JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1601–1609
© 2001 The Authors
Received February 21, 2001
Accepted July 7, 2001
ISSN 0022-3573

Synthesis, antiplatelet and vasorelaxing effects of monooxygenated flavones and flavonoxypropanolamines

Mei-Ing Chung, Feng-Wen Han, Hsien-Cheng Lin, Shorong-Shii Liou, Pao-Ling Huang, Horng-Huey Ko, Ya-Ling Chang, Jaw-Jou Kang, Che-Ming Teng and Chun-Nan Lin

School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China

Mei-Ing Chung, Feng-Wen Han, Horng-Huey Ko, Chun-Nan Lin

School of Technology of Medical Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China

Hsien-Cheng Lin

Ta-Jen Institute of Technology, Ping Tung Hsieng, Taiwan 907, Republic of China

Shorong-Shii Liou, Pao-Ling Huang

Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan 100, Republic of China

Ya-Ling Chang, Che-Ming Teng

Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan 100, Republic of China

Jaw-Jou Kang

Correspondence: M.-I. Chung, School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China. E-mail: meinch@cc.kmu.edu.tw

Funding: This work was supported by the National Science Council of the Republic of China (NSC89-2314-B-037-024).

Abstract

A series of flavones and flavonoxypropanolamines were synthesized and tested in-vitro for their ability to inhibit aggregation of washed rabbit platelets and human platelet-rich plasma (PRP), and for vasoconstriction of rat thoracic aorta. The various substituted positions of the hydroxyl group in flavone ring B and the various oxypropanolamine side chains substituted at position C-2′ of flavone modified the antiplatelet effects. All the compounds tested in human PRP showed significant inhibition of secondary aggregation induced by adrenaline (epinephrine), suggesting that the antiplatelet effect of these compounds is mainly due to an inhibitory effect on thromboxane formation. Compounds **11** and **12** also had potent vasorelaxant effects in rat thoracic aorta. Phenylephrine- and high-K^+-induced $^{45}Ca^{2+}$ influx in aorta were both inhibited by the selected compound **11**. This result indicates that the inhibitory effect of **11** on the contractile response caused by high-K^+ medium and noradrenaline (norepinephrine) in rat thoracic aorta is mainly due to inhibition of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels.

Introduction

Flavonoids isolated from natural sources have been shown to be potent inhibitors of platelet aggregation (Teng et al 1988; Lin et al 1996) and also vasorelaxing agents (Ko et al 1991). In the course of continued screening work on antithrombotic herbs, especially those having antiplatelet and vasorelaxing effects, a potent antiplatelet agent, 2′-hydroxyflavone (**1**) from *Polygonatum alte-lobatum* has been isolated (Huang et al 1997). As part of ongoing work on the discovery of drugs with biological activity, we synthesized 2′-hydroxyflavone (**1**) and its analogues and evaluated their antiplatelet and vasorelaxing actions.

Materials and Methods

Platelet aggregation

Washed rabbit platelets were obtained from ethylene diamine tetraacetate (EDTA)-anticoagulated platelet-rich plasma (PRP) according to procedures described

previously (Teng et al 1987). Human PRP was obtained from the supernatant after centrifugation of a 1:9 mixture of blood and sodium citrate solution (3.8%). Platelet numbers were counted by using a Coulter Counter (Model ZM) 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 (BSA). All glassware was siliconized. PRP or the platelet suspension was stirred at 1200 rev min^{-1} for 1 min before addition of the aggregation inducer. Aggregation was measured by a turbidimetric method (O'Brien 1962). The absorbance of platelet-poor plasma or platelet-free Tyrode's 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.

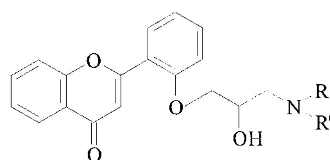
Aortic contraction

Wistar rats of either sex, 250–300 g, were killed by a blow to the head. The thoracic aorta was isolated and excess fat and connective tissue removed. The aorta was placed in an 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. The aorta was 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 dimethyl sulfoxide (DMSO) was fixed at 0.1%.

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

Ca^{2+} influx was measured in a manner similar to that described by Meisheri et al (1980). Aortic rings were placed in test tubes containing Krebs solution with 1 μCi mL^{-1} of $^{45}Ca^{2+}$ in the presence of DMSO (0.1%) or various concentrations of **11** (Table 1) 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

Table 1 Flavonoxypropanolamines.



Compound	R	R′	mp(L) °CL	Crystallization solvent	% yield	Formula	Anal.
4	$-CH_3$	$-CH_3$	Oil	—	53	$C_{20}H_{21}NO_4$	C, H, N
5	-H	$-CH_2CH_3$	190–192	C_6H_{12}	40	$C_{20}H_{21}NO_4 \cdot 2H_2O$	C, H, N
6	-H	$-(CH_2)_2CH_3$	Oil	—	53	$C_{21}H_{23}NO_4$	C, H, N
7	-H	—◁	91–92	C_6H_{12}–Me_2CO	57	$C_{21}H_{21}NO_4 \cdot H_2O$	C, H, N
8	-H	—CH<	95–96	C_6H_{12}	44	$C_{21}H_{23}NO_4$	C, H, N
9	-H	$-(CH_2)_3CH_3$	Oil	—	45	$C_{22}H_{25}NO_4 \cdot H_2O$	C, H, N
10	-H	$—CH_2CH$<	Oil	—	67	$C_{22}H_{25}NO_4$	C, H, N
11	-H	$—CH(CH_3)CH_2CH_3$	175–177	MeOH	35	$C_{22}H_{25}NO_4$	C, H, N
12	-H	$-C(CH_3)_3$	156–157	MeOH	40	$C_{22}H_{25}NO_4$	C, H, N

tubes containing 2 mL of ice-cold Ca^{2+}-free Krebs solution with 2 mM EGTA for 40 min 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 ± s.e.m. A one-way analysis of variance was used for multiple comparison. If there was significant variation between treatment groups, 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.

Chemistry

Monooxygenated flavones (**1**–**3**; Figure 1) and a series of new flavonoxypropanolamines (**4**–**12**; Table 1) were synthesized by general synthetic routes as shown in Figure 2 for 2′-hydroxyflavone (**1**). In general, monooxygenated flavones were synthesized from Baker-Venkataraman rearrangement of 2′-acetylphenylmonomethoxygenated benzoate which was prepared from 2′-hydroxyacetophenone and appropriate monomethoxygenated benzoyl chloride, to the diketone compounds. Cyclization of the diketone compounds yielded monomethoxygenated flavones. After demethylation, monohydroxyflavones were obtained. 2′-Hydroxyflavone was allowed to react with excess epichlorohydrin to yield the epoxide derivative. Ring opening of epoxide with the corresponding amines afforded the corresponding 2′-flavonoxypropanolamines (Wu et al 1989).

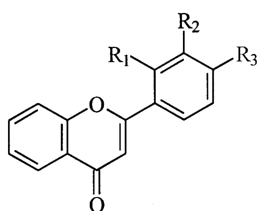


Figure 1 Structure of monooxygenated flavones **1**–**3**.

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 (400 or 200 MHz) and ^{13}C (100 or 50 MHz) NMR spectra (δ (ppm), J (Hz)) were determined with a Varian Unity-400 spectrometer. Mass spectra were determined with a Jeol JMS-D-100 mass spectrometer. Elemental analyses were within ± 0.4% of the theoretical values, unless otherwise noted. Chromatography was performed using a flash-column technique on silica gel 60 supplied by E. Merck.

2′-Hydroxyflavone (**1**), 3′-hydroxyflavone (**2**), and 4′-hydroxyflavone (**3**) were synthesized by genenal synthetic method (Wu et al 1989) and the synthetic products were identical by spectral methods.

General procedure for synthesis of flavonoxypropanolamines ***4–12***

To a solution of 0.1g (2 mmol) sodium hydroxide in water (1 mL) were added 2-propanol (20 mL) and 2′-hydroxyflavone (**1**; 0.5 g, 2 mmol). To the above mixture was then added excess of the appropriate epichlorohydrin (2 mL, 20 mmol), and the mixture was heated at 70°C for 2 h with stirring. The hot reaction mixture was filtered to remove a dimeric byproduct (a glycidyl ether). The clear filtrate, on cooling, yielded a solid (**1′**) (Liou et al 1994), purified by column chromatography (silica gel and $CHCl_3$).

To the former purified solid (**1′**), (0.6 g, 2 mmol) in absolute ethanol (60 mL) the appropriate amine (40 mmol) was added and the mixture was heated at 50–55°C for 5 h with stirring. The reaction mixture was clarified by filtration and the filtrate was concentrated under reduced pressure. The product was filtered, washed with absolute ethanol, and purified by column chromatography, yielding the flavonoxypropanolamines, **4**–**12**. The yields of **4**–**12** are reported in Table 1.

*2′-(2,3-Epoxypropoxy)flavone (**1′**).* White power (0.4 g, 1.4 mmol, yield 60%). IR (KBr) 1638, 1258 cm^{-1}; ^1H NMR($CDCl_3$) δ 2.77 (dd, J = 4.8, 2.4 Hz, 1H, C*H*H in the epoxide ring), 2.91 (t, J = 4.8 Hz, 1H, CH*H* in the epoxide ring), 3.39 (m, 1H, CH of epoxide ring), 4.13 (dd, J = 11.6, 5.6 Hz, 1H, OC*H*H), 4.37 (dd, J = 11.6, 3.2 Hz, 1H, OCH*H*), 7.02 (s, 1H, H-3), 7.07 (m, 1H, H-3′), 7.14 (dt, J = 7.6, 12 Hz, 1H, H-5′), 7.41 (m, 1H, H-6), 7.48 (m, 1H, H-4′), 7.53 (m, 1H, H-8), 7.68 (m, 1H, H-7), 7.84 (dd, J = 8.0, 2.0 Hz, 1H, H-6′), 8.24 (dd, J = 8.0, 2.0 Hz, 1H, H-5);^{13}C NMR ($CDCl_3$) δ 44.7 (CH_2 in the epoxide ring), 49.9 (CH in the epoxide ring), 69.5 (OCH_2), 112.7 (C-3), 113.3 (C-3′), 118.0 (C-8), 121.4

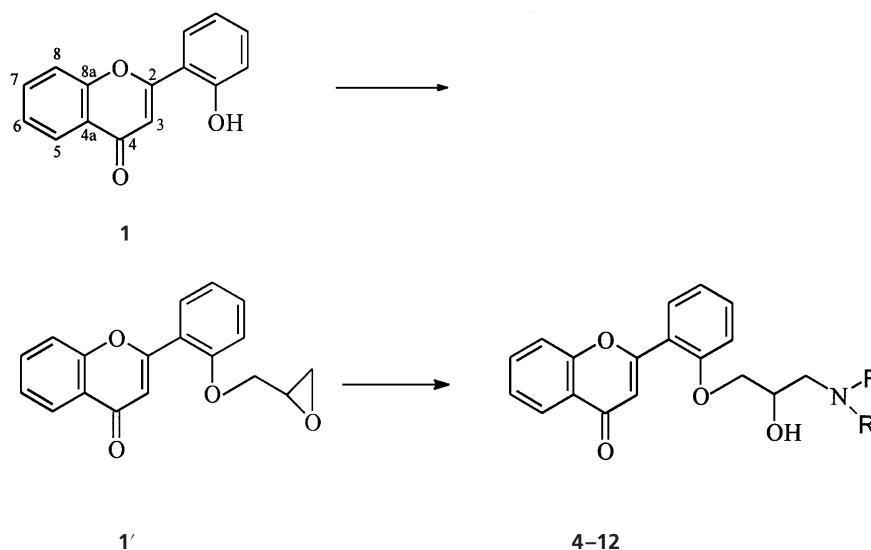


Figure 2 Synthesis of flavonoxypropanolamines **4**–**12**.

(C-5′), 121.7 (C-1′), 123.9 (C-4a), 124.9 (C-6), 125.7 (C-5), 129.6 (C-6′), 132.4 (C-4′), 133.6 (C-7), 156.5 (C-8a), 156.7 (C-2′), 161.2 (C-2), 178.6 (C-4); MS *m/z* 294 (M^+, 49). Anal. ($C_{18}H_{14}O_4$), C, H.

*2′-[3-(*N, N-*Dimethylamino)-2-hydroxypropoxy]flavone (**4**).* IR (KBr) 3450, 1658 cm^{-1}; ^1H NMR (CD_3OD) δ 2.29 (s, 6H, -N(C*H*$_3$)$_2$), 2.52 (m, 2H, -C*H*$_2$N(CH_3)$_2$), 4.01 (dd, $J = 9.6, 6.0$ Hz, 1H, -OC*H*H-), 4.08 (dd, $J = 9.6, 4.0$ Hz, 1H-OCH*H*-), 4.14 (m, 1H, -*H*COH-), 7.06 (m, 2H, H-3′ and -5′), 7.13 (s, 1H, H-3), 7.42 (m, 2H, H-6 and -4′), 7.56 (m, 1H, H-8), 7.71 (m, 1H, H-7), 7.82 (dd, $J = 8.0, 1.6$ Hz, 1H, H-6′), 8.05 (dd, $J = 8.0, 1.6$ Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 46.2 (Me × 2), 63.4 (-CH_2=), 68.2 (-CHOH-), 72.6 (-OCH_2-), 112.8 (C-3), 114.1 (C-3′), 119.4 (C-8), 121.5 (C-1′), 122.1 (C-5′), 124.2 (C-4a), 126.0 (C-6), 126.4 (C-5), 130.2 (C-6′), 134.1 (C-4′), 135.4 (C-7), 157.8 (C-8a), 158.7 (C-2′), 163.3 (C-2), 180.8 (CO); MS *m/z* 340 ($(M+1)^+$, 3).

*2′-[3-(Ethylamino)-2-hydroxypropoxy]flavone (**5**).* IR (KBr) 3410, 1629 cm^{-1}; ^1H NMR (CD_3OD) δ 1.26 (t, $J = 7.6$ Hz, 3H, -CH_2C*H*$_3$), 2.99 (m, 2H, -C*H*$_2$$CH_3$), 3.06 (dd, $J = 12.8, 9.6$ Hz, 1H, -C*H*HNHCH_2-), 3.16 (dd, $J = 12.8, 3.4$ Hz, 1H- CH*H*NHCH_2-), 4.19 (m, 2H, -OCH_2-), 4.30 (m, 1H, -*H*COH-), 7.18 (s, 1H, H-3), 7.20 (m, 2H, H-3′ and -5′), 7.53 (m, 2H, H-6 and -4′), 7.70 (m, 1H, H-8), 7.83 (m, 1H, H-7), 7.96 (dd, $J = 8.0, 2.0$ Hz, 1H, H-6′), 8.15 (dd, $J = 8.0, 2.0$ Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 11.5 (CH_3), 43.5 (-C*H*$_2$$CH_3$), 55.6 (-$CH_2$NH-), 65.5 (-$CH_2$*C*HOH-), 71.8 (-$OCH_2$-), 112.9 (C-3), 114.3 (C-3′), 119.6 (C-8), 122.0 (C-1′), 122.7 (C-5′), 124.4 (C-4a), 126.1 (C-6), 126.7 (C-5), 130.7 (C-6′), 134.3 (C-4′), 135.8 (C-7), 158.1 (C-8a), 158.3 (C-2′), 163.7 (C-2), 180.9 (CO); MS *m/z* 340 ($(M+1)^+$, 5).

*2′-[3-(n-Propylamino)-2-hydroxypropoxy]flavone (**6**).* IR (KBr) 3410, 1621 cm^{-1}; ^1H NMR (CD_3OD) δ 0.94 (t, $J = 7.6$ Hz, 3H, -CH_3), 1.57 (m, 2H, -C*H*$_2$$CH_3$), 2.70 (m, 2H, -NHC*H*$_2$-), 2.88 (dd, $J = 12.4, 8.8$ Hz, 1H, -C*H*HNH-), 2.97 (dd, $J = 12.4, 3.8$ Hz, 1H, -CH*H*NH-), 4.15 (d, $J = 5.2$ Hz, 2H, -OCH_2-), 4.22 (m, 1H, -*H*COH-), 7.17 (m, 2H, H-3′ and -5′), 7.16 (s, 1H, H-3), 7.52 (m, 2H, H-6 and –4′), 7.68 (m, 1H, H-8), 7.81 (m, 1H, H-7), 7.93 (dd, $J = 8.0, 2.0$ Hz, 1H, H-6′), 8.14 (dd, $J = 8.0, 2.0$ Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 11.7 (-CH_3), 22.6 (-C*H*$_2$$CH_3$), 51.9 (-NH-$CH_2$-), 52.6 (-CHOH-C*H*$_2$-), 68.5 (-CHOH-), 72.5 (-OCH_2-), 112.8 (C-3), 114.3 (C-3′), 119.5 (C-8), 121.9 (C-1′), 122.4 (C-5′), 124.4 (C-4a), 126.1 (C-6), 126.6 (C-5), 130.6 (C-6′), 134.2 (C-4′), 135.7 (C-7), 158.0 (C-8a), 158.6 (C-2′), 163.8 (C-2), 181.0 (CO); MS *m/z* 353 (M^+, 7).

2′-[3-(Cyclopropylamino)-2-hydroxypropoxy]flavone (7). IR (KBr) 3280, 1620 cm^{-1}; ^1H NMR (CD_3OD) δ 0.33 (m, 2H, -C*H*H- and -C*H*H in the cyclopropyl ring), 0.44 (m, 2H, -CH*H*- and -CH*H*- in the cyclopropyl ring), 2.18 (m, 1H, -CH- in the cyclopropyl ring), 2.81 (dd, $J = 12.6, 8.2$ Hz, 1H, -C*H*HNH-), 2.92 (dd, $J = 12.6, 3.8$ Hz, 1H, -CH*H*NH-), 4.13 (m, 2H, -OCH_2-),

4.18 (m, 1H, -C*H*OH-), 7.17 (s, 1H, H-3), 7.18 (m, 2H, H-3′ and -5′), 7.50 (m, 2H, H-6 and -4′), 7.69 (m, 1H, H-8), 7.82 (m, 1H, H-7), 7.92 (dd, $J = 8.0$, 1.6 Hz, 1H, H-6′), 8.15 (dd, $J = 8.0$, 1.6 Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 6.08 (-CH_3), 6.34 (-CH_2-), 31.2 (=CHNH-), 53.1 (-CH_2NH-), 69.3 (-CHOH-), 72.8 (-OCH_2-), 112.8 (C-3), 114.3 (C-3′), 119.5 (C-8), 121.9 (C-1′), 122.2 (C-5′), 124.4 (C-4a), 126.1 (C-6), 126.6 (C-5), 130.6 (C-6′), 134.2 (C-4′), 135.6 (C-7), 158.1 (C-8a), 158.8 (C-2′), 164.0 (C-2), 181.0 (CO); MS m/z 351 (M^+, 4).

*2′-[3-(Isopropylamino)-2-hydroxypropoxy]flavone (**8**).* IR (KBr) 3275, 1620 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.06 (d, $J = 6.4$ Hz, 6H, -CH(CH_3)$_2$), 2.83 (dd, $J = 12.6$, 6.0 Hz, 1H, -C*H*HNH-), 2.88 (dd, $J = 12.6$, 3.6 Hz, 1H, -CH*H*NH-), 4.15 (m, 3H, -OCH_2C*H*OH-), 7.16 (s, 1H, H-3), 7.18 (m, 2H, H-3′ and -5′), 7.50 (m, 2H, H-6 and -4′), 7.69 (m, 1H, H-8), 7.82 (m, 1H, H-7), 7.93 (dd, $J = 8.0$, 1.6 Hz, 1H, H-6′), 8.16 (dd, $J = 8.0$, 1.6 Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 22.4 (2 ×-CH_3), 49.9 (-CH=), 50.8 (-CH_2NH-), 69.5 (-CHOH-), 72.8 (-OCH_2-), 112.8 (C-3), 114.3 (C-3′), 119.5 (C-8), 122.0 (C-1′), 122.3 (C-5′), 124.4 (C-4a), 126.1 (C-6), 126.6 (C-5), 134.2 (C-4′), 135.6 (C-7), 158.1 (C-8a), 158.8 (C-2′), 164.0 (C-2), 181.0 (CO); MS m/z 353 (M^+, 1).

*2′-[3-(n-Butylamino)-2-hydroxypropoxy]flavone (**9**).* IR (KBr) 3390, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, $J = 7.2$ Hz, 3H, -CH_2CH_3), 1.31 (m, 2H, -C$H_2$$CH_3$), 1.47 (m, 2H, -$CH_2CH_2$$CH_2$), 2.64 (m, 2H, -NH$CH_2$), 2.81 (dd, $J = 12.0$, 8.0 Hz, 1H, -CHOHC*H*HNH-), 2.90 (dd, $J = 12.0$, 3.8 Hz, 1H, -CHOHCH*H*NH), 3.40 (bs, 1H, NH), 4.12 (m, 2H, -OCH_2-), 4.13 (m, 1H, -C*H*OH-), 7.06 (m, 2H, H-3′ and -5′), 7.10 (s, 1H, H-3), 7.74 (m, 2H, H-6 and -4′), 7.50 (m, 1H, H-8), 7.66 (m, 1H, H-7), 7.82 (dd, $J = 8.0$, 1.6 Hz, 1H, H-6′), 8.18 (dd, $J = 8.0$, 1.6 Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$) δ 13.8 (-CH_3), 20.3 (-C$H_2$$CH_3$), 31.5 (-C$H_2$$CH_2$$CH_3$), 49.4 (-NH$CH_2$), 51.9 (-CHOHC$H_2$NH-), 67.6 (-CHOH-), 71.3 (-OCH_2-), 112.4 (C-3), 112.8 (C-3′), 118.0 (C-8), 121.0 (C-1′ and -5′), 123.6 (C-4a), 125.0 (C-6), 125.6 (C-5), 129.3 (C-6′), 132.5 (C-4′), 133.7 (C-7), 156.4 (C-8a), 157.1 (C-2′), 161.4 (C-2), 178.9 (CO); MS m/z 367 (M^+, 5).

*2′-[3-(Isobutylamino)-2-hydroxypropoxy]flavone (**10**).* IR (KBr) 3410, 1620 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.95 (d, $J = 6.7$ Hz, 6H, -CH(CH_3)$_2$), 1.86 (m, 1H, -C*H*(CH_3)$_2$), 2.60 (m, 2H, -NHCH_2CH-), 2.97 (m, 2H, -CHOHCH_2NH-), 4.17 (m, 2H, -OCH_2-), 4.23 (m, 1H, -C*H*OH-), 7.17 (s, 1H, H-3), 7.19 (m, 2H, H-3′ and -5′), 7.55 (m, 2H, H-6 and -4′), 7.71 (dd, $J = 8.0$, 1.6 Hz, 1H, H-8), 7.84 (m, 1H, H-7), 7.95 (dd, $J = 8.0$, 1.6 Hz, 1H, H-6′), 8.17 (dd, $J = 8.0$, 1.6 Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 20.7 (-CH_3), 20.8 (-CH_3), 28.5 (-*C*H(CH_3)$_2$), 52.9 (-NH-CH_2), 58.0 (-CHOHCH_2NH-), 68.6 (-CHOH-), 72.5 (-OCH_2-), 112.8 (C-3), 114.3 (C-3′), 119.5 (C-8), 121.9 (C-1′), 122.4 (C-5′), 124.4 (C-4a), 126.1 (C-6), 126.6 (C-5), 130.6 (C-6′), 134.2 (C-4′), 135.7 (C-7), 158.1 (C-8a), 158.7 (C-2′), 163.9 (C-2), 181.0 (CO); MS m/z 367 (M^+, 2).

*2′-[3-(*sec*-Butylamino)-2-hydroxypropoxy]flavone (**11**).* Compound **11** was prepared from (±) *sec*-butylamine. IR (KBr) 3300, 1630 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.88 (t, $J = 7.2$ Hz, 3H, -CH_2 CH_3), 1.05 (d, $J = 6.4$Hz, 3H, -CHCH_3), 1.31 (m, 1H, -C*H*HCH_3), 1.55 (m, 1H, -CH*H*CH_3), 2.64 (m, 1H, =C*H*HCH_3), 2.78 (m, 1H, -C*H*HNH-), 2.90 (m, 1H, -CH*H*NH-), 4.12 (m, 3H, -OCH_2- and -C*H*OH-), 7.04 (m, 2H, H-3′ and -5′), 7.09 (s, 1H, H-3), 7.40 (m, 2H, H-6 and -4′), 7.50 (d, $J = 8.0$ Hz, 1H, H-8), 7.69 (m, 1H, H-7), 7.78 (dd, $J = 7.6$, 1.6 Hz, 1H, H-6′), 8.01 (dd, $J = 7.6$, 1.6 Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 10.2 (-CH_3), 18.2 (=CHCH_3), 55.4 (-NH*C*HCH_3-), 49.8 (-CH_2NH-), 67.9 (-CHOH-), 72.2 (-OCH_2-), 113.0 (C-3), 113.6 (C-3′), 118.7 (C-8), 121.2 (C-5′), 121.5 (C-1′), 124.4 (C-4a), 125.3 (C-6), 125.6 (C-5), 132.9 (C-4′), 134.0 (C-7), 156.8 (C-8a), 163.8 (C-2′), 178.1 (CO); MS m/z 367 (M^+, 2).

*2′-[3-(*tert*-Butylamino)-2-hydroxypropoxy]flavone (**12**).* IR (KBr) 3390, 1636 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.27 (s, 9H, 3 × Me), 2.97 (dd, $J = 12.0$, 9.2 Hz, 1H, -C*H*HNH-), 3.10 (dd, $J = 12.0$, 3.2Hz, 1H, -CH*H*NH-), 4.16 (m, 2H, -OCH_2-), 4.06, (m, 1H, -C*H*OH-), 7.09 (m, 2H, H-3′ and -5′), 7.09 (s, 1H, H-3), 7.42 (m, 2H, H-6 and -4′), 7.55 (dd, $J = 8.4$, 0.4 Hz, 1H, H-8), 7.72 (m, 1H, H-7), 7.80 (dd, $J = 8.0$, 1.6 Hz, 1H, H-6′), 8.02 (dd, $J = 8.0$, 1.6 Hz, 1H, H-5); ^{13}C NMR (CD_3OD) δ 26.8 (-C(CH_3)$_3$), 45.9 (-CH_2NH-), 55.8 [-*C*(CH_3)$_3$], 68.1 (-CHOH-), 72.1 (-OCH_2-), 112.6 (C-3), 114.3 (C-3′), 119.4 (C-8), 121.4 (C-1′), 122.3 (C-5′), 124.1(C-4a), 125.9 (C-6), 126.5 (C-5), 130.3 (C-6′), 134.2 (C-4′), 135.6 (C-7), 157.7 (C-8a), 158.4 (C-2′), 163.3 (C-2), 180.7 (CO); MS m/z 367 (M^+, 2).

Results and Discussion

The antiplatelet effects of **1**–**12** were studied in the aggregation of washed rabbit platelets induced by thrombin (0.1 IU mL^{-1}), arachidonic acid (100 μM), collagen (10 μg mL^{-1}), and platelet-activating factor

Table 2 Effect of flavones and flavonoxypropanolamines on the aggregation induced by thrombin, arachidonic acid, collagen and platelet-activating factor (PAF) in washed rabbit platelets.

Compound	Aggregation (%)			
	Thrombin (0.1 IU mL^{-1})	Arachidonic acid (100 μM)	Collagen (10 μg mL^{-1})	PAF (2 ng mL^{-1})
Control	91.5 ± 0.4	85.8 ± 0.6	88.6 ± 0.7	91.0 ± 0.4
1	86.2 ± 2.1 (100)	0.0 ± 0.0*** (100)	8.3 ± 4.48*** (100)	82.4 ± 3.5* (100)
2	88.0 ± 2.3	0.0 ± 0.0***	14.0 ± 2.5***	11.4 ± 4.7***
3	85.9 ± 2.4*	0.0 ± 0.0***	10.1 ± 7.5***	61.4 ± 2.2***
4	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0*** (100)	0.0 ± 0.0***
5	0.0 ± 0.0***	0.0 ± 0.08***	0.0 ± 0.0***(100)	0.0 ± 0.0***
6	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0*** (50)	0.0 ± 0.0***
7	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
8	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
9	1.2 ± 1.1***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
10	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0*** (100)	0.0 ± 0.0***
11	5.1 ± 4.2***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
12	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Aspirin	91.9 ± 2.5	0.0 ± 0.0***	85.4 ± 3.9	90.5 ± 1.2

Platelets were pre-incubated with DMSO (0.5%, control), aspirin (50 μM) or compounds **1**–**12** (300 μM) at 37°C for 3 min, and the inducer was then added. Percentages of aggregation are presented as means ± s.e.m., n = 3–5. When the concentration of compounds was different from 300 μM, the corrected dose is given in parentheses. *$P < 0.05$, ***$P < 0.001$ as compared with the respective control values.

Table 3 IC50 values (μM) of flavones and flavonoxypropanolamines on the aggregation induced by thrombin, arachidonic acid, collagen and platelet-activating factor (PAF) in washed rabbit platelets.

Compound	Thrombin (0.1 IU mL^{-1})	Arachidonic acid (100 μM)	Collagen (10 μg mL^{-1})	PAF
1	> 100 (10.1)	18.9	47.2	> 100 (8.4)
2	> 300 (3.0)	170	< 300 (84.5)	220
3	> 300 (5.3)	37.5	< 300 (88.8)	< 300 (31.8)
4	> 100 (4.6)	85.6	43.9	153
5	> 100 (4.3)	49.0	46.0	139
6	> 100 (3.2)	93.5	20 (5.4)	221
7	> 100 (2.3)	18.0	38.0	154
8	183	56.9	58.8	71.8
9	164	61.4	38.7	51.1
10	> 100 (7.5)	48.9	41.6	90.7
11	< 300 (94.4)	35.1	< 300 (100)	54.2
12	< 300 (100)	48.5	< 300 (100)	62.7

When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. IC50, 50% inhibition concentration.

(PAF, 2 ng mL^{-1}). As shown in Table 2, monohydroxyflavones **1**–**3**, at a concentration of 100 or 300 μM, showed a potent antiplatelet effect on the aggregation induced by arachidonic acid and collagen while having no significant antiplatelet effect on the aggregation induced by thrombin. It clearly indicated that the substituted position of the B-ring hydroxyl group of **1**–**3** did not alter the antiplatelet effect at high concentration on the aggregation induced by thrombin.

Compound **2** (300 μM) strongly inhibited the platelet aggregation induced by PAF while **1** (100 μM) and **3** (300 μM) did not. This indicated that the flavone with a hydroxyl group substituted at C-3′ enhanced the antiplatelet effect at high concentration on the aggregation

Table 4 Effect of selected flavones and flavonoxypropanolamines on aggregation of human platelet-rich plasma (PRP) induced by ADP, collagen or adrenaline.

Compound		Aggregation (%)		
		ADP	Adrenaline	Collagen
Control		91.8 ± 2.6	93.0 ± 0.4	91.2 ± 2.8
1	(150 μM)	–	–	94.7 ± 1.6
	(300 μM)	87.3 ± 2.3*	53.7 ± 1.9***	97.6 ± 1.2
	(450 μM)	–	48.9 ± 3.6***	–
2	(300 μM)	83.5 ± 1.5	91.1 ± 0.7*	80.0 ± 7.1
	(450 μM)	–	57.6 ± 3.7***	–
3	(300 μM)	78.0 ± 3.4*	90.8 ± 1.5*	85.6 ± 1.8
	(450 μM)	–	46.9 ± 5.5***	–
7	(150 μM)	85.0 ± 0.3	91.3 ± 3.4	94.0 ± 2.7
	(300 μM)	88.6 ± 3.5	54.3 ± 2.2***	88.4 ± 1.6
Aspirin	(50 μM)	84.4 ± 1.2	39.6 ± 15.4*	74.0 ± 3.2

Platelets were pre-incubated with **1**–**3**, **7**, aspirin or DMSO (0.5%, control) at 37°C for 3 min, then ADP (20 μM), adrenaline (5 μM) or collagen (10 μg mL^{-1}) was added. Percentages of aggregation are presented as means ± s.e.m., n = 3–5. –, not determined. *$P < 0.05$, ***$P < 0.001$ as compared with the respective control values.

induced by PAF. All the 2′-oxypropanolamines (**4**–**12**) strongly inhibited the platelet aggregation at high concentration induced by various inducers as shown in Table 2. This showed that the amino side chains of **4**–**12** did not alter the antiplatelet effects at high concentration on the platelet aggregation induced by various inducers. It also clearly indicated that an oxypropanolamine side chain substituted at C-2′ of the flavone significantly enhanced the antiplatelet effects on platelet aggregation induced by thrombin, collagen and PAF (Table 2). Aspirin was used in this study as a positive control. It was found that aspirin (50 μM) completely inhibited the platelet aggregation induced by arachidonic acid but not by thrombin, collagen or PAF. More experiments were performed to study the potency of **1**–**12** on thrombin-, arachidonic-acid-, collagen- and PAF-induced washed rabbit platelet aggregation. As shown in Table 3, in thrombin-induced platelet aggregation, none of the compounds at low concentration showed any antiplatelet effect. In arachidonic-acid-induced platelet aggregation, compounds **1** and **7** were the most potent. This indicated that a 3-cyclopropylamino-2-hydroxypropoxy side chain substituted at C-2′ of **1** significantly enhanced the antiplatelet effect. In collagen-induced platelet aggregation, **1**–**3**, **6**–**8**, **11** and **12** showed a less potent antiplatelet effect than in arachidonic-acid-induced platelet aggregation while **4**, **5**, **9** and **10** showed a more potent antiplatelet effect than in arachidonic-acid-induced platelet aggregation. Compounds **4**, **5**, **7**–**9** and **10**, with an oxypropanolamine side chain substituted at C-2′ of **1**, at low concentration significantly enhanced the antiplatelet effect on collagen-induced platelet aggregation. In PAF-induced platelet aggregation, none of the compounds at low concentration had a potent antiplatelet effect.

The antiplatelet effects of selected compounds **1**, **2**, **3** and **7** were also tested on the aggregation of human PRP induced by ADP (20 μM), adrenaline (5 μM) and collagen (10 μg mL^{-1}). As shown in Table 4, all selected compounds showed significant antiplatelet effects on

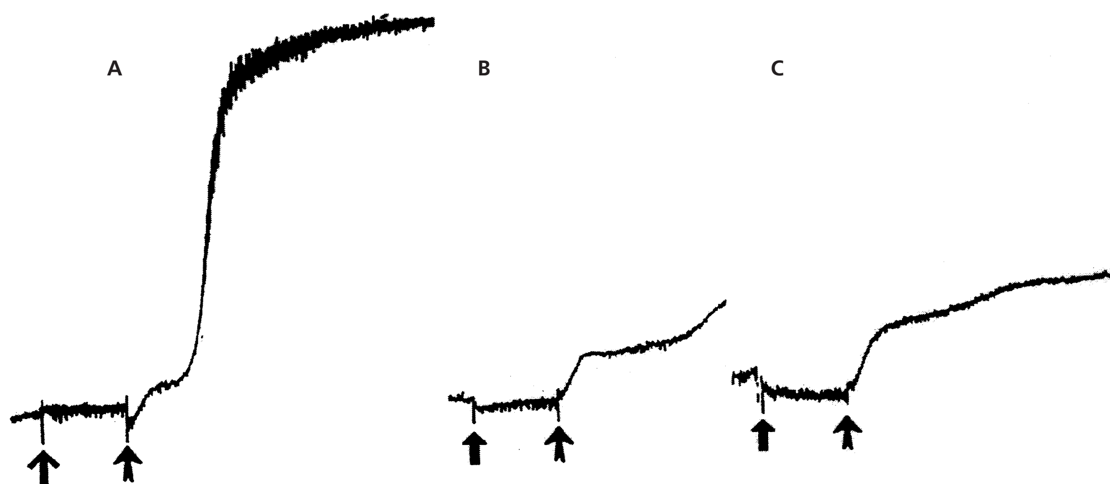


Figure 3 Effect of 2′-hydroxyflavone (**1**) on aggregation of human platelet-rich plasma (PRP) induced by adrenaline. PRP was incubated with dimethylsulfoxide (0.5%) (A), or 2′-hydroxyflavone, 300 μM (B), 450 μM (C) for 3 min; the inducer adrenaline (5 μM) was then added to trigger the aggregation.

Table 5 Effect of selected flavonoxypropanolamines on high-K^+- and Ca^{2+}-induced and noradrenaline-induced contraction of rat thoracic aorta.

Compound		K^+(80 mM) + Ca^{2+} (1.9 mM)	Noradrenaline	
			(3 μM) Phasic	(3 μM) Tonic
Control		100 ± 5.8	100 ± 3.9	100 ± 3.8
11	(15 μM)	–	96.5 ± 2.5	80.8 ± 0.7*
	(45 μM)	68.4 ± 2.1*	0.0 ± 0.0***	25.0 ± 1.3**
	(150 μM)	24.9 ± 0.5**	–	–
12	(15 μM)	–	82.1 ± 6.2	81.9 ± 1.0*
	(45 μM)	76.4 ± 6.2	0.0 ± 0.0***	10.9 ± 2.7**
	(150 μM)	24.6 ± 8.6*	–	–

Rat aorta was pre-incubated with **11** and **12** or DMSO (0.1 %, control) at 37°C for 15 min, then high K^+ (80 μM) and Ca^{2+} (1.9 mM) or noradrenaline (3 μM) was added. Percentages of contraction were calculated and presented as means ± s.e.m., n = 3. –, not determined. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared with the respective control values.

adrenaline-induced platelet aggregation. Aspirin was also used in this study as a positive control. It was found that aspirin (50 μM) inhibited the platelet aggregation induced by adrenaline but not by ADP and collagen. In human PRP, these compounds prevented secondary aggregation induced by adrenaline (e.g. **1**; Figure 3). We conclude that their mechanism of action is chiefly due to the inhibition of thromboxane formation (Weiss 1983b).

In rat thoracic aorta, **11** and **12** (Table 5) markedly depressed the contractions induced by Ca^{2+} (1.9 mM) in high-K^+ (80 mM) medium and the noradrenaline (3 μM)-induced phasic and tonic contractions, but the tonic contractions were more resistant to **11** and **12**.

Compound **11** inhibited, in a concentration-dependent manner, phenylephrine- and high-K^+-induced $^{45}Ca^{2+}$ influx of rat aorta (Figure 4).

The tonic contraction in response to noradrenaline results primarily from Ca^{2+} entry through receptor-activated Ca^{2+} channels with little requirement for Ca^{2+} through voltage-dependent Ca^{2+} channels (Bohr 1963; Hudgins & Weiss 1968; Weiss 1983a). Noradrenaline-induced tonic contraction and phenylephrine-induced $^{45}Ca^{2+}$ influx were both inhibited by **11** (Table 5, Figure 4), indicating that **11** 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 (Karaki & Weiss 1979). Since **11** inhibited the Ca^{2+}-dependent contraction and $^{45}Ca^{2+}$ influx in high-K^+ medium, it may be a blocker of voltage-dependent Ca^{2+} channels (Table 5, Figure 4). As shown in Figure 4, high K^+-induced $^{45}Ca^{2+}$ influx was more easily inhibited by verapamil, a relatively selective inhibitor of voltage-dependent Ca^{2+} channels (Karaki et al 1984). The results from this study indicate that **11** is a relatively selective inhibitor of receptor-operated Ca^{2+} channels and its inhibitory effect on the contractile response caused by high K^+ and noradrenaline in rat thoracic aorta is mainly due to the inhibition of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. Thus, **11** and **12** possess antiplatelet and vasorelaxing actions similar to those of norathyriol and apigenin. The activity in PRP represents the functional antagonist property in a physiologically relevant medium and the activity in the rat thoracic aorta represents antagonism on a non-platelet side of the vascular system. These dual activities show that these compounds may be developed as anti-thrombotic agents.

Conclusion

These results indicate that most of the synthesized compounds possessed potent antiplatelet effect on arachidonic-acid- and collagen-induced platelet aggregation. Their antiplatelet effect is mainly due to an inhibitory effect on thromboxane formation. The selected compound **11** is a relatively selective inhibitor of receptor-operated Ca^{2+} channels and its vasorelaxing effect on the contraction caused by high K^+ and noradrenaline in rat thoracic aorta is mainly due to the inhibition of Ca^{2+} influx through both voltage-dependent and receptor-operated Ca^{2+} channels. Thus this series of compounds with novel antiplatelet and vasorelaxing effects might be developed as antithrombotic agents.

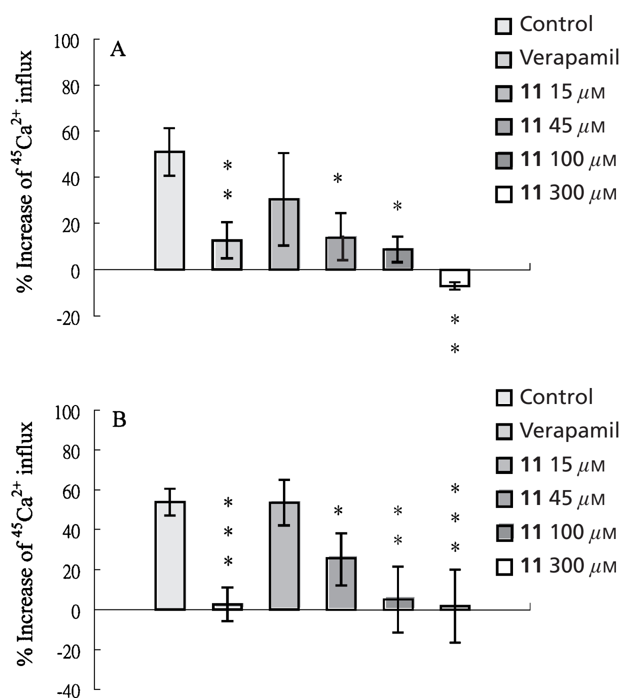


Figure 4 Effect of 2′-[3-(*sec*-Butylamino)-2-hydroxypropoxy]flavone (**11**) on the $^{45}Ca^{2+}$ influx induced by phenylephrine and KCl in rat aortic rings. Aortic rings were placed in test-tubes containing Krebs solution with $^{45}Ca^{2+}$ (1 μCi mL^{-1}) and incubated for 20 min with DMSO (0.1 %) or verapamil (2 μM) or various concentrations of **11**, then phenylephrine (3 μM, A), KCl (60 mM, 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 ± s.e.m., n = 3–4, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the respective control (phenylephrine or K^+ alone).

References

Bohr, D. H. (1963) Vascular smooth muscle: dual effect of calcium. *Science* **139**: 597–599

Huang, P. L., Gan, K. H., Wu, R. R., Lin, C. N. (1997) Benzoquinones, a homoiso-flavanone and other constituents from *Polygonatum alte-lobatum*. *Phytochemistry* **44**: 1369–1373

Hudgins, P. M., Weiss, G. B. (1968) Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmacol. Exp. Ther.* **159**: 91–99

Karaki, H., Weiss, G. B. (1979) Alterations in high and low affinity binding of ^{45}Ca in rabbit aortic smooth muscle by norepinephrine and potassium after exposure to lanthanum and low temperature. *J. Pharmacol. Exp. Ther.* **211**: 86–92

Karaki, H., Nakagawa, H., Urakawa, N. (1984) Comparative effects of verapamil and sodium nitroprusside on contraction and ^{45}Ca uptake in the smooth muscle of rabbit aorta, rat aorta, and guinea-pig taenia coli. *Br. J. Pharmacol.* **81**: 393–400

Ko F. N., Huang T. F., Teng C. M. (1991) Vasodilatory action mechanisms of apigenin isolated from *Apium graveolens* in rat thoracic aorta. *Biochim. Biophys. Acta.* **1115**: 69–74

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

Liou, S. S., Teng, C. M., Ko, F. N., Lin, C. N. (1994) Gamma-Pyrone compounds. 5. Synthesis and antiplatelet effects of xanthonoxypropanolamines and related compounds. *J. Pharm. Sci.* **83**: 391–395

Meisheri, K. D., Palmer, R. F., Van Breemen, C. (1980) The effects of amrinone on contractility, Ca^{2+} uptake and cAMP in smooth muscle. *Eur. J. Pharmacol.* **61**: 159–165

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

Teng, C. M., Chen, W. Y., Ko, W. C., Ouyang, C. (1987) Antiplatelet effect of butylidenephthalide. *Biochim. Biophys. Acta* **46**: 375–382

Teng, C. M., Lee, L. G., Ko, F. N., Huang, T. H. (1988) Inhibition of platelet aggregation by apigenin from *Apium graveolens*. *Asia Pacific J. Pharmacol.* **3**: 85–89

Weiss, G. B. (1983a) Delineation of actions of calcium antagonists in vascular smooth muscle. In: Merrill, G. F., Weiss, H. R. (eds) *Ca^{2+} entry blockers, adenosine and neurohumors*. Urban and Schwarzenberg, Baltimore, p. 63

Weiss, H. J. (ed.) (1983b) Antiplatelet drugs: pharmacological aspects. In: *Platelets: pathophysiology and antiplatelet drug therapy*. Alan R Liss Inc., New York, p. 46

Wu, E. S. C., Cole, T. E., Davidson, T. A., Dailey, M. A., Doring, K. G., Fedorchuk, M., Loch, J. T., Thomas, T. L., Blosser, J. C., Borrelli, A. R., Kinsolving, C. R., Parker, R. B., Strand, J. C., Watkins, B. E. (1989) Flavones. 2. Synthesis and structure-activity relationship of flavodiol and its analogues, a novel class of antihypertensive agents with catecholamine depleting properties. *J. Med. Chem.* **32**: 183–192