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2′,5′-Dihydroxychalcone as a Potent Chemical Mediator and Cyclooxygenase Inhibitor

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Abstract

Eleven chalcone derivatives have been tested for their inhibitory effects on platelet aggregation in rabbit platelet suspension and the activation of mast cells and neutrophils.

Arachidonic acid-induced platelet aggregation was potently inhibited by almost all the compounds and some also had a potent inhibitory effect on collagen-induced platelet aggregation and cyclooxygenase. Some hydroxychalcone derivatives showed strong inhibitory effects on the release of β-glucuronidase and lysozyme, and on superoxide formation by rat neutrophils stimulated with the peptide fMet-Leu-Phe (fMLP). We found that the anti-inflammatory effect of 2′,5′-dihydroxychalcone was greater than that of trifluoperazine. 2′,5′-Dihydroxy and 2′,3,4,4′-tetrahydroxyl chalcones, even at low concentration (50 μM), tested in platelet-rich plasma from man almost completely inhibited secondary aggregation induced by adrenaline.

These results suggest that the anti-platelet effects of the chalcones are mainly a result of inhibition of thromboxane formation.

Because platelet aggregation is an important pathogenic factor in the development of atherosclerosis and associated thrombosis (Kikimoto et al 1990), one rational approach in the research for antithrombotic drugs is to search for inhibitors of platelet aggregation.

Mast cells play a central role in the pathogenesis of diseases such as allergic asthma, rhinoconjunctivitis, urticaria, anaphylaxis and systemic mastocytosis and might be important in other chronic inflammatory disorders (Middleton & Kandaswami 1992). The neutrophil is an important inflammatory cell. It can be triggered by a variety of inflammatory stimuli to produce highly reactive oxygen species which have potent microbiocidal and inflammatory effects (Babior 1978; Tauber & Babior 1985). Inhibition of chemical mediators released from mast cell and neutrophil degranulation is, therefore, a rational therapeutic approach to the treatment of a variety of inflammatory and allergic diseases.

We have reported that broussochalcone A, a natural chalcone of *Broussonetia papyrifera*, has a potent antiplatelet effect (Lin et al 1996) and is a potent inhibitor of cyclooxygenase; at 3 mg mL^{-1} it had a significant inhibitory effect on the release of β-glucuronidase (% inhibition, 51·1 ± 12·6) and lysozyme (% inhibition, 68·0 ± 13·4) from rat neutrophils stimulated with fMLP. Some synthetic chalcones had potent inhibitory effects on 5-lipoxygenase, with antioxidative effects, and some also inhibited cyclooxygenase (Sogawa et al 1993).

In this paper we describe the synthesis and anti-platelet and anti-inflammatory effects, in vitro, of a variety of chalcone derivatives and discuss their structure–activity relationships. The compounds tested are listed in Table 1.

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Materials and Methods

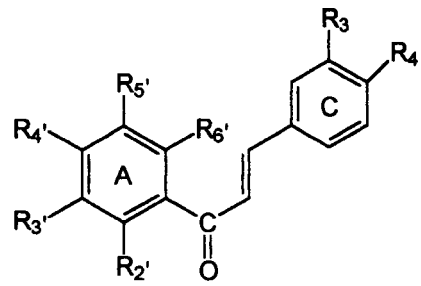
Platelet aggregation

Washed rabbit-platelets were obtained from ethylenediaminetetraacetic acid (EDTA)-anticoagulated platelet-rich plasma (PRP) according to procedures described previously (Teng et al 1987). PRP from man was obtained from the supernatant after the centrifugation of a 1 : 9 mixture of blood and sodium citrate solution (3·8%). Platelet numbers were counted by use of a Coulter Counter (Model ZM) and adjusted to $4·5 \times 10^8$ 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. All glassware was siliconized. PRP or the platelet suspension was stirred at 1200 rev min^{-1} one min before addition of the aggregation inducer. Aggregation was measured by a turbidimetric method (O'Brien 1962). The absorbance of PRP or platelet-poor plasma or platelet-free Tyrode solution was taken as 100% aggregation. The aggregation was measured by means of a Lumi-aggregometer (Chrono-Log Co., USA) connected to dual channel recorders.

Measurement of cyclooxygenase activity

Prostaglandin endoperoxide synthase purified from ram seminal vesicles, activity was measured by a modification of a procedure described elsewhere (Egan et al 1976). Briefly, the tested compounds were pre-incubated with prostaglandin endoperoxide synthase in the reaction mixture containing 0·1 M Tris-HCl, pH 8·0, 8 mM haematin and 5 mM tryptophan at 30°C for 2 min before addition of 50 mM arachidonate. Oxygen consumption in the reaction mixture was continuously monitored with a Clark-type oxygen electrode using a YSI oxygen monitor (Model 5300).

Table 1. The structures of chalcone derivatives **1–11**.



No.	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$	R_3	R_4	mp (°C)	Recryst. solvent	Yield (%)	Formula	Anal.
1	H	H	OMe	H	H	OMe	OMe	92–93	MeOH	67	$C_{18}H_{18}O_4$	C,H
2	OH	H	H	H	H	H	H	83–84	Benzene	72	$C_{15}H_{12}O_2$	C,H
3	H	H	OH	H	H	H	H	172–173	Benzene	83	$C_{15}H_{12}O_2$	C,H
4	OH	H	OH	H	H	H	H	145–146	$CHCl_3$	78	$C_{15}H_{12}O_3$	C,H
5	OH	H	H	OH	H	H	H	165–166	$CHCl_3$	80	$C_{15}H_{12}O_3$	C,H
6	OH	H	H	H	OH	H	H	155–156	$CHCl_3$	69	$C_{15}H_{12}O_3$	C,H
7	H	H	H	H	H	OH	OH	203–204	$CHCl_3$	75	$C_{15}H_{12}O_3$	C,H
8	OH	OH	OH	H	H	H	H	157–158	$CHCl_3$	76	$C_{15}H_{12}O_4{\cdot}H_5O$	C,H
9	H	H	OH	H	H	OH	OH	218–219	$CHCl_3$	83	$C_{15}H_{12}O_4$	C,H
10	OH	H	OH	H	H	OH	OH	251–252	$CHCl_3$	72	$C_{15}H_{12}O_5$	C,H
11	OH	H	H	OH	H	OH	OH	204–205	$CHCl_3$	80	$C_{15}H_{12}O_5{\cdot}½H_5O$	C,H

Mast cell degranulation

Heparinized Tyrode solution was injected into the peritoneal cavity of exsanguinated rats (Sprague–Dawley, 250–300 g). After abdominal massage, the cells in the peritoneal fluid was harvested and then separated through 38% bovine serum albumin. The cells were washed and suspended in Tyrode solution. The cell suspension was pre-incubated with DMSO or drugs at 37°C for 3 min. β-Glucuronidase (phenolphthalein-β-D-glucuronide as substrate, 550 nm) and histamine (*o*-phthaldialdehyde condensation, 350 and 450 nm) in the supernatant were determined 15 min after addition of compound 48/80 (10 mg mL^{-1}). The total content was measured after treatment of the cell suspension with Triton X-100. The percent released was determined (Wang et al 1994).

Neutrophil degranulation

Blood was withdrawn from the rat and mixed with EDTA. After dextran sedimentation, Ficoll-hypaque separation and hypotonic lysis of the residual erythrocytes, neutrophils were washed and suspended in Hank's balanced salt solution (Boyum 1968). The cell suspension was pre-incubated with dimethylsulphoxide (DMSO) or drugs at 37°C for 3 min, then challenged with fMLP (1 mM). Lysozyme (*Micrococcus lysodeikticus* as substrate, 450 nm) and β-glucuronidase in the supernatant were determined 45 min later (Smith & Iden 1979).

Superoxide-radical formation

The neutrophil suspension was pre-incubated with DMSO or drugs at 37°C for 3 min, then superoxide dismutase and Hank's balanced salt solution were added into the blank and test tubes, respectively. After addition of cytochrome c, reaction was initiated by challenge with fMLP (0·3 mM). After 30 min the reaction was terminated by centrifugation and superoxide in the supernatant was determined by spectrophotometry at 550 nm (Market et al 1984).

Chemistry: synthetic methods

Melting points (uncorrected) were determined with a Yanaco Micro-Melting Point apparatus. IR spectra were determined with a Hitachi model 260-30 IR spectrophotometer. ^1H and ^{13}C NMR spectra [δ (ppm), J (Hz)] were determined with a Varian Gemini 200 MHz FTNMR spectrometer. Results from elemental analysis were within ± 0·4% of the theoretical values, unless otherwise noted. Flash chromatography was performed on silica gel 60 supplied by E. Merck.

General procedure for obtaining chalcones **2–11** *(Sogawa et al 1993)*

2′,5′-Dihydroxychalcone (**5**). 2,5-Dihydroxyacetophenone (3·8 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were stirred for 0·5 h in dichloromethane (80 mL) and then 3,4-dihydro-α-pyran at room temperature for 4 h. The reaction mixture was washed twice with water, dried, and evaporated in vacuo. The residue yielded crude 2′,5′-bis(tetrahydropyran-2-yloxy) acetophenone (**5a**). Crude **5a**, benzaldehyde (2·67 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were dissolved in MeOH (100 mL) and the mixture was stirred for 12 h at 40°C and then evaporated in-vacuo. Water (100 mL) was added and the mixture was neutralized with HCl (1 M; 35 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried, and evaporated in-vacuo. This residue yielded crude 2′,5′-bis(tetrahydropyran-2-yloxy)chalcone (**5c**). Crude **5c** and *p*-toluenesulphonic acid (0·2 g, 1·05 mmol) were dissolved in MeOH. The reaction mixture was stirred for 4 h at room temperature and then evaporated in-vacuo. Water (100 mL) was added and the mixture was neutralized with 5% $NaHCO_3$ (50 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried, and evaporated in-vacuo. The residue was chromatographed on silica gel with cyclohexane-EtOAc (2 : 1) as mobile phase to give 5 (4·81 g, 20 mmol, 80%): mp 165–166°C ($CHCl_3$); ^1H NMR (CD_3OD) δ 6·85 (d, 1H, J = 8·9 Hz, H-3′), 7·04 (dd, 1H, J = 8·9,

2·9 Hz, H-4′), 7·41–7·45 (m, 4H), 7·71–7·76 (m, 2H), 7·74 (d, 1H, J = 15·6 Hz, H-a), 7·87 (d, 1H, J = 15·6 Hz, H-b); ^{13}C NMR (CD_3OD) δ 115·5 (C-6′), 119·7 (C-3′), 121·2 (C-1′), 121·8 (C-a), 125·9 (C-4), 129·8 (C-2 and C-6), 130·0 (C-3 and C-5), 131·9 (C-4), 136·6 (C-1), 146·3 (C-b), 150·6 (C-5′), 157·6 (C-2′), 195·0 (CO) (Agrawal 1989); IR (KBr) 3400, 1670 cm^{-1}; MS m/z 240 (M^+, 56).

3,4,4′-Trimethoxychalcone (**1**). *p*-Methoxyacetophenone (3·75 g, 25 mmol), 3,4-dimethoxybenzaldehyde (4·2 g, 25 mmol), and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** to give **1** (5·0 g, 16·75 mmol, 67%): mp 92–93°C (MeOH); ^{1}H NMR ($CDCl_3$) δ 3·86 (s, 3H, OMe), 3·90 (s, 3H, OMe), 3·92 (s, 3H, OMe), 6·87 (d, 1H, J = 8·3 Hz, H-5), 6·95 (dd, 2H, J = 6·9, 2·1 Hz, H-3′ and H-5′), 7·14 (d, 1H, J = 2·0 Hz, H-2), 7·20 (dd, 1H, J = 8·3, 2·0 Hz, H6), 7·39 (d, 1H, J = 15·6 Hz, H-a), 7·73 (d, 1H, J = 15·6 Hz, H-b), 8·02 (dd, 2H, J = 6·8, 2·0 Hz, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$) δ 55·4 (OMe), 55·9 (2 × OMe), 110·0 (C-2 and C-5), 113·7 (C-3′ and C-5′), 119·7 (C-6), 122·9 (C-a), 128·1 (C-1), 130·6 (C-2′ and C-6′), 131·2 (C-1′), 144·0 (C-b), 149·1 (C-3), 151·3 (C-4), 163·2 (C-4′), 188·6 (CO) (Agrawal 1989); IR (KBr) 1660 cm^{-1}; MS m/z 298 (M^+, 97).

2′-Hydroxychalcone (**2**). 2-Hydroxyacetophenone (3·4 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2′-(tetrahydropyran-2-yloxy)acetophenone (**2a**). Crude **2a**, benzaldehyde (2·67 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **2** (4·04 g, 18 mmol, 72%): mp 83–84°C (benzene); ^{1}H NMR (CD_3OD) δ 6·93–7·01 (m, 2H), 7·40–7·54 (m, 4H), 7·71–7·76 (m, 2H), 7·84 (d, 1H, J = 15·6 Hz, H-a), 7·88 (d, 1H, J = 15·6 Hz, H-b); ^{13}C NMR (CD_3OD) δ 119·0 (C-3′), 120·1 (C-5′), 121·3 (C-1′), 121·5 (C-a), 129·8 (C-2 and C-6), 130·0 (C-3 and C-5), 131·6 (C-6′), 131·9 (C-4), 136·0 (C-1), 137·4 (C-4′), 146·5 (C-b), 164·3 (C-2′), 195·0 (CO) (Agrawal 1989); IR (KBr) 1650 cm^{-1}; MS m/z 224 (M^+, 57).

4′-Hydroxychalcone (**3**). 4′-Hydroxyacetophenone (3·4 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 4′-(tetrahydropyran-2-yloxy)acetophenone (**3a**). Crude **3a**, benzaldehyde (2·67 g, 25 mmol), and barium hydroxide octahydrate (0·15 g, 25 mmol) were treated as for **5c** and **5** to give **3** (4·65 g, 20·75 mmol, 83%): mp 172–173°C (benzene); ^{1}H NMR (CD_3OD) δ 6·90 (dd, 2H, J = 6·8, 2·1 Hz, H-3′ and H-5′), 7·43 (m, 3H), 7·73 (m, 4H), 8·03 (dd, 2H, J = 6·8, 2·1 Hz, H-2′ and H-6′); ^{13}C NMR (CD_3OD) δ 116·8 (C-3′ and C-5′), 123·3 (C-a), 129·9 (C-3 and C-5), 130·3 (C-2 and C-6), 131·2 (C-1′), 131·8 (C-4), 132·7 (C-2′ and C-6′), 136·7 (C-1), 145·5 (C-b), 164·3 (C-4′), 191·1 (CO) (Agrawal 1989); IR (KBr) 3300, 1670 cm^{-1}; MS m/z 224 (M^+, 100).

2′,4′-Dihydroxychalcone (**4**). 2,4-Dihydroxyacetophenone (3·8 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2,4-bis(tetrahydropyran-2-yloxy)acetophenone (**4a**). Crude **4a**, benzaldehyde (2·67 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **4** (4·69 g, 19·5 mmol, 78%): mp 145–146°C ($CHCl_3$); ^{1}H NMR (CD_3OD) δ 6·30 (d, 1H, J = 2·4 Hz, H-3′), 6·43 (dd, 1H, J = 8·9, 2·4 Hz, H-5′), 7·40–7·43 (m, 3H), 7·70–7·80 (m, 4H), 7·97 (d, 1H, J = 8·9 Hz, H-6′); ^{13}C NMR (CD_3OD) δ 104·1 (C-3′), 109·6 (C-5′), 114·9 (C-1′), 122·1 (C-a), 129·6 (C-2 and C-6), 130·0 (C-3), 130·3 (C-5), 131·9 (C-4), 133·8 (C-6′), 136·6 (C-1), 145·4 (C-b), 166·9 (C-2′), 167·9 (C-4′), 193·6 (CO) (Agrawal 1989); IR (KBr) 3400, 1630 cm^{-1}; MS m/z 240 (M^+, 48).

2′,6′-Dihydroxychalcone (**6**). 2,6-Dihydroxyacetophenone (3·8 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2′,6′-bis(tetrahydropyran-2-yloxy)acetophenone (**6a**). Crude **6a**, benzaldehyde (2·67 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **6** (4·14 g, 17·25 mmol, 69%): mp 155–156°C ($CHCl_3$); ^{1}H NMR (CD_3OD) δ 6·39 (d, 1H, J = 8·2 Hz, H-3′ and H-5′), 7·24 (t, 1H, J = 8·2 Hz, H-4′), 7·40–7·43 (m, 3H), 7·62–7·65 (m, 2H), 7·78 (d, 1H, J = 15·7 Hz, H-a), 8·14 (d, 1H, J = 15·7 Hz, H-b); ^{13}C NMR (CD_3OD) δ 108·7 (C-3′ and C-5′), 112·4 (C-1′), 109·6 (C-5′), 129·1 (C-a), 129·7 (C-3 and C-5), 130·2 (C-2 and C-6), 131·6 (C-4′), 137·0 (C-1), 137·2 (C-4), 144·3 (C-b), 163·6 (C-2′ and C-6′), 197·0 (CO) (Agrawal 1989); IR (KBr) 3400, 1690 cm^{-1}; MS m/z 240 (M^+, 41).

3,4-Dihydroxychalcone (**7**). 3,4-Dihydroxyacetophenone (3·45 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 3,4-bis(tetrahydropyran-2-yloxy)acetophenone (**7b**). Crude **7b**, acetophenone (3·0 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **7** (4·50 g, 18·75 mmol, 75%): mp 203–204°C ($CHCl_3$); ^{1}H NMR (CD_3OD) δ 6·84 (d, 1H, J = 8·1 Hz, H-5), 7·10 (dd, 1H, J = 2·4, 8·1 Hz, H-6), 7·18 (d, 1H, J = 2·4 Hz, H-2), 7·47 (d, 1H, J = 15·6 Hz, H-a), 7·48–7·59 (m, 3H), 7·68 (d, 1H, J = 15·6 Hz, H-b), 8·02 (dd, 2H, J = 1·6, 6·6 Hz, H-2 (and C-6′); ^{13}C NMR (CD_3OD) δ 115·7 (C-2), 116·6 (C-5), 119·6 (C-a), 123·7 (C-6), 128·1 (C-1), 129·4 (C-3′ and C-5′), 129·7 (C-2′ and C-6′), 133·8 (C-4′), 139·7 (C-1′), 146·8 (C-b), 147·4 (C-3), 150·1 (C-4), 192·7 (CO) (Agrawal 1989); IR (KBr) 3500, 1640 cm^{-1}; MS m/z 240 (M^+, 51).

2′,3′,4′-Trihydroxychalcone (**8**). 2,3,4-Trihydroxyacetophenone (4·2 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2′,3′,4′-tris(tetrahydropyran-2-yloxy)acetophenone (**8a**). Crude **8a**, benzaldehyde (2·67 g, 25 mmol) and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **8** (4·87 g, 19 mmol, 76%): mp 157–158°C ($CHCl_3$); ^{1}H NMR (CD_3OD) δ 6·48 (d, 1H, J = 8·9 Hz, H-5′), 7·41–7·46 (m, 3H), 7·58 (d, 1H, J = 8·9 Hz, H-6′), 7·72–7·82 (m, 4H); ^{13}C NMR (CD_3OD) δ 108·7 (C-5′), 115·1 (C-1′), 121·9 (C-a), 123·5 (C-2 and C-6), 129·7 (C-3), 130·0 (C-5), 131·6 (C-4), 133·7 (C-6′), 136·3 (C-1), 145·0 (C-b), 153·6 (C-2′ and C-3′), 159·4 (C-4′), 193·9 (CO) (Agrawal 1989); IR (KBr) 3200, 1640 cm^{-1}; MS m/z 256 (M^+, 23).

3,4,4′-Trihydroxychalcone (**9**). 4-Hydroxyacetophenone (3·4 g, 25 mmol) or 3,4-dihydroxybenzaldehyde (3·45 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 4′-(tetrahydro-

pyran-2-yloxy) acetophenone (**9a**) or 3,4-bis(tetrahydropyran-2-yloxy)benzaldehyde (**9b**). Crude **9a**, crude **9b**, and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **9** (5·32 g, 20·75 mmol, 83%): mp 218–219°C ($CHCl_3$); ^1H NMR (CD_3OD) δ 6·81 (d, 1H, J = 8·1 Hz, H-5), 6·89 (dd, 2H, J = 2·0, 8·9 Hz, H-3′ and H-5′), 7·09 (dd, 1H, J = 2·0, 8·1 Hz, H-6), 7·17 (d, 1H, J = 2·0 Hz, H-2), 7·50 (d, 1H, J = 15·6 Hz, H-α), 7·65 (d, 1H, J = 15·6 Hz, H-β), 8·00 (dd, 2H, J = 2·0, 8·9 Hz, H-2′ and H-6′); ^{13}C NMR (CD_3OD) δ 115·6 (C-5), 116·3 (C-3′ and H-5′), 116·2 (C-2), 119·5 (C-6), 123·4 (C-α), 128·4 (C-1), 131·2 (C-1′), 132·1 (C-2′ and C-6′), 146·2 (C-β), 146·7 (C-3), 149·7 (C-4), 163·6 (C-4′), 191·0 (CO) (Agrawal 1989); IR (KBr) 3400, 1640 cm^{-1}; MS m/z 256 (M^+, 96).

2′,3,4,4′-Tetrahydroxychalcone (**10**). 2,4-Dihydroxyacetophenone (3·8 g, 25 mmol) or 3,4-dihydroxybenzaldehyde (3·45 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2,4-bis(tetrahydropyran-2-yloxy)acetophenone (**10a**) or 3,4-bis(tetrahydropyran-2-yloxy)benzaldehyde (**10b**), respectively. Crude **10a**, crude **10b**, and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **10** (4·9 g, 18 mmol, 72%): mp 251–252°C ($CHCl_3$); ^1H NMR (CD_3OD) δ 6·30 (d, 1H, J = 2·4 Hz, H-3′), 6·42 (dd, 1H, J = 2·4, 9·0 Hz, H-5′), 6·82 (d, 1H, J = 8·2 Hz, H-5), 7·12 (dd, 1H, J = 2·0, 8·2 Hz, H-6), 7·19 (d, 1H, J = 2·0 Hz, H-2), 7·53 (d, 1H, J = 15·3 Hz, H-α), 7·73 (d, 1H, J = 15·3 Hz, H-β), 7·94 (d, 1H, J = 9·0 Hz, H-6′); ^{13}C NMR (CD_3OD) δ 104·1 (C-3′), 109·5 (C-5′), 115·0 (C-1′), 116·1 (C-2), 116·9 (C-5), 118·6 (C-α), 123·9 (C-6), 128·7 (C-1), 133·3 (C-6′), 146·3 (C-β), 147·1 (C-3), 150·2 (C-4), 166·6 (C-2′), 167·7 (C-4′), 193·8 (CO) (Agrawal 1989); IR (KBr) 3300, 1650 cm^{-1}; MS m/z 272 (M^+, 93).

2′,3,4,5′(-Tetrahydroxychalcone (**11**). 2,5-Dihydroxyacetophenone (3·8 g, 25 mmol) or 3,4-dihydroxybenzaldehyde (3·45 g, 25 mmol) and pyridinium *p*-toluenesulphonate (0·15 g, 0·6 mmol) were treated as for **5a** to give crude 2,5-bis(tetrahydropyran-2-yloxy)acetophenone (**11a**) or 3,4-bis-(tetrahydropyran-2-yloxy)benzaldehyde (**11b**), respectively. Crude **11a**, crude **11b**, and barium hydroxide octahydrate (8·15 g, 25 mmol) were treated as for **5c** and **5** to give **11** (3·06 g, 11·25 mmol, 45%): mp 204–205°C ($CHCl_3$); ^1H NMR (CD_3OD) δ 6·81 (d, 1H, J = 8·9 Hz, H-3′), 6·83 (dd, 1H, J = 8·2 Hz, H-5), 7·02 (dd, 1H, J = 2·9, 8·9 Hz, H-4′), 7·12 (dd, 1H, J = 2·0, 8·3 Hz, H-6), 7·20 (d, 1H, J = 2·0 Hz, H-2), 7·39 (d, 1H, J = 2·9 Hz, H-6′), 7·55 (d, 1H, J = 15·6 Hz, H-α), 7·74 (d, 1H, J = 15·6 Hz, H-β); ^{13}C NMR (CD_3OD) δ 115·3 (C-6′), 115·8 (C-2), 116·6 (C-5), 118·1 (C-3′), 119·6 (C-1′), 121·3 (C-α), 123·9 (C-6), 125·4 (C-4′), 128·1 (C-1), 146·9 (C-β), 147·4 (C-3), 150·3 (C-4), 150·5 (C-5′), 157·5 (C-2′), 195·0 (CO) (Agrawal 1989); IR (KBr) 3300, 1660 cm^{-1}; MS m/z 272 (M^+, 20).

Results and Discussion

The aggregation of washed rabbit-platelets induced by thrombin (0·1 unit mL^{-1}), arachidonic acid (100 mM), collagen (10 mg mL^{-1}) and platelet-activating factor (PAF) (2 ng mL^{-1}) was used to study the antiplatelet effects of **1–11**. As shown in Table 2, all the synthetic chalcone derivatives had potent antiplatelet effects on arachidonic acid-induced aggregation. Compound **9** had potent antiplatelet effects on arachidonic acid- and collagen-induced aggregation, whereas the *o*-methylated product of **9** (i.e. **1**) did not enhance the antiplatelet effects or inhibition of cyclooxygenase activity. It is clear that phenolic groups of chalcone derivatives might be important in the inhibition of cyclooxygenase. The hydroxylated products of **2**, with hydroxylation at C-4′ and C-6′ (**4** and **6**, respectively), did not enhance the antiplatelet effects on arachidonic acid- and collagen-induced aggregation, whereas hydroxylation at C-5′ and at C-3′ and C-4′ (**5** and **8**, respectively) very markedly enhanced the antiplatelet effects on arachidonic acid-induced aggregation. The hydroxylated product of **5**, with hydroxylation at C-3 and C-4 (**11**), did not enhance the anti-

Table 2. Inhibition of platelet aggregation and cyclooxygenase activity (IC50, μM).

Compound	IC50 (μM)*				
	Thrombin	Arachidonic acid	Collagen	PAF	Cyclooxygenase
1	> 300 (14)	42·6	> 100	> 300 (33·5)	> 300 (16)
2	103·3	16·1	18·6	90·2	> 100 (13)†
3	> 300	42·8	> 100	> 300 (2)	> 100 (20)†
4	> 300	15·2	> 100	> 300 (20)	280†
5	> 100	0·3	> 100	151·1	37·5
6	> 300 (14)	48·9	85·0	> 100 (1)	221·6
7	> 300	6·8	13·1	> 300 (2)	34†
8	> 100	7·4	14·6	> 100 (4)	> 300 (12)
9	> 300 (3)	12·3	85·6	> 300 (28)	40·4
10	> 300 (2)	9·6	> 100	> 300 (10)	209·4
11	> 300 (40)	9·2	17·0	> 100 (20)	170†
Indomethacin	− 0·1‡	100‡	22‡	3·8‡	6·2

Platelets were incubated with different concentrations of chalcone derivatives or DMSO (0·5%) at 37°C for 3 min, and thrombin (0·1 units mL^{-1}), arachidonic acid (100 μM), collagen (10 mg μL^{-1}) or PAF (2 ng mL^{-1}) was then added to trigger the aggregation. Chalcone derivatives were pre-incubated with enzyme at 30°C for 3 min before addition of arachidonate to start the reaction. Analysis of the regression line was performed to calculate IC50 values. *When 50% inhibition could not be reached at the highest concentration, the % inhibition is given in parentheses. †Data from Sogawa et al (1993). ‡Values are percent inhibition compared with control at 20 mM.

platelet effect on arachidonic acid-induced aggregation but markedly enhanced the antiplatelet effect on collagen-induced aggregation. The hydroxylated product of **7**, with hydroxylation at C-2′ and C-4′ (**10**), did not enhance the antiplatelet effect on arachidonic acid-induced aggregation but reduced the antiplatelet effect on collagen-induced aggregation.

These results showed an interesting structure–activity correlation, clearly indicating that appropriate variation in the A ring only, except for **2**, **8** and **11** (see Table 2) resulted in good selectivity in inhibitory effect on arachidonic acid-induced aggregation. Appropriate variation in the B ring only, for example **7**, resulted in good selectivity in inhibitory effects on arachidonic acid- and collagen-induced aggregation.

Indomethacin was used in this study as positive control. Indomethacin (20 mM) completely inhibited the platelet aggregation induced by arachidonic acid, slightly inhibited that induced by collagen, but did not affect that induced by thrombin or PAF (Table 2).

The antiplatelet effects of **2**, **4**, **5** and **7–11** on platelet aggregation induced by adrenaline (5 μM) in human PRP were also studied. As shown in Table 3, **5** and **10** had potent antiplatelet effect on adrenaline-induced platelet aggregation. This effect appeared to be concentration-dependent. In adrenaline-induced platelet aggregation, **2** and **4** at low concentration did not prevent secondary aggregation but when present at high concentration (150 μM) they completely abolished aggregation; **7–9** and **11**, on the other hand, did not prevent secondary aggregation even when present at high concentration.

When the activity of fatty acid cyclooxygenase from ram vesicular glands was measured in the presence of the compounds, **4**, **7** and **9–11** inhibited the enzyme in a dose-dependent manner. As shown in Table 2, the IC50 values depended on the oxygenated substitution of the chalcone.

The antiplatelet effect of the natural chalcone broussochalcone A (a 3,4,4′-trihydroxychalcone derivative; Scheme 1) an inhibitor of arachidonic acid- and collagen-induced platelet aggregation, markedly inhibited cyclooxygenase (Lin et al 1996) but when present at high concentration (450 μM) significantly prevented secondary aggregation induced by adrenaline in human PRP (Lin et al 1996). Compounds **7** and **9** inhibited arachidonic acid- and collagen-induced platelet aggregation and also markedly inhibited cyclooxygenase (Table 2) but did not significantly prevent secondary aggregation induced by adrenaline in human PRP, even when present at high concentration (300 μM) (Table 2). This could be because of the higher binding capacity of plasma for these inhibitors or the low stability of the compounds in PRP solution. Further experiments are need to elucidate their exact mechanism of action (Lin et al 1996).

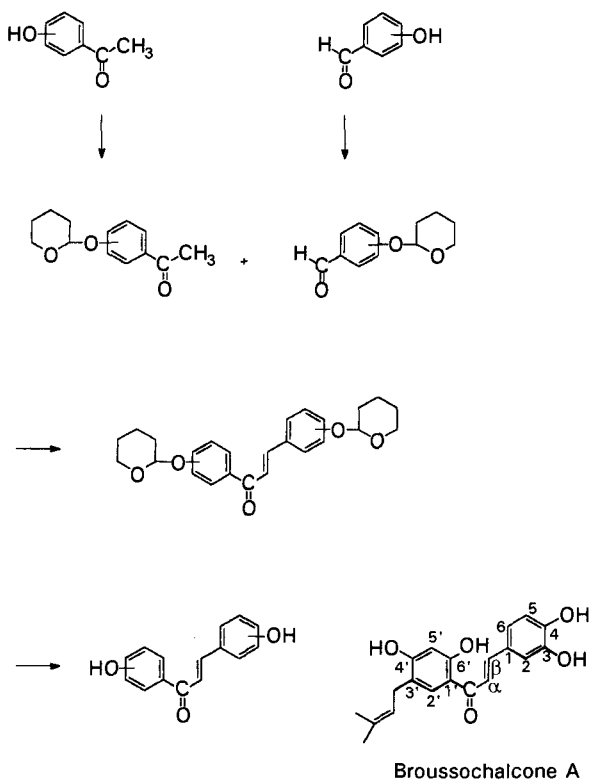
Compounds **5** and **10** are potent inhibitors of arachidonic acid-induced platelet aggregation and secondary aggregation induced by adrenaline in human PRP (Tables 2 and 3 and Fig. 1) but compound **5** inhibited cyclooxygenated strongly whereas **10** inhibited it weakly.

The antiplatelet action of **5** is mainly a result of inhibition of cyclooxygenase activity and reduced thromboxane formation, and the antiplatelet action of **10** is possibly a result of inhibition of thromboxane synthase leading to reduced thromboxane formation (Mitchell & Sharp 1964; Mustard et al 1975; Weiss 1983). Compounds **2** and **4** significantly prevent the secondary aggregation induced by adrenaline in human PRP (Table 3) but do not markedly inhibit cyclooxygenase (Table 2). This indicates that the antiplatelet actions of these compounds might be a result of inhibition of thromboxane synthase leading to reduced thromboxane formation (Mitchell & Sharp 1964; Mustard et al 1975; Weiss 1983). The exact mechanisms of action of these two chalcones are different because **2** strongly inhibits arachidonic acid- and collagen-induced platelet aggregation and significantly inhibits PAF-induced platelet

Table 3. Antiplatelet effects of **2**, **4**, **5**, and **7–11** on adrenaline (5 μM)-induced aggregation in human PRP.*

Treatment	Dose (μM)	Aggregation (%)
Control	–	99·9 ± 2·2
2	150	9·6 ± 1·5†
	100	99·1 ± 1·7
4	150	8·6 ± 1·1
	100	94·7 ± 2·9
5	150	17·7 ± 1·6†
	75	23·8 ± 2·2
	50	24·0 ± 3·0
7	300	95·2 ± 0·7
8	300	95·7 ± 0·8
9	300	96·3 ± 1·2
10	150	15·6 ± 1·1†
	75	23·8 ± 1·6†
	50	28·0 ± 2·6†
11	300	94·8 ± 0·3
Aspirin	50	39·6 ± 15·4‡

*PRP was pre-incubated with DMSO (0·5%, control), **2**, **4**, **5** and **7–11**, or aspirin at 37°C for 3 min, and adrenaline was then added. Values are presented as mean ± s.e.m. (n = 3–5). †$P < 0.001$, ‡$P < 0.05$, significant compared with control values.



SCHEME 1. General synthesis of chalcone derivatives and structure of Aroussochalcone.

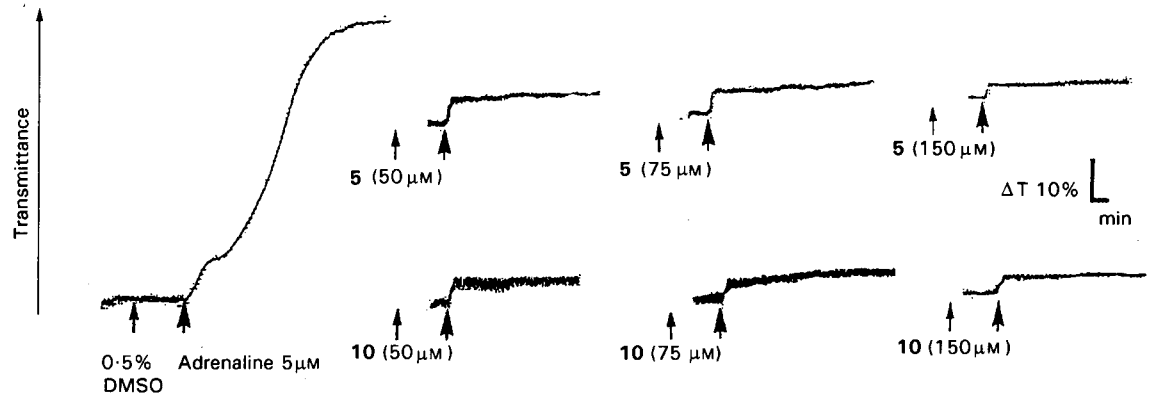


FIG. 1. Inhibitory effect of **5** and **10** on the adrenaline-induced aggregation of human PRP. PRP was incubated with DMSO (0·5%) or different concentrations of **5** or **10** for 3 min then adrenaline was added to trigger the aggregation.

aggregation but **4** strongly inhibits arachidonic acid-induced platelet aggregation only. Compounds **8** and **11** at high concentration (300 μM) do not significantly prevent the secondary aggregation induced by adrenaline in human PRP. Further experiments are need to elucidate their exact mechanism of action.

The anti-inflammatory activity of **1–11** was studied by investigation of their inhibitory effect on the activation of mast cells and neutrophils. Compound 48/80 (10 μg mL^{-1}) induced the release of histamine and β-glucuronidase from rat peritoneal mast cells. Compound **5** caused strong and dose-dependent inhibition of mast-cell degranulation induced by compound 48/80 (Table 4).

fMLP (1 mM) induced the release of β-glucuronidase and lysozyme from rat neutrophils. Compounds **4**, **5**, **7**, **10**, and **11** had strong and dose-dependent inhibitory effects on the fMLP-induced release of β-glucuronidase and lysozyme from rat neutrophils (Table 5). As shown in Table 4, the hydroxylation of **3** at C-2 (**4**) enhanced the inhibitory effect on the fMLP-induced activation of neutrophils. The hydroxylated product of **4** or **7**, with hydroxylation at C-3 and C-4 (**10**) or at C-2′ and C-4′ (**10**) enhanced the inhibitory effect on the fMLP-induced release of lysozyme from rat neutrophils, but did not enhance the inhibitory effect on the fMLP-induced release of β-glucuronidase. The inhibitory effects of **5** and **10** on the fMLP-induced activation of neutrophils were greater than those of trifluoperazine. Trifluoperazine was used in this study as a positive control and caused dose-dependent inhibition of fMLP-induced neutrophil degranulation.

fMLP (0·3 mM) also induced superoxide formation by rat neutrophils. Compounds **5** and **7** had inhibitory effects on this superoxide formation (Table 6); the sizes of the effects of **5** and **7** were greater than that of trifluoperazine.

The hydroxylated products of **5** and **7**, with hydroxylation at C-3 and C-4 (**11**) or C-2′ and C-4′ (**10**) did not enhance the inhibition of fMLP-induced superoxide formation in rat neutrophils, but the hydroxylated product of **3** and **4**, with hydroxylation at C-2′ (**4**) or C-3 and C-4 (**10**) enhanced the

Table 4. The concentration-dependent inhibition by chalcone derivatives on the release of β-glucuronidase and histamine from rat peritoneal mast cells stimulated with compound 48/80.

Compound	IC50 (μM)*	
	β-Glucuronidase	Histamine
1	48·8 ± 7·4	53·4 ± 9·0
2	–	–
3	–	–
4	–	–
5	20·8 ± 0·84	30·1 ± 2·96
6	> 300 (−15·7 ± 14·4)	> 300 (9·9 ± 14·8)
7	–	–
8	> 100 (44·2 ± 8·3)	–
9	> 100 (19·6 ± 8·4)	> 30 (30·7 ± 10·6)
10	> 30 (3·7 ± 3·7)	> 30 (6·3 ± 2·8)
11	–	–
Mepacrine	12·4 ± 1·6	8·4 ± 1·8

Release reaction was triggered by the addition of compound 48/80 (10 mg mL^{-1}) to the mast cell suspension, which was pre-incubated with DMSO or compounds **1**, **5**, **6**, **8–10** or mepacrine at 37°C for 3 min. After a further 15 min incubation the reaction was terminated, and histamine and β-glucuronidase in the supernatant were determined. *When 50% inhibition could not be reached at the highest concentration, the % inhibition is given in parentheses.

Table 5. The concentration-dependent inhibition by chalcone derivatives of the release of β-glucuronidase and lysozyme from rat neutrophils stimulated with fMLP.

Compound	IC50 (μM)*	
	β-Glucuronidase	Lysozyme
1	> 100 (17·8 ± 6·1)	> 100 (19·3 ± 2·5)
2	> 100 (38·4 ± 1·8)	> 100 (31·4 ± 10·0)
3	47·3 ± 8·2	50·0 ± 3·6
4	11·6 ± 1·4	20·5 ± 0·5
5	2·3 ± 0·2	3·6 ± 0·3
6	> 100 (11·9 ± 14·1)	> 100 (18·5 ± 3·4)
7	8·3 ± 0·4	20·5 ± 1·5
8	> 100 (38·3 ± 10·0)	91·8 ± 4·1
9	65·2 ± 7·1	62·2 ± 6·7
10	10·5 ± 0·6	11·5 ± 0·7
11	10·6 ± 1·0	22·5 ± 0·3
Trifluoperazine	22·2 ± 0·6	17·1 ± 1·0

Release was triggered by the addition of fMLP (1 mM) to the polymorphonuclear leukocyte suspension, which was pre-incubated with DMSO or with compounds **1–11** or trifluoperazine at 37°C for 3 min. After incubation for a further 45 min the β-glucuronidase and lysozyme in the supernatant were determined. *When 50% inhibition could not be reached at the highest concentration, the % inhibition is given in parentheses.

Table 6. The concentration-dependent inhibition by chalcone derivatives of superoxide formation by rat neutrophils stimulated with fMLP.

Compound	IC50 (μM)*
1	40·2 ± 5·7
2	> 100 (46·0 ± 2·7)
3	36·6 ± 4·1
4	> 30 (46·5 ± 7·4)
5	2·6 ± 0·1
6	> 100 (24·7 ± 10·5)
7	11·3 ± 0·6
8	> 100 (48·7 ± 5·5)
9	51·2 ± 8·4
10	22·0 ± 1·5
11	51·0 ± 3·5
Trifluoperazine	14·8 ± 1·7

*PMN leukocyte suspension, pre-incubated for 3 min at 37°C with DMSO or compounds **1–11** or trifluoperazine, superoxide dismutase and Hank's balanced salt solution were added into the blank and test tubes. After addition of cytochrome c, reaction was initiated by challenge with fMLP (0·3 μM). The reaction was terminated after 10 min and the amount of superoxide radical generated was determined (nmol $O_2^-/10^6$ cells). *When 50% inhibition could not be reached at the highest concentration, the % inhibition is given in parentheses.

compounds' inhibitory effects. These data indicate that the anti-inflammatory effect of these compounds might be mediated through the suppression of chemical mediators released from mast cell and neutrophil degranulation. Further experiments are need to evaluate their anti-inflammatory effect and elucidate their exact mechanism of action.

In summary, compounds **5** and **7** had potent anti-cyclooxygenase and anti-inflammatory activity. Because of this dual activity these compounds might be developed as anti-thrombotic or anti-inflammatory agents.

Although compounds **4**, **10** and **11** had potent inhibitory effects on the fMLP-induced activation of neutrophils, they did not show potent anti-cyclooxygenase activity. Thus a relationship between anti-cyclooxygenase activity and anti-inflammatory activity was not recognized.

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References

Agrawal, P. K. (1989) Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam, pp. 376–382

Babior, B. M. (1978) Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. 298: 659–668

Boyum, A. (1968) Isolation of mononuclear cells and granulocytes from blood. Scand. J. Clin. Invest. 97 (Suppl.): 77–89

Egan, R. W., Paxten, J., Kurhl, F. A. (1976) Mechanism for irreversible self-deactivation of prostaglandin synthase. J. Biol. Chem. 251: 7329–7335

Kikimoto, R., Hara, H., Ninomiya, K., Osakabe, M., Sugano, M., Fukami, H., Tamao, Y. (1990) Syntheses and platelet aggregation inhibitory and antithrombotic properties of [2-[(ω-aminoalkoxy)-phenyl]ethyl]benzenes. J. Med. Chem. 33: 1818–1823

Lin, C. N., Lu, C. M., Fang, S. C., Lin, H. C., Hsu, M. F., Wang, J. P., Ko, F. N., Shieh, B. J., Teng, C. M. (1996) Novel antiplatelet constituents of Formosan moraceous plants. J. Nat. Prod. 59: 834–838

Market, M., Andrews, P. C., Barior, B. M. (1984) Measurement of O_2^- production by human neutrophils. The preparation and assay of NADPH oxidase-containing particles from human neutrophils. Methods Enzymol. 105: 358–365

Middleton Jr, E., Kandaswami, C. (1992) Effects of flavonoids on immune and inflammatory cell functions. Biochem. Pharmacol. 43: 1167–1179

Mitchell, J. R. A, Sharp, A. A. (1964) Platelet clumping in vitro. Br. J. Haematol. 10: 78–93

Mustard, J. F., Perry, W. D., Kinlough-Rathbone, R. L., Packham, M. A. (1975) Factors responsible for ADP-induced release reaction of human platelets. Am. J. Physiol. 228: 1757–1765

O'Brien, J. R. (1962) Platelet aggregation: some results from a new method of study. J. Clin. Pathol. 15: 452–455

Smith, R. J., Iden, S. S. (1979) Phorbol myristate acetate-induced release of granule enzymes from human neutrophils: inhibition by the calcium antagonist, 8-(*N*,*N*-diethylamino)-octyl 3,4,5-trimethoxybenzoate hydrochloride. Biochem. Biophys. Res. Commun. 91: 263–271

Sogawa, S., Nihro, Y., Ueda, H., Izumi, A., Miki, T., Matsumoto, H., Satoh, T. (1993) 3,4-Dihydroxychalcones as potent 5-lipoxygenase and cyclooxygenase inhibitors. J. Med. Chem. 36: 3904–3909

Tauber, A. I., Babior, B. M. (1985) Neutrophil oxygen reduction: the enzymes and the products. Free Radical Biol. Med. 1: 265–307

Teng, C. M., Chen, W. Y., Ko, W. C., Ouyang, C. (1987) Antiplatelet effect of butylidenephthalide. Biochem. Biophys. Acta. 924: 375–382

Wang, J. P., Raung, S. L., Lin, C. N., Teng, C. M. (1994) Inhibitory effect of norathyriol, a xanthone from *Tripterospermum lanceolatum*, on cutaneous plasma extravasation. Eur. J. Pharmacol. 251: 35–42

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