone derivatives.

Materials and Methods

Platelet aggregation

Rabbit washed platelets were obtained from ethylenediaminetetraacetic acid (EDTA)-anticoagulated platelet-rich plasma (PRP). Platelet numbers were counted and adjusted to 4.5×10^8 platelets mL^{-1}. The platelet pellets were suspended in Tyrode's solution containing (mM): NaCl 136·8, KCl 2·8, $NaHCO_3$ 11·9, $MgCl_2$ 2·1, NaH_2PO_4 0·33, $CaCl_2$ 1·0 and glucose 11·2 with 0·35% bovine serum albumin. Human PRP was obtained from the supernatant after the centrifugation of venous blood mixed with 3·8% sodium citrate (1:9 to blood). All glassware was siliconized. One minute before the addition of the aggregation inducer, PRP or the platelet suspension was stirred at 1200 rev min^{-1}. Aggregation was measured by turbidimetry. The absorbance of PRP or the platelet suspension was taken as 0% aggregation and the absorbance of platelet-poor plasma or platelet-free Tyrode's solution as 100% aggregation. The aggregation was measured by a Lumi-aggregometer (Chrono-Log Co., USA) connected to dual-channel recorders.

Correspondence: Chun-Nan Lin, School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan 807, Republic of China.

Antithrombotic activity in-vivo (Di Minno & Silver 1983)

Male Wistar mice, 25–30 g were used. Solutions (0.1 mL) of platelet-aggregating mixtures [40 μg collagen (Sigma Chemical Co.) plus 3·6 μg adrenaline (Sigma Chemical Co.) diluted in 0·154 M NaCl were injected into one mouse tail-vein at a rate of about 20 $\mu L\ s^{-1}$. The mice were held at an ambient air temperature of 27°C for 15–30 min before tail-vein injections. Death of animals or paralysis for more than 15 min of the hind limbs were considered as thrombotic effects. The ability of test compounds or reference drugs to protect mice from the lethal or paralytic effect of thrombotic agents was studied by intraperitoneal administration of agents in 0·3 mL 0·154 M NaCl 1 h before the thrombotic challenge.

Chemistry

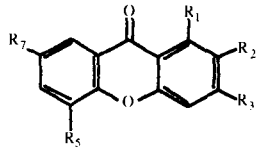
Synthetic methods. All melting points are uncorrected. IR spectra were recorded on a Hitachi model 260-30 IR spectrophotometer. ^1H and ^{13}C NMR spectra [δ(ppm), J (Hz)] were obtained on a Varian Gemini 200 MHz FT-NMR spectrometer. Mass spectra were determined on a Jeol JMS-D-100 mass spectrometer.

Elemental analyses were within ± 0·4% of the theoretical values, unless otherwise noted.

2-Hydroxyxanthone (**1**), 1,7-dihydroxyxanthone (**6**), 2,5-dihydroxyxanthone, (**8**), norathyriol peracetate (**10**), 3-[3-(cyclopropylamino)propoxy]xanthone (**11**), 3-[3-(cyclopropylamino) hexoxy] xanthone (**12**), 3,4-(2-ethoxycarbonyl-1,4-ethylenedioxy) xanthone (**13**) were synthesized and characterized as reported previously (Lin et al 1995, 1996).

2-Hydroxyxanthone acetate (2). Compound **1** (0·2 g, 0·94 mmol) was treated as previously reported (Lin et al 1993) to yield **2** as colourless needles (CH_3OH)(0.22 g, 0·87 mmol, 92%); mp 158159C; MS m/z (%) 254 (7)(M^+); IR (KBr): 1760,

Table 1. Effect of xanthone derivatives on the rabbit washed platelet aggregation induced by thrombin, arachidonic acid, collagen and platelet-activating factor (PAF).



1: R_1 = H, R_2 = OH, R_3 = R_5 = R_7 = H
3: R_1 = R_3 = R_5 = R_7 = H, R_2 = OCH_2CH = $C(CH_3)_2$
4: R_1 = H, R_2 = $OCH_2CH(OH)CH_2NH(CH_2)_2CH_3$, R_3 = R_5 = R_7 = H
5: R_1 = H, R_2 = $OCH_2CH(OH)CH_2NHCH(CH_3)_2$, R_3 = R_5 = R_7 = H
6: R_1 = R_7 = OH, R_2 = R_3=R_5 = H
8: R_1 = H, R_2 = R_5 = OH, R_3 = R_7 = H

Compound	Platelet Aggregation (%)			
	Thrombin	Arachidonic acid	Collagen	PAF
Control	93.6 ± 1.5	91.3 ± 1.3	91.3 ± 1.3	94.1 ± 1.4
1	88.3 ± 0.7**	36.5 ± 2.1***	44.0 ± 4.2***	65.6 ± 6.1***
2 (**1** acetate)	90.7 ± 1.8	0.0 ± 0.0***	80.5 ± 7.1	71.2 ± 4.8**
3	81.3 ± 2.5*	0.0 ± 0.0***	0.0 ± 0.0***	69.7 ± 5.5**
4	6.5 ± 3.7***	8.5 ± 4.3***	0.0 ± 0.0***	1.1 ± 0.9***
5	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
6	25.0 ± 11.5***	0.0 ± 0.0***	0.0 ± 0.0***	5.3 ± 4.6***
7 (**6** diacetate)	89.6 ± 1.0	77.7 ± 2.3***	80.5 ± 0.4***	79.9 ± 1.5***
8	91.8 ± 2.1	0.0 + 0.0***	0.0 ± 0.0[c]	64.0 ± 8.3**
9 (**8** diacetate)	89.6 ± 1.1	0.0 ± 0.0***	56.1 ± 9.5***	63.8 ± 5.9**
Aspirin	91.9 ± 2.5	0.0 ± 0.0***	85.4 ± 3.9	90.5 ± 1.2

Platelets were preincubated with **1, 2, 3, 4, 5,6, 8,9** (each at 300 μM), **7** (100 μM), aspirin (50 μM) or dimethylsulphoxide(0.5% control), respectively, at 37°C for 3 min, then thrombin(0.1 units mL^{-1}), arachidonic acid (100 μM), collagen (l0 g mL^{-1}) or PAF(2 ng mL^{-1}) was added. Percentages of aggregation are presented as means ± s.e.m. (n = 3-6). *P < 0.05, **P < 0.01, ***P < 0.001 compared with the respective control values.

1660, 1630 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2·23 (s, 3H, *O*Ac), 7·37 (m, 1H, H-7), 7·45 (dd, J = 9·0, 2·5 Hz, 1H, H-3), 7·47 (d, J = 7.8 Hz, 1H, H-5)7·49 (m, 1H, H-4), 7·72 (m, 1H, H-6), 8·01 (d, J2·5 Hz, 1H, H-1), 8·32 (dd, J = 8·0, 1·6 Hz, 1H, H-8); Anal ($C_{15}H_{10}O_4$) C, H.

2-Prenyloxyxanthone (3). To a solution of **2** (0.6 g, 2.8 mmol) in anhydrous methanol was added methanolic solution of sodium methoxide (15 mL, 30%). Prenyl bromide (2 mL) was added to the above mixture in an ice-bath and then refluxed for 4 h. After removal of the solvent, excess of water was poured into the mixture and then acidified with concentrated HCl. The solid was precipitated, collected and chromatographed on silica gel. Elution with $CHCl_3$ yielded a yellow powder. Recrystallized from $CHCl_3$ to give **3** as pale yellow needles (0·25 g, 0.92 mmol, 27%); mp 69-70°C; MS m/z (%): 28(3)(M^+), 212(100)(M-CH_2 $CHC(CH_3)_2$ + H)$^+$; IR (KBr): 1660, 1620 cm^{-1}; ^1H NMR(CD_3OD): δ 1·79 (s, 6H, 2 × CH_3), 4·57 (d, J = 6·6 Hz, 2H, –*CH*$_2$–), 5·46 [(m, 1H, –CH_2 $CH=C(CH_3)_2$] 7·27-7·53 (m, 5H, aromatic H), 7·70–7·79 (m, 1H, aromatic H), 8·17 (dd, J = 8·0, 1·7 Hz, H-8); ^{13}CNMR (CD_3OD): δ 18·3 (CH_3) 25·9 (CH_3), 66·5 (CH_2), 107·7 (C-1), 119·2 (C-5) 120·6 (C-4), 120·7 (–*C* H = $C(CH_3)_2$, 122·0 (C-8a), 122·8 (C-9a), 125·0 (C-7), 126·6 (C-8), 127·1 (C-3), 136·2 (C-6), 139·5 (–CH = *C* $(CH_3)_2$), 152·0 (C-4a), 156·7 (C-10a), 157·5 (C-2), 178·6 (CO); Anal ($C_{18}H_{16}O_3$)C, H.

2-[3-(Propylamino)-2-hydroxypropoxy]xanthone, hydrochloride salt (**4**). Compound **1** (0·5 g, 2·36 mmol) was treated as previously reported (Lin et al 1993) to yield **4** as colourless needles (CH_3OH) (0·15 g, 0.41 mmol, 50%); mp 208°C; MS m/z (%):327(2)(M^+); ^1H NMR (CD_3OD):δ 1.05 (t, J = 7.6 Hz, 3H, CH_3), 1.17 (m, 2H, –*CH*$_2$ CH_3), 3·06(t, J = 7.6 Hz, 2H, -NHC*H*$_2$ CH_2-), 3·21(dd, J = 12.6, 7·2 Hz, 1H, –CHOHC*H*$_2$ NH-), 3·34 (dd, J = 12·6, 3·2 Hz, 1H, –CHOHC*H*$_2$ NH–), 4.16 (m, 2H, –OCH_2–), 4·32 (m, 1H, –*CH* OH–), 7·44-7·53 (m, 2H, aromatic H), 7·61 (m, 2H, aromatic H), 7·72 (d, J = 1·6 Hz, H-1), 7·85 (m, 1H, aromatic H), 8·28 (dd, J = 8·0, 1·6 Hz, 1H, H-8); ^{13}C NMR (CD_3OD):δ 11·0 (CH_3), 19·1 (–CH_2CH_3), 49·0 (–CHOH*C* H_2NH–), 49·5 (–NH-*C*H_2CH_2-), 65·1 (OCH_2), 70·5 (–*C* HOH–), 107·0 (C-1), 118.2 (C-5), 119·9 (C-4), 120·5 (C-8a), 121·5 (C-9a), 124·3 (C-3), 125·0 (C-7), 125·9 (C-8), 135·5 (C-6), 150·4 (C-4a), 154·8 (C-2), 155·5 (C-10a), 175·8 (CO); Anal ($C_{19}H_{21}O_4NHCl$) C, H, N.

2-[3-(Isopropylamino)-2-hydroxypropoxy]xanthone hydrochloride salt (**5**). Compound **1** (0·5 g, 2·36 mmol) was treated as previously reported (Lin et al 1993) to yield **5** as a colourless powder (0·21 g, 0·58 mmol, 72%); mp 169–170°C; MS m/z (%):327 (7)(M^+); ^1H NMR (CD_3OD):δ 1.39 (d, J = 6·4 Hz, 3H, CH_3), 1·40 (d, J = 6·4 Hz, 3H, CH_3), 3·20 (dd, J = 12·6, 9·2 Hz, 1H, –CHOHC*H*$_2$ NH–), 3·34 (dd, J = 12·6, 3·2 Hz, 1H, –CHOHC*H*$_2$ NH–), 3·48(m, 1H, –*CH* $(CH_3)_2$), 4·17 (m, 2H, –O*CH*$_2$–), 4·30 (m, 1H, –*CH* OH–), 7·44-7·54 (m, 2H, aromatic H), 7·60 (m, 2H, aromatic H), 7·73 (d, J = 1·6 Hz, H-1), 7·84 (m, 1H, aromatic H), 8·28 (dd, J = 8·O, 1·6 Hz, 1H, H-8); ^{13}C NMR (CD_3OD): δ 18·8 (CH_3) 19·4 (CH_3), 48·3 (–CH_2NH–), 52·1 (–C H(CH_3)$_2$), 66·8 (–OCH_2-), 71·7 –CHOH–), 108·1(C-1), 119·3 (C-5), 121·0 (C-4), 122·0 (C-8a),

Table 2. IC50 (μM) values of xanthone derivatives on the rabbit washed platelet aggregation induced by arachidonic acid, collagen and PAF and on the aggregation of human platelet-rich plasma (PRP) induced by adrenaline and ADP.

Compound	Arachidonic acid	Collagen	PAF	Adrenaline	ADP
2	91.3	—	—	—	—
3	10.2	96.3	—	—	—
4	—	—	247.3	4.4	74.4
5	89.1	—	104.6	9.7	310.5
8	47.8	—	—	37.7	—
9	90	—	—	—	—

123·0 (C-9a), 125·3 (C-3), 126·4 (C-7), 127·2 (C-8), 136·5 (C-6), 152·7 (C-4a), 156·4 (C-2), 157·6 (C-10a), 178·6 (CO); Anal ($C_{19}H_{21}O_4NHCl$) C, H, N.

1,7-Dihydroxyxanthone diacetate (7). Compound **6** (0·2 g, 0·88 mmol) was treated as previously reported (Lin et al 1993) to yield **7** as colourless needles (MeOH) (0·23 g 0·77 mmol, 83%); mp 185-186°C; MS m/z (%):312(1)(M^+); ^{1}H NMR ($CDCl_3$):δ 2·32, 2·48 (2s, 6H, 2 OAc); Anal ($C_{17}H_{12}O_6$) C, H.

2,5-Dihydroxyxanthone diacetate (9). Compound **8** (0·2 g, 0·88 mmol) was treated as previously reported (Lin et al 1993) to yield **9** as colourless needles (CH_3OH) (0.23 g, 0.74 mmol, 84%) mp 148-149°C; MS m/z (%):312(14)(M^+); ^{1}H NMR ($CDCl_3$):δ 2·32, 2.48 (2s, 6H, 2 OAc); Anal ($C_{17}H_{12}O_6$) C, H.

Results and Discussion

The antiplatelet effects of **1–9** were studied on the aggregation of rabbit washed platelets induced by thrombin (0·1 units mL^{-1}) arachidonic acid (100 μM), collagen (10 $\mu g\ mL^{-1}$) and platelet-activating factor (PAF) (2 ng mL^{-1}). As shown in Tables 1 and 2, **2–6, 8** and **9** (each at 300 μM) completely inhibited the platelet aggregation induced by arachidonic acid while **3-6** and **8** (each at 300 μM) also completely inhibited those induced by collagen. Compound **3** showed the most potent inhibition of platelet aggregation induced by arachidonic acid (IC50 = 10·2 μM) and collagen (IC50 = 96·3 μM). In thrombin- and PAF-induced platelet aggregation, **4-6** (300 μM) showed complete or nearly complete inhibition. This clearly indicates that *O*-prenylation of **1** markedly enhances the antiplatelet effects on arachidonic acid- and collagen-induced aggregation and an oxypropanolamine side chain substituted at C-2 of **1** (300 μM) markedly enhances the antiplatelet effects on thrombin-, arachidonic acid-, collagen- and PAF-induced aggregation. As an oxypropanolamine side chain substituted at C-3 of 3-hydroxyxanthone did not show significant enhancement of antiplatelet effects (Lin et al 1993), we suggest that the oxygenated group of C-2 in the xanthone skeleton is the important moiety related to the antiplatelet effects of 5-xanthonoxypropanolamines. Esterification of **1, 6** and **8** did not enhance the antiplatelet effects except for **1,** which showed an enhancement of antiplatelet effect on arachidonic acid. Aspirin was used in this study as a positive control. It was found that aspirin (50 μM) inhibited completely the platelet aggregation induced by arachidonic acid but not that induced by thrombin, collagen or PAF (Table 1).

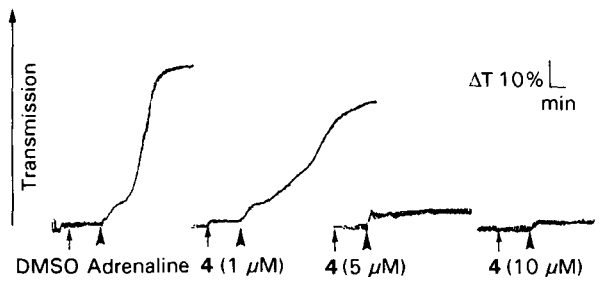


FIG. 1. Inhibitory effect of **4** on the aggregation of human platelet-rich plasma (PRP) induced by adrenaline (5 μM).

Table 3. Protection of mice from thrombotic challenge.

Treatment	Dose (mg kg^{-1})	Killed or paralysed/tested	Protection (%)
Control		20/22	9
Indomethacin	2	1/8***	88
Aspirin	20	1/8***	88
4	50	0/9***	100
	30	1/9***	89
	15	2/9***	78
	10	4/9*	56
	5	5/9	45
5	30	2/10***	80
	20	4/10**	60
	10	6/10	40
10	30	1/8*	88
	15	3/5	40
11	50	1/8***	88
	30	2/8**	75
	15	4/10**	60
	5	9/11	18
12	100	2/8**	75
	50	7/8	13
13	50	5/10*	50

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with controls using chi-squared test.

Antiplatelet effects of **4, 5** and **8** were also found in the aggregation of human PRP induced by ADP (20 μM) and adrenaline (5 μM). As shown in Table 2, compounds **4** and **5** showed potent antiplatelet effects when adrenaline was used as the aggregation agents. This indicates that an ω-aminoalkoxyl side chain substituted at C-2 of the xanthone skeleton may show the most potent antiplatelet effect on adrenaline-induced platelet aggregation (Lin et al 1995). In adrenaline-induced platelet aggregation, **4** and **5** prevented secondary aggregation at low concentration and completely abolished the aggregation at high concentration (Fig. 1). This suggests that their mechanism of action may be chiefly by the inhibition of thromboxane formation (Weiss 1983).

Table 3 shows that **4, 5** and **10–13** had good (50-84%) to excellent (89-100%) protection in the mice against the thrombotic challenge. Compound **4** shows the most potent protection, and it was found to be comparable with aspirin as an antithrombotic agent.

Acknowledgements

The authors are indebted to financial support from the Department of Health, Executive Yuan, ROC (DOH 84-HR-218).

References

Chen, I. J., Liou, S. J., Liou, S. S., Lin, C. N. (1993) Xanthonol: a calcium channel and β-adrenoceptor. Gen. Pharmacol. 24: 1425–1433

Di Minno, G., Silver, M. J. (1983) Mouse antithrombotic assay: A simple method for the evaluation of antithrombotic agents in vivo. Potentiation of antithrombotic activity by ethyl alcohol. J. Pharmacol. Exp. Ther. 225: 57-60

Lin, C. N., Liou, S. S., Ko, F. N., Teng, C. M. (1993) γ-Pyrone compounds. 4. Synthesis and antiplatelet effects of monooxygenated and dioxygenated xanthones and xanthonoxypropanolamines. J. Pharm. Sci. 82: 11-16

Lin, C. N., Liou, S. S., Lai, S. C., Lin, H. C., Ko, F. N., Liu, H. W., Teng, C. M. (1995) Synthesis and antiplatelet effects of ω-aminoalkoxylxanthones. J. Pharm. Pharmacol. 47: 588–594

Lin, C. N., Chung, M. I., Liou, S. J., Lee, T. H., J. P. Wang (1996) Synthesis and antiinflammatory effects of xanthone derivatives. J. Pharm. Pharmacol. 48: 532–538

Weiss, H. J. (1983) Antiplatelet drugs: Pharmacological aspects, In: Platelets: Pathophysiology and Antiplatelet Drug Therapy. Alan R Liss, Inc., New York, P. 46