Synthesis and Anti-platelet, Anti-inflammatory and Anti-allergic Activities of Methoxyisoflavanquinone and Related Compounds

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In a continuation of our search for novel anti-platelet agents, isoflavone quinone and isoflavanquinone were selected as lead compounds and the synthesis of their methoxy derivatives was carried out. Among them, the 4′- and 7-methoxy derivatives were successfully prepared, whereas the attempt to obtain 3′-methoxy derivatives resulted in their isomers, 3′-methoxyflavone quinone and 3′-methoxyflavanquinone, instead.

After screening for their anti-platelet, anti-inflammatory and anti-allergic activities, a preliminary structure–activity relationship was established.

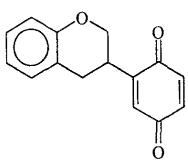
Compounds 6c, 7a—c, 8c and 9a—c were found to exhibit significant activities. In particular, compound 7c demonstrated very potent anti-platelet, anti-inflammatory and anti-allergic activities and was then recommended for further pharmacological investigation.

Key words methoxyisoflavanquinone; methoxyflavanquinone; anti-platelet activity; anti-inflammatory activity; anti-allergic activity; structure–activity relationship

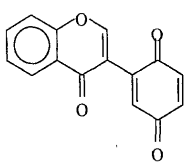
In a previous paper,[1] we synthesized isoflavanquinone (A) and its related compounds and discovered that both compound A and isoflavone quinone (B) exhibited potent biological activities.

For this reason, we selected both A and B as lead compounds and then synthesized their methoxy derivatives by incorporating methoxy groups, which are found commonly in natural products,[2] into various positions on the structure of compounds A and B to form our target compounds.

This report describes the synthesis and biological activity of these target compounds and related compounds.



(A)



(B)

Results and Discussion

Chemistry The synthesis of 7 (or 4′)-methoxyisoflavanquinone (**7a** or **7b**) and 7 (or 4′)-methoxyisoflavone quinones (**9a** or **9b**) are illustrated schematically in Chart 1. As shown, chalcones **2a, b** were first obtained from condensation of 2-hydroxy-4-substituted acetophenones in alkali with 2-benzyloxy-4-substituted benzaldehydes **1a, b** which were prepared from 2-hydroxy-4-substituted benzaldehydes.

Chalcones **2a, b** were then acetylated with Ac_2O in pyridine to protect the 2′-hydroxy group. The acetylated chalcones **3a, b** were subsequently treated with thallium nitrate in trimethyl orthoformate [$Tl(NO_3)_3/CH(OCH_3)_3$] at room temperature, according to the method published in a previous paper,[1] to yield the corresponding acetals **4a, b**.

The acetals **4a, b** were heated on a warm bath with dil. HCl in methyl alcohol to give the corresponding isoflavones **5a, b**. These products were then reduced by catalytic hydrogenation on palladium/charcoal to yield the corresponding isoflavans **6a, b** with the loss of their benzyl groups.

Finally, the isoflavans **6a, b** were oxidized with potassium nitrosodisulfonate (Fremy's salt) to afford the corresponding isoflavanquinones **7a, b**.

On the other hand, debenzylation of compounds **5a, b** with 47% HBr yielded the corresponding 2′-hydroxyisoflavones **8a, b** which were subsequently oxidized with Fremy's salt to the corresponding isoflavone quinones **9a, b**.

However, as indicated in Chart 2 and detailed below, the attempt to synthesize 3′-methoxyisoflavanquinone (**7x**) following an analogous procedure for the preparation of isoflavanquinone **7a, b**, failed to furnish the target compound **7x**.

First, 2-hydroxyacetophenone was condensed with 2-benzyloxy-3-methoxybenzaldehyde (**1c**) to give chalcone **2c** successfully. When compound **2c** was acetylatyed with Ac_2O and subsequently treated with $Tl(NO_3)_3/CH(OCH_3)_3$, the molecular formula of the resulting major product **5c** was determined to be $C_{23}H_{18}O_4$, based on its mass spectrum (*m/z* 358, M^+) from electron impact mass (EI-MS) and on its data from elemental analysis. While its molecular formula agreed well with our expectation for compound **5x**, its ^{1}H-NMR spectrum did not show the same result.

Due to the complexity of the ^{1}H-NMR spectrum of compound **5c**, the complete assignment of the signals is still lacking. Despite this, compound **5c** was subjected to catalytic hydrogenation to give the flavan type product **6c** with saturation at the 2 and 3 positions. Further oxidation of compound **6c**, with Fremy's salt, converted its B-ring to a quinone moiety forming a flavanquinone type product **7c**. After comparing the ^{1}H-NMR spectrum of **7c** with the above synthesized 4′-methoxyisoflavanquinone (**7a**), we noticed the presence of a set of mutually coupled signals associated with H-2, 3, 4 in the spectrum of product **7c** that differed from the corresponding pattern of compound **7a**. The spectrum of **7c** exhibited H-3ax, H-3eq, H-4ax, H-4eq and H-2 signals at δ 1.72—1.85 (1H, m), 2.30—2.38 (1H, m), 2.75 (1H, ddd), 3.00 (1H, ddd) and 5.12 (1H, dd) respectively. This spectrum lead us to the

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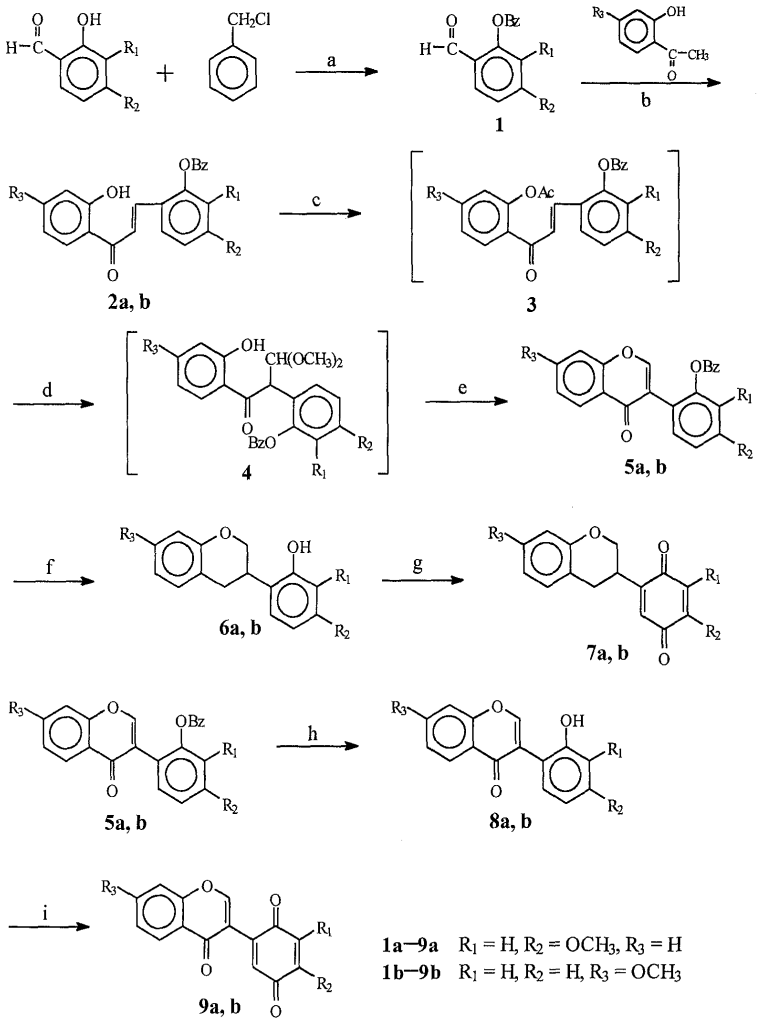


reagents: (a) K_2CO_3/KI/Acetone; (b) NaOH/EtOH/H_2O; (c) Acetic anhydride/Pyridine; (d) $Tl(NO_3)_3/CH(OCH_3)_3$; (e) dil. HCl/CH_3OH; (f) H_2/Pd(C)/CH_3COOH; (g) $K(SO_3)_2NO$/Acetone/CH_3OH; (h) 47% HBr; (i) $K(SO_3)_2NO$/Acetone/CH_3OH

Chart 1

assignment that the quinone moiety (B-ring) for **7c** was attached to the 2-position of the benzopyran and thus it is a flavanquinone, rather than the isoflavanquinone isomer.

All of the remaining spectral data of **7c** were in agreement with our structure assignment. Thus, it was apparent that the treatment of acetylated **2c** with $Tl(NO_3)_3/CH(OCH_3)_3$, followed the cyclization route to form flavone type 2′-benzoxy-3′-methoxyflavone (**5c**), instead of undergoing rearrangement, as in acetylated **2a** or **2b**, to give isoflavone type compound **5x**.

So far, the exact mechanism is still unclear. Under the same reaction conditions [$Tl(NO_3)_3/CH(OCH_3)_3$], compound **2c** did not follow the rearrangement route and instead cyclized directly to a flavone type product, whereas compound **2a**, **2b** and the previously reported[1,3,4] 2-benzyloxy-2′-hydroxy chalcones all underwent rearrangement to form isoflavone type products. We are now working on providing the answer in the near future.

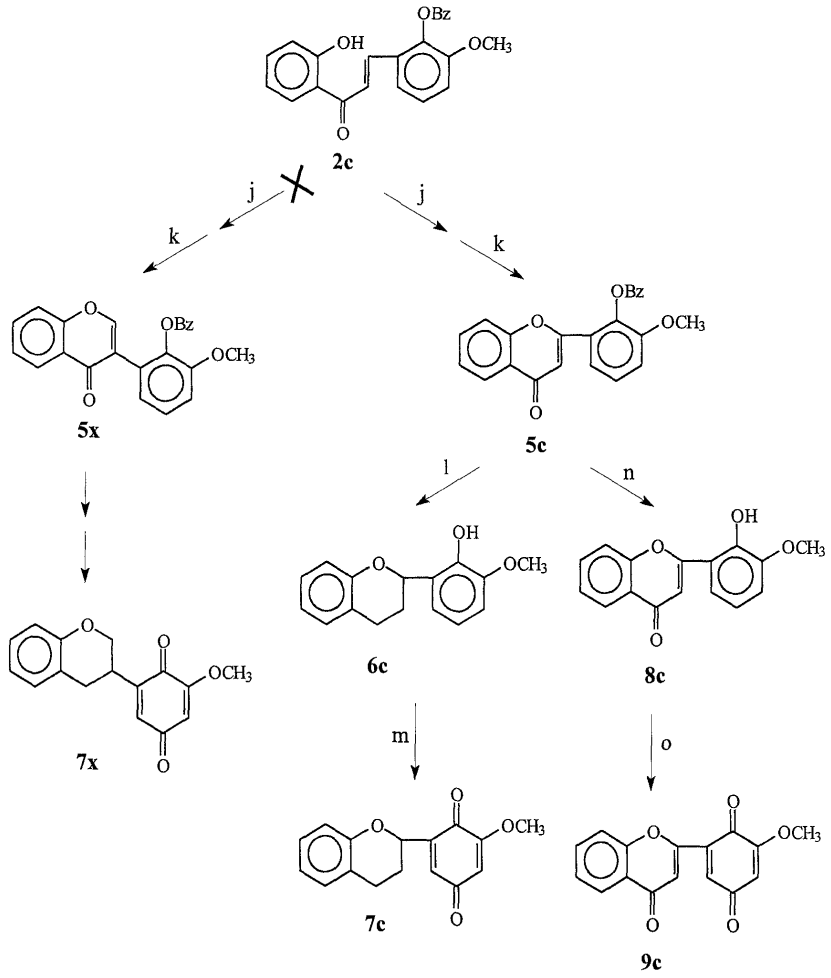
Meanwhile, in order to expand our knowledge base to include the biological activities of compounds related to our target compounds, structure modification was also conducted on the unexpected **5c**. The debenzylation of compound **5c** with 47% HBr, followed by oxidation with Fremy's salt, produced 3′-methoxyflavone quinone (**9c**) which was also tested for its biological activities.

Biological Activity. Anti-platelet Activity The anti-platelet activities of all the target compounds are summarized in Table 1. Among the three chalcones **2a**—**c** tested, compound **2c** was the only one displaying significant activity equal to about 2 fold the inhibitory potency of aspirin against arachidonic acid (AA)-induced platelet-aggregation.

When the chalcones were first converted to flavones **5a**—**c** with a benzyl protection group still on the 2′-OH of their B-ring, we also tested their activity and found only the 4′-methoxy derivatives **5a** exhibited moderate activity.

Debenzylation of the above flavones resulted in compounds **8a**—**c** with free 2′-OH, and elevated activities. In particular, the most potent flavone **8c** demonstrated an IC_{50} value of 3.6 μM which is about 5.5 times the inhibitory potency of aspirin against AA-induced platelet aggregation.

Furthermore, the oxidation of the B-ring of compound **8a**—**c** to quinones **9a**—**c** respectively resulted in potent ac-



Reagents : (j) $Tl(NO_3)_3/CH(OCH_3)_3$; (k) dil.HCl/CH_3OH; (l) $H_2/Pd(C)/CH_3COOH$;

(m) $K(SO_3)_2NO$/Acetone; (n) 47% HBr; (o) $K(SO_3)_2NO$/Acetone/CH_3OH

Chart 2

tivity for isoflavone quinone **9a, b** and flavone quinone **9c**. Especially in the flavone quinone type **9c** which displayed complete inhibition of platelet aggregation stimulated by thrombin, AA, collagen and platelet activating factor (PAF) at the concentration of 25 μg/ml. Its IC_{50} value was 11.3 μM which equaled about 2 times the potency of aspirin against AA-induced aggregation.

Among the flavan and isoflavan type of compounds **6a—c** synthesized above, **6c** was the most potent one showing complete inhibition against AA-, collagen- and PAF-induced platelet aggregation at the concentration of 100 μg/ml. Its IC_{50} value was 5.1 μM, equal to about 4 times the potency of aspirin against AA-induced aggregation.

Conversion of flavone and isoflavones into their corresponding flavanquinone **7c** or isoflavanquinones **7a, b** further elevated their activities. Especially so in the flavanquinone type **7c** which displayed complete inhibition against platelet aggregation induced by thrombin, AA, collagen and PAF at the concentration of 100 μg/ml. As a comparison, the inhibitory potency of **7c** against AA-induced platelet aggregation was about 10 fold stronger than aspirin.

From the above results, it appeared that flavonoids (**8c**, **9c**) and flavanoids (**6c**, **7c**) were generally superior to their corresponding isoflavonoids (**8a, b**, **9a, b**) and isoflavanoids (**6a, b**, **7a, b**) in terms of their platelet activities.

Anti-inflammatory Activity Effect on Neutrophil Degranulation: The activities of our target compounds toward neutrophil degranulation were also examined. As summarized in Table 2, in all the compounds tested, only those with quinone type B-ring (**7a—c**, **9a—c**) exhibited potent activities. Among them, 3′-methoxy flavanquinone (**7c**) note worthly demonstrated the highest potency (IC_{50}=0.19 μM), equivalent to about 30 times the potency of the positive control trifluoperazine (IC_{50}=5.8 μM).

As was also implicated from the data in Table 2, in general, the flavanquinone type compound (**7c**) was more potent than the isoflavanquinone type (**7a, b**), and the flavone quinone type (**9c**) was more potent than the isoflavone quinone type (**9a, b**). This seems to follow the same trend of relative efficacy as their antiplatelet activities.

Effect on Neutrophil Superoxide Formation: Table 3 shows that, with the exception of 3′-methoxyflavanquinone (**7c**) which exhibited very potent inhibitory effect (IC_{50}=0.44 μM) on formyl-Met-Leu-Phe (fMLP)-induced neutrophil superoxide formation, none of the remaining target compounds demonstrated significant effects.

Table 1. The Inhibitory Effects of Compounds on Platelet Aggregation Induced by Thrombin, AA, Collagen and PAF (*in Vitro*)

	Compounds (μg/ml)	Percent aggregation			
		Thrombin	AA	Collagen	PAF
2a	Control	92.0± 0.7	88.1± 0.5	89.5± 0.6	90.6± 0.9
	100	85.3± 3.3*	61.9± 1.1***	48.1± 8.7***	64.3± 3.1***
2b	Control	92.0± 0.7	88.1± 0.5	89.5± 0.6	90.6± 0.9
	100	73.9± 5.8*	46.1± 7.3***	53.0± 7.9***	54.2± 1.8***
2c	Control	89.1± 1.4	84.9± 0.9	84.5± 0.6	86.4± 2.6
	100	74.9± 3.5***	0.0± 0.0***	0.0± 0.0***	58.2± 2.0***
	20			0.0± 0.0***	
	10		0.0± 0.0***	2.7± 2.3***	
	5		40.0± 20.0*	72.0± 1.1***	
	2		78.6± 2.3*	82.2± 1.9	
	IC_{50}		12.1 μM	14.9 μM	
5a	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	69.3± 2.3***	0.0± 0.0***	0.0± 0.0***	64.2± 1.1***
	50		0.0± 0.0***		
	20		0.0± 0.0***		
	10		80.1± 2.4***		
	IC_{50}		37.8 μM	N.D.	
5b	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	72.9± 1.1**	64.9± 1.7***	63.0± 4.9***	60.5± 7.4***
5c	Control	91.5± 0.2	86.0± 0.6	84.5± 0.7	92.5± 0.2
	100	84.5± 0.3**	15.2± 1.6***	26.7± 1.2***	72.6± 0.6***
6a	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	85.9± 1.2*	0.0± 0.0***	2.8± 2.3***	0.0± 0.0***
	50		0.0± 0.0***	83.4	±3.5***
	20		5.4± 2.8***		
	10		84.3± 2.1*		
	IC_{50}		61.8 μM	N.D.	N.D.
6b	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	87.3± 2.1	1.4± 1.2***	17.4± 0.9***	77.5± 3.2***
	50		2.8± 2.3***		
	20		56.2± 3.3***		
	10		82.2± 2.0*		
	IC_{50}		96.5 μM	N.D.	
6c	Control	91.5± 0.2	86.0± 0.6	84.5± 0.7	92.5± 0.2
	100	79.7± 1.2***	0.0± 0.0***	0.0± 0.0***	0.0± 0.0***
	50				82.8± 1.1***
	0.5		0.0± 0.0***		
	0.2		75.3± 1.6***		
	0.1		81.0± 0.6**		
	IC_{50}		5.1 μM	N.D.	N.D.
7a	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	50	28.1± 3.0***	0.0± 0.0***	0.0± 0.0***	4.2± 2.0***
	20		0.0± 0.0***		78.7± 2.7***
	10		0.0± 0.0***		
	5		0.0± 0.0***		
	2		83.5± 1.5***		
	IC_{50}	N.D.	11.3 μM	N.D.	N.D.
7b	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	<50	28.2± 2.4***	0.0± 0.0***	0.0± 0.0***	1.4± 1.2***
	<20		0.0± 0.0***		81.8± 2.8*
	<10		0.0± 0.0***		
	<5		0.0± 0.0***		
	<2		81.8± 3.9*		
	IC_{50}	N.D.	11.2 μM	N.D.	N.D.
7c	Control	91.5± 0.2	86.0± 0.6	84.5± 0.7	92.5± 0.2
	100	0.0± 0.0***	0.0± 0.0***	0.0± 0.0***	0.0± 0.0***
	10				0.0± 0.0***
	5				72.2± 1.6***
	2		0.0± 0.0***		83.5± 1.1***
	1		21.3± 1.3***		
	0.5		60.3± 1.5***		
	0.2		74.4± 1.1***		
	0.1		81.8± 0.7***		
	IC_{50}	N.D.	1.96 μM	N.D.	19.2 μM
8a	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	89.4± 1.2	0.0± 0.0***	2.8± 2.3***	84.2± 1.0**
	50		0.0± 0.0***		
	20		12.1± 6.4***		
	10		79.2± 3.1***		
	IC_{50}		54.3 μM	N.D.	
8b	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	100	86.6± 2.1	0.0± 0.0***	6.5± 3.7***	80.2± 5.2
	50		0.0± 0.0***		
	20		49.1± 6.4***		
	10		89.0± 1.1		
	IC_{50}		81.1 μM	N.D.	
8c	Control	88.7± 1.1	85.9± 1.0	85.4± 0.4	88.1± 0.8
	100	86.7± 3.1	0.0± 0.0***	0.0± 0.0***	79.3± 5.5
	50			0.0± 0.0***	
	20			6.0± 5.2***	
	10			27.6± 9.1***	
	5		0.0± 0.0***	59.4± 8.6**	
	2		10.9± 9.4***	84.5± 0.4	
	1		33.8± 17.0**		
	0.5		80.5± 0.8**		
	0.2		85.8± 1.3		
	IC_{50}		3.6 μM	28.6 μM	
9a	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	<50	44.3± 7.0***	0.0± 0.0***	0.0± 0.0***	16.4± 0.9***
	<20		0.0± 0.0***		
	<10		77.8± 1.5***		
	IC_{50}		47.4 μM		
9b	Control	90.4± 1.1	90.0± 0.7	90.1± 1.0	89.5± 0.9
	50	35.6± 2.7***	0.0± 0.0***	0.0± 0.0***	16.2± 2.3***
	20		0.0± 0.0***		86.5± 1.5
	10		2.8± 2.3***		
	5		74.8± 2.7***		
	IC_{50}	N.D.	24.8 μM	N.D.	N.D.
9c	Control	90.4± 1.1	87.2± 1.1	86.7± 1.7	90.5± 1.8
	25	0.0± 0.0***	0.0± 0.0***	0.0± 0.0***	0.0± 0.0***
	20	13.9± 7.1***	0.0± 0.0***	0.0± 0.0***	3.0± 2.6***
	10	34.1± 8.3***	0.0± 0.0***	2.4± 2.1***	26.1±13.8***
	5	55.8± 8.0***	18.1±15.7***	15.5± 8.6***	47.6±13.2**
	2	89.4± 0.6	74.9± 6.0	66.7± 6.8**	85.9± 1.9
	1		85.4± 1.8	84.9± 1.4	
	IC_{50}	25.2 μM	11.3 μM	12.0 μM	21.3 μM
Aspirin	IC_{50}	N.D.	20.0 μM	N.D.	N.D.

Platelets were incubated with tested sample or 0.5% dimethylsulfoxide (DMSO) at 37 °C for 1 min, then thrombin (0.1 U/ml), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added to trigger the aggregation. Values are presented as mean±S.E., n=3—5. *: p<0.05, **: p<0.01, ***: p<0.001 N.D.=not determined.

Anti-allergic Activity Effect on Mast Cell Degranulation: As summarized in Table 4, in all the compounds tested, only those with quinone type B-ring (**7a—c**, **9a—c**) exhibited appreciable activities. Among them, 3′-methoxyflavanquinone (**7c**) displayed the highest potency (IC_{50} 1.59 μM) equivalent to about 13 times the potency of the positive control mepacrine (IC_{50}=21.2 μM).

It was again observed that the flavanquinone type of compound (**7c**) appeared more potent than its isoflavanquinone counterparts (**7a, b**), and the flavone quinone type (**9c**) appeared more potent than their isoflavone quinone counterparts (**9a, b**). Such a trend of their relative efficacy paralleled exactly their activities against neutrophil degranulation mentioned above.

In summary, we have synthesized methoxy derivatives of flavanquinone, flavone quinone, isoflavone quinone as well as isoflavanquinone, and have evaluated their anti-platelet, anti-

Table 2. The Inhibitory Effect of Compounds on Neutrophil Degranulation (*in Vitro*)

	Compounds (μM)	Percent release			
		β-Glucuronidase	% Inhibition	Lysozyme	% Inhibition
2a	Control	26.4± 1.8		50.1± 2.6	
	100	22.6± 1.9	20.2± 0.8	45.5± 7.1	6.8± 7.1
	30	21.9± 2.8	23.3± 4.4	48.3± 6.8	0.9± 6.2
2b	Control	26.4± 1.8		50.1± 2.6	
	100	19.5± 2.8**	32.0± 4.5	43.4± 4.3	10.3± 2.0
	30	22.7± 2.6	20.2± 2.5	47.6± 5.9	1.9± 3.8
2c	Control	23.3± 2.5		43.4± 3.0	
	30	22.0± 0.7	2.8± 9.6	44.1± 7.4	0.5±10.7
	10	22.7± 1.4	1.3± 5.6	46.4± 5.7	−5.9± 6.4
5a	Control	45.8± 2.0		66.7± 4.1	
	30	19.9± 3.4**	57.1± 6.0	41.6± 9.7	39.0±11.2
	10	27.7± 4.3**	40.2± 6.5	48.5± 6.2	27.7± 5.8
	3	32.7± 3.7*	29.1± 5.1	53.9± 6.8	19.7± 5.7
	IC_{50}	21.7 μM			
5b	Control	45.8± 2.0		66.7± 4.1	
	100	37.5± 1.5	18.2± 1.4	60.2± 6.6	10.2± 6.0
	30	29.0± 1.0**	36.7± 1.6	54.7± 8.9	18.8± 9.5
5c	Control	19.7± 0.1		42.7± 0.2	
	30	16.2± 1.0	17.8± 5.5	38.0± 1.3	10.8± 3.6
	10	17.0± 2.6	13.6±13.8	42.4± 1.0	0.5± 2.4
	3	17.4± 1.4	11.6± 7.9	41.3± 2.4	3.3± 5.4
6a	Control	45.8± 2.0		66.7± 4.1	
	30	10.1± 1.4**	78.0± 2.6	32.5± 6.1*	52.0± 6.5
	10	26.3± 7.4*	43.8±13.3	43.7± 7.2	35.0± 7.8
	3	33.0± 3.2*	28.2± 4.2	58.4± 3.9	12.1± 5.7
	IC_{50}	13.7 μM		26.7 μM	
6b	Control	45.8± 2.0		66.7± 4.1	
	100	24.3± 0.9	47.1± 0.8	55.7± 6.4	16.9± 5.7
	30	32.0± 2.4*	30.2± 7.2	57.3± 6.7	14.5± 6.2
6c	Control	19.7± 0.1		42.7± 0.2	
	30	20.1± 2.1	−1.9±10.7	46.5± 2.0	−8.8± 4.1
	10	15.1± 2.5	23.3±12.8	43.3± 2.5	−1.6± 6.0
	3	16.1± 1.2	18.3± 6.4	38.6± 3.5	9.6± 8.2
7a	Control	45.8± 2.0		66.7± 4.1	
	30	−0.1± 0.7**	100.1± 1.6	22.5± 2.7**	66.3± 2.7
	10	21.0± 3.2**	54.6± 5.7	56.0± 5.8	16.1± 5.4
	3	28.4± 5.0**	38.7± 8.0	59.9± 3.2	9.8± 5.4
	IC_{50}	7.4 μM		22.7 μM	
7b	Control	45.8± 2.0		66.7± 4.1	
	30	−0.8± 0.3**	101.8± 0.7	0.8± 0.4**	98.7± 0.6
	3	28.4± 3.9**	38.5± 6.2	46.0±11.4	32.7±13.6
	1	34.9± 4.2	24.4± 5.7	54.7± 8.6	19.0± 8.3
	IC_{50}	6.0 μM		10.6 μM	
7c	Control	18.2± 1.1		36.8± 3.8	
	1	−1.1± 0.6**	106.7± 3.6	−1.7± 1.0**	105.2± 3.7
	0.3	1.8± 0.4**	89.4± 3.1	10.8± 4.2**	70.9±10.4
	0.1	9.0± 0.3**	50.3± 3.7	33.9± 5.2	8.7± 7.3
	IC_{50}	0.19 μM		1.37 μM	
8a	Control	45.8± 2.0		66.7± 4.1	
	100	28.9± 1.1**	36.9± 3.2	54.7± 8.8	18.7± 8.7
	30	30.0± 2.3**	35.3± 4.8	55.8± 7.8	17.0± 6.8
8b	Control	45.8± 2.0		66.7± 4.1	
	100	29.1± 1.2**	36.6± 1.5	57.7± 5.2	13.8± 3.0
	30	31.6± 0.8**	31.1± 1.0	59.8± 5.4	10.7± 3.8
8c	Control	25.6± 1.0		39.9± 0.5	
	30	25.6± 1.0	−1.5± 7.8	35.1± 1.7	12.3± 3.1
	10	25.1± 1.2	1.9± 6.8	35.9± 2.0	10.2± 4.1
9a	Control	45.8± 2.0		66.7± 4.1	
	30	0.5± 0.2**	98.9± 0.6	1.8± 0.6**	97.0± 1.1
	3	11.6± 1.9**	74.7± 4.3	54.6± 5.2	18.3± 4.6
	1	35.9± 3.8	22.1± 5.4	63.7± 3.9	4.0± 6.9
	IC_{50}	**6.3 μM**		**14.5 μM**	
9b	Control	45.8± 2.0		66.7± 4.1	
	30	0.7± 0.1**	98.4± 0.6	−4.8± 1.0**	107.1± 1.3
	3	4.0± 0.3**	91.1± 0.8	11.4± 1.0**	82.9± 1.4
	0.3	31.9± 2.2**	30.8± 1.5	58.6± 1.9	12.5± 1.3
	0.03	38.1± 2.9	17.2± 1.9	62.7± 1.2	6.4± 1.5
	IC_{50}	6.0 μM		7.8 μM	
9c	Control	21.7± 0.2		55.3± 4.9	
	3	−0.1± 1.5**	100.5± 0.9	2.6± 1.4**	95.6± 2.3
	1	10.1± 1.5**	53.6± 7.0	14.5± 2.5**	74.2± 2.2
	0.3	17.0± 1.2	21.9± 4.8	40.2± 4.7	27.5± 2.0
	IC_{50}	3.9 μM		3.2 μM	
Trifluoperazine IC_{50}		5.8 μM		5.9 μM	

The neutrophil suspension was preincubated at 37 °C with 0.5% DMSO or tested compound for 3 min in the presence of cytochalasin B (5 μg/ml). Forty-five minutes after the addition of fMLP (1 μM), β-glucuronidase and lysozyme in the supernatant was determined. Values are presented as mean±S.E., n=3—5, *: $p<0.05$, **: $p<0.01$.

Table 3. The Inhibitory Effect of Compounds on the Neutrophil Superoxide Formation (*in Vitro*)

	Compounds (μM)	Superoxide formation	
		n mol/10^6 cells	% Inhibition
2a	Control	1.72±0.07	
	100	0.93±0.13**	46.2± 7.5
	30	1.08±0.12**	37.4± 6.0
2b	Control	1.72±0.07	
	100	1.62±0.19	−0.7±11.6
	30	1.47±0.11	8.2± 9.6
2c	Control	1.84±0.09	
	30	1.30±0.10	26.7± 2.7
	10	1.41±0.07	19.5± 8.8
5a	Control	0.93±0.07	
	100	0.99±0.03	−0.9±10.1
	30	0.89±0.06	4.3± 3.8
5b	Control	0.93±0.07	
	100	0.47±0.10**	51.1± 6.4
	30	0.76±0.09*	27.7± 3.3
	10	0.78±0.24	11.3± 1.6
	IC_{50}	84.3 μM	
5c	Control	1.14±0.03	
	30	0.67±0.08**	42.1± 8.7
	10	0.82±0.06**	29.1± 7.4
	3	0.80±0.05**	31.8± 3.2
6a	Control	0.93±0.07	
	100	0.81±0.06	12.1± 6.6
	30	0.75±0.08	19.0±13.0
6b	Control	0.93±0.07	
	100	0.65±0.04*	29.7± 4.4
	30	0.52±0.03**	44.2± 2.9
6c	Control	1.14±0.03	
	30	1.14±0.04	−1.1± 7.7
	10	0.07±0.01	14.2± 4.3
	3	1.00±0.09	12.1± 8.3
7a	Control	0.93±0.07	
	100	3.78±0.09**	−314.8±34.5
	30	3.43±0.11**	−276.6±31.9
7b	Control	0.93±0.07	
	100	3.52±0.01**	−270.4±29.9
	30	3.35±0.08**	−251.8±22.1
7c	Control	1.58±0.12	
	0.3	0.43±0.15**	74.0± 8.7
	0.1	0.79±0.07**	48.8± 5.7
	0.03	1.04±0.15**	32.7±13.4
	IC_{50}	0.44 μM	
8a	Control	0.93±0.07	
	100	1.12±0.03	−18.1±11.6
	30	0.90±0.12	3.7± 8.1
8b	Control	0.90±0.07	
	100	1.11±0.17	−19.1±15.2
	30	1.32±0.15	−10.0± 9.1
8c	Control	1.58±0.11	
	30	1.14±0.11	26.4±10.3
	10	1.08±0.19*	32.2± 8.9
	3	1.12±0.07	28.9± 1.4
8c	Control	0.93±0.07	
	100	3.45±0.12**	−277.1±27.8
	30	3.34±0.27**	−269.4±27.1
9a	Control	0.93±0.07	
	100	3.56±0.01**	−316.0±8.7
	30	1.92±0.11**	103.2±24.9
9c	Control	2.38±0.15	
	100	4.77±0.24**	−102.4±17.1
	30	5.04±0.31**	−125.9±15.6
Trifluoperazine IC_{50}		8.0 μM	

The neutrophil suspension was preincubated at 37 °C with 0.5% DMSO or tested compound for 3 min in the presence of cytochalasin B (5 μg/ml). Fifteen minutes after the addition of fMLP (0.3 μM), the absorbance was determined at 550 nm. Values are presented as mean±S.E., n=3—5, *: $p<0.05$, **: $p<0.01$.

Table 4. The Inhibitory Effect of Compounds on Mast Cell Degranulation (*in Vitro*)

	Compound (μM)	Percent release			
		β-Glucuronidase	% Inhibition	Histamine	% Inhibition
2a	Control	53.5±2.9		51.5±3.0	
	100	48.4±2.0	7.3± 6.2	55.6±2.4	−1.5± 2.2
	30	45.5±3.5	12.4± 0.2	51.1±4.0	7.0± 3.4
2b	Control	53.5±2.9		51.5±3.0	
	100	49.1±5.1	7.0± 6.8	52.9±5.1	3.8± 4.3
	30	50.9±6.4	4.2± 6.1	53.4±5.7	3.3± 5.3
2c	Control	15.4±2.7		27.6±4.3	
	30	9.7±1.8*	44.8± 5.1	15.7±1.7*	41.1± 6.4
	10	10.0±1.8	35.3± 6.2	18.7±3.8	32.0± 9.9
5a	Control	34.9±1.9		64.6±2.1	
	100	32.2±2.0	7.4± 2.0	57.6±1.2	10.2± 4.1
	30	33.0±1.4	4.9± 2.9	61.0±1.7	4.9± 3.8
5b	Control	34.9±1.9		64.6±2.1	
	100	32.9±3.3	6.0± 2.5	61.6±6.0	5.1± 3.2
	30	32.6±2.6	6.6± 0.2	60.7±5.1	6.1± 1.9
5c	Control	16.8±0.1		40.5±2.5	
	30	21.3±1.0	−27.0± 5.2	39.2±1.7	−1.3± 0.4
	10	19.6±1.4	−16.7± 7.7	37.4±1.3	3.1± 5.0
6a	Control	34.9±1.9		64.6±2.1	
	100	17.4±1.6**	49.4± 6.1	24.9±1.8**	61.1± 3.4
	30	28.5±3.1	18.5± 2.1	55.3±4.4	14.5± 1.4
6b	Control	34.9±1.9		64.6±2.1	
	100	31.7±2.2	8.7± 2.8	58.8±5.0	9.2± 2.1
	30	30.9±3.6	11.5± 3.3	58.0±4.7	10.4± 1.6
6c	Control	16.8±0.1		40.5±2.5	
	10	17.2±1.2	−2.8± 8.4	28.8±2.6	25.8± 0.2
	3	14.1±0.4	16.5± 2.8	34.4±5.8	12.1± 7.2
7a	Control	34.9±1.9		64.6±2.1	
	10	8.4±2.4**	76.0± 6.7	12.5±1.9**	80.5± 3.4
	3	22.2±1.4**	36.4± 0.8	50.3±2.1	22.2± 1.1
	1	27.7±2.2	20.6± 3.8	57.2±1.5	11.5± 0.6
	IC_{50}	5.6 μM		6.2 μM	
7b	Control	34.9±1.9		64.6±2.1	
	30	4.3±0.4**	87.4± 1.9	0.02±0.9**	99.8± 1.4
	10	8.8±0.9**	74.6± 3.2	13.6±1.8**	78.8± 3.3
	3	22.8±1.4**	33.7± 8.1	49.7±1.6	23.1± 0.4
	IC_{50}	7.3 μM		9.1 μM	
7c	Control	26.2±2.8		37.6±3.4	
	(1)	−4.8±3.0**	124.2± 14.4	0.7±5.3**	97.2± 15.8
	(0.3)	13.2±2.5	34.3± 15.0	24.7±7.3	30.9± 14.2
	(0.1)	18.9±1.7	6.6± 11.9	34.8±5.9	4.6± 11.7
	IC_{50}	1.59 μM		1.96 μM	
8a	Control	34.9±1.9		64.6±2.1	
	100	30.5±2.1	12.4± 2.0	60.3±3.8	6.5± 0.1
	30	31.8±2.4	8.7± 3.5	62.6±5.8	3.4± 2.8
8b	Control	34.9±1.9		64.6±2.1	
	100	30.1±2.7	13.8± 1.1	52.0±3.8	19.6± 1.1
	30	32.1±3.0	8.2± 2.5	56.3±3.8	12.9± 0.4
8c	Control	32.7±0.7		30.9±2.2	
	30	26.3±1.3	19.5± 4.0	27.7±3.4	10.7± 4.4
	10	27.7±0.2	15.0± 2.7	29.6±3.0	3.9± 7.5
9a	Control	34.9±1.9		64.6±2.1	
	100	6.0±0.8**	82.8± 1.0	6.8±0.7**	89.3± 1.5
	10	8.2±0.5**	72.4± 4.0	14.1±4.2**	77.4± 7.7
	3	30.5±3.2	12.8± 6.1	60.9±2.8	5.5± 6.5
	IC_{50}	31.8 μM		31.7 μM	
9b	Control	34.9±1.9		64.6±2.1	
	30	7.7±1.8**	78.5± 4.1	10.7±1.5**	83.2± 2.9
	10	26.4±1.9	24.3± 2.7	53.8±2.2	16.5± 4.5
	3	30.5±2.2	12.3± 5.1	57.8±0.3	10.4± 2.9
	IC_{50}	19.0 μM		18.8 μM	
9c	Control	41.4±1.2		71.2±1.6	
	10	4.5±0.5**	89.0± 1.4	3.5±0.4**	94.9± 0.7
	3	11.1±1.1**	73.1± 2.7	17.9±1.8**	74.6± 2.8
	1	29.2±1.5**	29.0± 4.5	58.8±2.6	18.8± 2.4
	IC_{50}	10.3 μM		12.1 μM	
Mepacrine IC_{50}		21.2 μM		18.4 μM	

The mast cell suspension was preincubated at 37 °C with 0.5% DMSO or tested compound for 3 min. Fifteen minutes after the addition of compound 48/80 (10 μg/ml), β-glucuronidase and histamine in the supernatant was determined. Values are presented as mean±S.E., n=3—5, *: $p<0.05$, **: $p<0.01$.

inflammatory and anti-allergic activities. Consequently, we have established their preliminary structure–activity relationships. We found that selected compounds **6c**, **7a—c**, **8c** and **9a—c**, exhibited significant activities. Worthy of special mentioning was compound **7c** which showed the most potent activities and was therefore selected for further studies of action mechanism and *in vivo* activity.

Experimental

Chemistry All melting points are uncorrected. IR spectra were recorded on Shimadzu IR-440 and Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR spectrometer in $CDCl_3$ or DMSO-d_6. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. MS spectra were measured with an HP 5995 GC-MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV-Vis recording spectrophotometer as methanolic solutions. Elemental analyses (C, H) were performed on a Perkin-Elmer 240 elemental analyzer and the results were within ±0.4% of the calculated values.

2-Benzyloxy-4-methoxybenzaldehyde (**1a**): 2-Hydroxy-4-methoxybenzaldehyde (15.2 g, 100 mmol) was dissolved in acetone (150 ml). Benzyl chloride (15 ml, 130 mmol), anhydrous K_2CO_3 (20 g) and anhydrous KI (26 g) were added. The mixture was stirred under reflux (50—60 °C) for 6 h, and then evaporated *in vacuo*. Water (100 ml) was added and the mixture was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and evaporated *in vacuo*. The residue was eluted through a silica gel column with *n*-hexane/$CHCl_3$ (2 : 1) to give compound **1a** (20.3 g, 84%) as a pale orange oil (Table 5).

2-Benzyloxybenzaldehyde (**1b**): 2-Hydroxybenzaldehyde (12.2 g, 100 mmol) was treated in the same manner as described for compound **1a**. The residue was eluted through a silica gel column with *n*-hexane/EtOAc (8 : 2) to give compound **1b** (18.4 g, 87%) as a pale orange oil (Table 5).

2-Benzyloxy-3-methoxybenzaldehyde (**1c**): 2-Hydroxy-3-methoxybenzaldehyde (15.2 g, 100 mmol) was treated in the same manner as described for compound **1a**. The residue was eluted through a silica gel column with benzene to give compound **1c** (21.5 g, 89%) as a pale orange oil (Table 5).

2-Benzyloxy-2′-hydroxy-4-methoxy-chalcone (**2a**): 2′-Hydroxyacetophenone (6.83 g, 50 mmol) and compound **1a** (12.1 g, 50 mmol) were dissolved in 150 ml of EtOH, and then 25 ml 50% NaOH solution was added. The mixture was stirred for 2 h at 30—40 °C and then evaporated *in vacuo*. Water (100 ml) was added and the mixture was acidifed with 2 N HCl (50 ml) and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed through a silica gel column with *n*-hexane/$CHCl_3$ (2 : 1) and recrystallized from EtOH to afford compound **2a** (14.4 g, 80%) as yellow prism crystals (Table 5).

2-Benzyloxy-2′-hydroxy-4′-methoxy-chalcone (**2b**): Compound **1b** (10.6 g, 50 mmol) was reacted with 2′-hydroxy-4′-methoxyacetophenone (8.3 g, 50 mmol) following the above procedure for the preparation of compound **2a** to afford **2b** (15.3 g, 85%) as yellow prism crystals (Table 5).

2-Benzyloxy-2′-hydroxy-3-methoxy-chalcone (**2c**): Compound **1c** (12.1 g, 50 mmol) was reacted with 2′-hydroxyacetophenone (6.83 g, 50 mmol) as described for the preparation of compound **2a**. The residue was chromatographed through a silica gel column with benzene and recrystallized from EtOH to afford compound **2c** (15.3 g, 85%) as yellow prism crystals (Table 5).

2′-Benzyloxy-4′-methoxyisoflavone (**5a**): Compound **2a** (10.8 g, 30 mmol) was suspended in 300 ml acetic anhydride/pyridine (1 : 1) and stirred for 24 h at room temperature. The reaction mixture was evaporated *in vacuo*. Water (100 ml) was added and the mixture was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and evaporated *in vacuo* to give compound **3a**. Crude compound **3a** was suspended in trimethyl orthoformate (80 ml) and then thallium nitrate trihydrate (13.33 g, 30 mmol) in trimethyl orthoformate (40 ml) was added dropwise. The mixture was stirred at 25±2 °C for 9 h, poured into saturated brine solution, and extracted with $CHCl_3$. The organic extract was dried over $MgSO_4$, and evaporated *in vacuo* to give compound **4a**. Compound **4a** (8.4 g, 20 mmol) was suspended in 400 ml MeOH/2 N HCl (9 : 1) and stirred for 9 h at 30—40 °C. The reaction mixture was evaporated *in vacuo*, water (100 ml) was added and the mixture was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and evaporated *in vacuo*. The residue was eluted through a silica gel column with $CHCl_3$, and recrystallized from EtOH to give compound **5a** (6.1 g,

Table 5. Physical and Spectral Data of the Methoxyisoflavanquinones and Related Compounds

Compd No.	R_1	R_2	R_3	Yield (%)	mp (°C)	MS (M^+) (*m/z*)	UV, λ_{max} (log ε)	IR $\nu_{C=O}$ cm^{-1}	^{1}H-NMR (ppm)[a]	Analysis (%) Calcd. (Found) C	H
1a	H	OCH_3		84		242	273.4 (4.42)	1675	3.85 (3H, s, $-OCH_3$), 5.16 (2H, s, $-OCH_2-$), 6.52 (1H,d, *J*=2.2 Hz, H-3), 6.56 (1H, dd, *J*=8.8, 0.56 Hz, H-5), 7.38—7.45 (5H, m, H-2′, 3′, 4′, 5′, 6′), 7.85 (1H, d, *J*=8.6 Hz, H-6), 10.39 (1H, s, –CHO)	74.36 (74.12)	5.82 (5.81)
1b	H	H		87		212	253.6 (4.00)	1685	5.20 (2H, s, $-OCH_2-$), 7.04—7.06 (2H, m, H-3, 5), 7.35—7.46 (5H, m, H-2′, 3′, 4′, 5′, 6′), 7.53 (1H, ddd, *J*=8.4, 7.5, 1.7 Hz, H-4), 7.86 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 10.56 (1H, s, –CHO)	79.23 (78.93)	5.70 (5.71)
1c	OCH_3	H		89		242	259.6 (3.90)	1693	3.94 (3H, s, $-OCH_3$), 5.18 (2H, s, $-OCH_2-$), 7.10—7.20 (2H, m, H-4, 5), 7.33—7.40 (6H, m, H-6, 2′, 3′, 4′, 5′, 6′), 10.23 (1H, s, –CHO)	74.36 (74.21)	5.82 (5.83)
2a	H	OCH_3	H	80	166—167	360	383.0 (4.55)	1628	3.87 (3H, s, $-OCH_3$), 5.15 (2H, s, $-OCH_2-$), 6.56 (1H, d, *J*=1.6 Hz, H-5), 6.60 (1H, s, H-3), 6.72 (1H, ddd, *J*=7.6, 7.1, 1.1 Hz, H-5′), 6.97 (1H, d, *J*=8.3 Hz, H-3′), 7.35—7.50 (7H, m, H-6, 4′, 2″, 3″, 4″, 5″, 6″), 7.56 (1H, d, *J*=4.9 Hz, H-6′), 7.83 (1H, d, *J*=15.5 Hz, H-α), 8.02 (1H, d, *J*=15.5 Hz, H-β), 13.12 (1H, s, –OH)	76.65 (76.40)	5.59 (5.54)
2b	H	H	OCH_3	85	116—117	360	365.0 (4.23)	1628	3.85 (3H, s, $-OCH_3$), 5.18 (2H, s, $-OCH_2-$), 6.28 (1H, dd, *J*=8.9, 2.6 Hz, H-5′), 6.43 (1H, d, *J*=2.5 Hz,H-3′), 7.04 (1H, dd, *J*=7.3, 7.3 Hz, H-5), 7.06 (1H, d, *J*=8.1 Hz, H-3), 7.28 (1H, d, *J*=4.4 Hz, H-6), 7.35 (1H, dd, *J*=3.9, 2.2 Hz, H-4), 7.38—7.56 (5H, m, H-2″, 3″, 4″, 5″, 6″), 7.59 (1H, s, H-6′), 7.85 (1H, d, *J*=15.6 Hz, H-α), 8.04 (1H, d, *J*=15.6 Hz, H-β), 13.59 (1H, s, –OH)	76.65 (76.73)	5.59 (5.56)
2c	OCH_3	H	H	85	101—103	360	358.5 (4.34)	1635	3.93 (3H, s, $-OCH_3$), 5.09 (2H, s, $-OCH_2-$), 6.82 (1H, ddd, *J*=8.1, 7.2, 1.2 Hz, H-5′), 7.00 (1H, dd, *J*=8.4, 1.2 Hz, H-3′), 7.02 (1H, dd, *J*=7.8, 1.5 Hz, H-4), 7.12 (1H, dd, *J*=7.8, 7.8 Hz, H-5), 7.23 (1H, dd, *J*=7.8, 1.5 Hz, H-6), 6.30—7.49 (6H, m, H-4′, 2″, 3″, 4″, 5″, 6″), 7.64 (1H, dd, *J*=8.1, 1.8 Hz, H-6′), 7.70 (1H, d, *J*=15.6 Hz, H-α), 8.09 (1H, d, *J*=15.6 Hz, H-β), 12.88 (1H, s, –OH)	76.65 (76.52)	5.59 (5.59)
5a	H	OCH_3	H	85	127—128	358	274.0 (3.93)	1651	3.82 (3H, s, $-OCH_3$), 5.08 (2H, s, $-OCH_2-$), 6.58 (1H, d, *J*=2.4 Hz, H-5′), 6.62 (1H, s, H-3′), 7.27—7.38 (5H, m, H-2″, 3″, 4″, 5″, 6″), 7.42 (1H, d, *J*=1.0 Hz, H-6′), 7.45 (1H, dd, *J*=2.9, 1.0 Hz, H-6), 7.48 (1H, s, H-8), 7.67 (1H, ddd, *J*=7.8, 7.7, 1.7 Hz, H-7), 7.99 (1H, s, H-2), 8.32 (1H, dd, *J*=8.0, 1.7 Hz, H-5)	77.08 (77.27)	5.06 (5.07)
5b	H	H	OCH_3	80	163—164	358	266.8 (4.14)	1652	3.91 (3H, s, $-OCH_3$), 5.10 (2H, s, $-OCH_2-$), 6.85 (1H, d, *J*=2.4 Hz, H-8), 7.00 (1H, dd, *J*=8.9, 2.4 Hz, H-6), 7.05 (2H, m, H-3′, 5′), 7.27—7.39 (7H, m, H-4′, 6′, 2″, 3″, 4″, 5″, 6″), 7.94 (1H, s, H-2), 8.23 (1H, d, *J*=8.9 Hz, H-5)	77.08 (76.68)	5.06 (5.01)
5c	OCH_3	H	H	80	140—141	358	320.0 (4.33)	1705	3.84 (3H, s, $-OCH_3$), 5.09 (2H, s, $-OCH_2-$), 6.98 (1H, dd, *J*=8.0, 8.0 Hz, H-5′), 7.10 (1H, dd, *J*=8.0, 1.2 Hz, H-4′), 7.27 (1H, s, H-3), 7.29—7.40 (5H, m, H-2″, 3″, 4″, 5″, 6″), 7.46 (1H, d, *J*=2.1 Hz, H-6′), 7.49 (1H, ddd, *J*=8.5, 7.0, 1.0 Hz, H-6), 7.53 (1H, dd, *J*=8.7, 1.0 Hz, H-8), 7.75 (1H, ddd, *J*=8.7, 7.0, 1.5 Hz, H-7), 8.30 (1H, dd, *J*=8.5, 1.5 Hz, H-5)	77.08 (76.64)	5.06 (5.05)
6a	H	OCH_3	H	90	104—105	256	278.2 (3.73)		3.04 (1H, ddd, *J*=16.4, 5.5, 1.9 Hz, H-4ax.), 3.07 (1H, dd, *J*=16.4, 1.2 Hz, H-4eq.), 3.54—3.60 (1H, m, H-3), 3.77 (3H, s, $-OCH_3$), 4.08 (1H, dd, *J*=10.4, 10.4 Hz, H-2ax.), 4.35 (1H, ddd, *J*=10.4, 3.4, 1.9 Hz, H-2eq.), 5.15 (1H, s, –OH), 6.37 (1H, d, *J*=2.5 Hz, H-3′), 6.49 (1H, dd, *J*=8.5, 2.5 Hz, H-5′), 6.86 (1H, d, *J*=7.4 Hz, H-8), 6.88 (1H, ddd, *J*=8.2, 6.1, 1.2 Hz, H-6), 7.01—7.16 (3H, m, H-5, 7, 6′)	75.02 (75.18)	6.25 (6.30)
6b	H	H	OCH_3	90	130—131	256	276. 2 (3.81)		3.07 (1H, ddd, *J*=16.5, 5.4, 2.0 Hz, H-4ax.), 3.09 (1H, dd, *J*=16.5, 1.2 Hz, H-4eq.), 3.53—3.57 (1H, m, H-3), 3.77 (3H, s, $-OCH_3$), 4.07 (1H, dd, *J*=10.2, 10.2 Hz, H-2ax.), 4.37 (1H, ddd, *J*=10.2, 3.4, 2.0 Hz, H-2eq.), 5.16 (1H, s, –OH), 6.93 (1H, d, *J*=2.2 Hz, H-8), 6.96 (1H, dd, *J*=7.9, 1.2 Hz, H-6), 7.04—7.20 (4H, m, H-5, 3′, 4′, 5′), 7.35 (1H, dd, *J*=8.4, 1.7 Hz, H-6′),	75.02 (75.23)	6.25 (6.41)

Table 5. (Continued)

Compd No.	R_1	R_2	R_3	Yield (%)	mp (°C)	MS (M^+) (*m/z*)	UV, λ_{max} (log ε)	IR $\nu_{C=O}$ cm^{-1}	^{1}H-NMR (ppm)[a]	Analysis (%) Calcd. (Found) C	H
6c	OCH_3	H	H	90	71—73	256	279.7 (3.75)		2.98—3.08 (2H, m, H-3ax., 4ax.), 3.15—3.27 (2H, m, H-3eq., 4eq.), 3.90 (3H, s, $-OCH_3$), 5.10—5.20 (1H, m, H-2), 5.91 (1H, s, –OH), 6.79—6.86 (5H, m, H-6, 8, 4′, 5′, 6′), 7.09—7.17 (2H, m, H-5, 7)	75.02 (75.12)	6.25 (6.30)
7a	H	OCH_3	H	66	172—173	270	264. 8 (4.09)	1675 1644	2.80 (1H, dd, *J*=16.5, 6.7 Hz, H-4ax.), 3.12 (1H, dd, *J*=16.5, 6.9 Hz, H-4eq.), 3.46—3.54 (1H, m, H-3), 3.82 (3H, s, $-OCH_3$), 4.08 (1H, dd, *J*=10.5, 6.6 Hz, H-2ax), 4.28 (1H, dd, *J*=10.5, 2.3 Hz, H-2eq), 5.97 (1H, s, H-3′), 6.48 (1H, s, H-6′), 6.81 (1H, d, *J*=8.6 Hz, H-8), 6.87 (1H, dd, *J*=7.8, 7.3 Hz, H-6), 7.06 (1H, d, *J*=6.8 Hz, H-5), 7.11 (1H, dd, *J*=7.9, 7.4 Hz, H-7)	71.10 (70.89)	5.22 (5.21)
7b	H	H	OCH_3	65	176—178	270	236. 4 (4.18)	1675 1644	2.78 (1H, dd, *J*=16.5, 6.6 Hz, H-4ax.), 3.09 (1H, dd, *J*=16.5, 6.6 Hz, H-4eq.), 3.44—3.48 (1H, m, H-3), 3.79 (3H, s, $-OCH_3$), 4.05 (1H, dd, *J*=10.5, 6.7 Hz, H-2ax), 4.26 (1H, dd, *J*=10.5, 2.2 Hz, H-2eq), 5.98 (1H, s, H-6′), 6.46 (1H, dd, *J*=9.8, 2.0 Hz, H-4′), 6.53 (1H, d, *J*=9.8 Hz, H-3′), 7.03 (1H, d, *J*=2.4 Hz, H-8), 7.07 (1H, ddd, *J*=8.6, 2.2 Hz, H-6), 7.24 (1H, d, *J*=8.6 Hz, H-5)	71.10 (70.86)	5.22 (5.18)
7c	OCH_3	H	H	53	150—152	270	265.6 (4.16)	1685 1651	1.72—1.85 (1H, m, H-3ax.), 2.30—2.38 (1H, m, H-3eq.), 2.75 (1H, ddd, *J*=16.65, 4.58, 4.5 Hz, H-4ax), 3.00 (1H, ddd, *J*=16.65, 10.8, 5.85 Hz, H-4eq), 3.85 (3H, s, $-OCH_3$), 5.12 (1H, dd, *J*=9.45, 2.4 Hz, H-2),5.94 (1H, d, *J*=2.4 Hz, H-4′), 6.86—6.92 (3H, m, H-6′, 8, 6), 7.08 (1H, dd, *J*=7.2, 0.9 Hz, H-5), 7.13 (1H, ddd, *J*=8.4, 7.5, 0.9 Hz, H-7)	71.10 (70.95)	5.22 (5.30)
8a	H	OCH_3	H	86	166—167	268	276. 0 (4.06)	1721	3.83 (3H, s, $-OCH_3$), 6.56 (1H, dd, *J*=8.5, 2.6 Hz, H-5′), 6.67 (1H, d, *J*=2.5 Hz, H-3′), 7.09 (1H, d, *J*=8.5 Hz, H-6′), 7.51 (1H, ddd, *J*=8.1, 7.5, 1.0 Hz, H-6), 7.55 (1H, dd, *J*=9.0, 8.1 Hz, H-8), 7.78 (1H, ddd, *J*=7.8, 7.8, 1.7 Hz, H-7), 8.15 (1H, s, H-2), 8.37 (1H, dd, *J*=8.2, 1.6 Hz, H-5), 9.08 (1H, s, –OH)	71.64 (71.31)	4.51 (4.31)
8b	H	H	OCH_3	84	127—128	268	278. 0 (4.13)	1711	3.95 (3H, s, $-OCH_3$), 6.94 (1H, d, *J*=2.4 Hz, H-8), 6.99 (1H, dd, *J*=7.3, 1.3 Hz, H-6), 7.08 (1H, dd, *J*=9.0, 2.4 Hz, H-3′), 7.10 (1H, dd, *J*=8.1, 1.2 Hz, H-5′), 7.18 (1H, dd, *J*=7.7, 1.7 Hz, H-4′), 7.36 (1H, ddd, *J*=8.4, 7.8, 1.0 Hz, H-6′), 8.10 (1H, s, H-2), 8.27 (1H, d, *J*=9.0 Hz, H-5), 9.01 (1H, s, –OH)	71.64 (71.25)	4.51 (4.56)
8c	OCH_3	H	H	84	207—209	268	254.6 (4.40)	1701	3.84 (3H, s, $-OCH_3$), 6.92 (1H, dd, *J*=8.0, 8.0 Hz, H-5′), 7.06 (1H, dd, *J*=8.0, 1.3 Hz, H-4′), 7.27 (1H, s, H-3), 7.31 (1H, dd, *J*=7.5, 7.46 Hz, H-6), 7.54 (1H, d, *J*=8.9 Hz, H-8), 7.74—7.81 (3H, m, H-5, 7, 6′), 9.6 (1H, br, –OH)	71.64 (71.37)	4.51 (4.53)
9a	H	OCH_3	H	50	243—244	282	298. 0 (4.04)	1659 1628	3.83 (3H, s, $-OCH_3$), 6.23 (1H, s, H-3′), 7.08 (1H, s, H-6′), 7.54 (1H, ddd, *J*=8.2, 5.6, 0.9 Hz, H-6), 7.70 (1H, dd, *J*=8.2, 0.9 Hz, H-8), 7.85 (1H, ddd, *J*=8.4, 5.6, 1.0 Hz, H-7), 8.18 (1H, dd, *J*=8.2, 1.0 Hz, H-5), 8.47 (1H, s, H-2)	68.09 (67.73)	3.57 (3.76)
9b	H	H	OCH_3	45	214—216	282	298.2 (4.14)	1657 1636	3.82 (3H, s, $-OCH_3$), 6.88 (1H, dd, *J*=10.8, 1.7 Hz, H-4′), 6.94 (1H, d, *J*=10.8 Hz, H-3′), 7.00 (1H, d, *J*=1.7 Hz, H-6′), 7.11 (1H, dd, *J*=8.8, 1.6 Hz, H-6), 7.20 (1H, d, *J*=1.6 Hz, H-8), 7.93 (1H, dd, *J*=8.4, 1.9 Hz, H-5), 8.42 (1H, s, H-2)	68.09 (68.12)	3.57 (3.72)
9c	OCH_3	H	H	53	210—211	282	298. 8 (4.04)	1652 1644	3.84 (3H, s, $-OCH_3$), 6.18 (1H, d, *J*=2.5 Hz,H-4′), 6.98 (1H, d, *J*=2.5 Hz, H-6′), 7.34 (1H, s, H-3), 7.50 (1H, d, *J*=8.1 Hz, H-6), 7.65 (1H, dd, *J*=8.4, 1.7 Hz, H-8), 7.89 (1H, ddd, *J*=8.4, 7.1, 1.5 Hz, H-7), 8.29 (1H, dd, *J*=8.1, 1.5 Hz, H-5)	68.09 (67.98)	3.57 (3.70)

a) Compounds **7c**, **9a**, **9b**, **9c** were dissolved in DMSO-d_6 and the others in $CDCl_3$.

85%) as colorless needles (Table 5).

2′-Benzyloxy-7-methoxyisoflavone (**5b**): Compound **2b** (10.8 g, 30 mmol) was treated in the same manner as described for compound **5a** to afford **5b** (5.7 g, 80%) as colorless needles (Table 5).

2′-Benzyloxy-3′-methoxyflavone (**5c**): Compound **2c** (10.80 g, 30 mmol) was treated in the same manner as described for compound **5a**. The residue was eluted through a silica gel column with benzene, and recrystallized from EtOH to give compound **5c** (8.59 g, 80%) as colorless needles (Table 5).

2′-Hydroxy-4′-methoxyisoflavan (**6a**): Compound **5a** (1.1 g, 3 mmol) was suspended in AcOH (30 ml) containing 10% Pd/C (1.0 g) and shaken under an H_2 stream for 2 h at room temperature. After removing the catalyst by filtration, water (100 ml) was added to the filtrate, and the mixture was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$, and evaporated *in vacuo*. The residue was eluted through a silica gel column with benzene, and recrystallized from EtOH to give compound **6a** (0.7 g, 90%) as white prism crystals (Table 5).

2′-Hydroxy-7-methoxyisoflavan (**6b**): Compound **5b** (1.1 g, 3 mmol) was treated in the same manner as described for compound **6a** to afford **6b** (0.7 g, 90%) as white prism crystals (Table 5).

2′-Hydroxy-3′-methoxyflavan (**6c**): Compound **5c** (1.00 g, 2.8 mmol) was treated in the same manner as described for compound **6a** to afford **6c** (0.65 g, 90%) as white prism crystals (Table 5).

4′-Methoxyisoflavanquinone (**7a**): To a solution of compound **6a** (0.3 g, 1.3 mmol) in acetone/MeOH (4 : 1) (45 ml) was added 0.17 M KH_2PO_4 (45 ml) and potassium nitrosodisulfonate (3 g) in water (45 ml). The mixture was stirred at 25±3 °C for 4 h, poured into water (100 ml) and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was eluted through a silica gel column with $CHCl_3$ to yield compound **7a** (0.2 g, 66%) as a red-brown powder (Table 5).

7-Methoxyisoflavanquinone (**7b**): Compound **6b** (0.3 g, 1.3 mmol) was treated in the same manner as described for compound **7a** to afford **7b** (0.2 g, 65%) as a red-brown powder (Table 5).

3′-Methoxyflavanquinone (**7c**): Compound **6c** (0.31 g, 1.2 mmol) was treated in the same manner as described for compound **7a** to afford **7c** (0.17 g, 53%) as a red-brown powder (Table 5).

2′-Hydroxy-4′-methoxyisoflavone (**8a**): A suspension of compound **5a** (2.2 g, 6 mmol) in 47% HBr (80 ml) was stirred at 50±2 °C for 2 h. The reaction mixture was poured into water (100 ml) and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was eluted through a silica gel column with $CHCl_3$ to obtain compound **8a** (1.4 g, 86%) as colorless prism crystals (Table 5).

2′-Hydroxy-7-methoxyisoflavone (**8b**): Compound **5b** (2.2 g, 6 mmol) was treated in the same manner as described for compound **8a** to afford **8b** (1.4 g, 84%) as colorless prism crystals (Table 5).

2′-Hydroxy-3′-methoxyflavone (**8c**): Compound **5c** (2.00 g, 5.6 mmol) was treated in the same manner as described for compound **8a**. The residue was eluted through a silica gel column with benzene/EtOAc (9 : 1) to obtain compound **8c** (1.26 g, 84%) as colorless prism crystals (Table 5).

4′-Methoxyisoflavone quinone (**9a**): Compound **8a** (0.3 g, 1.25 mmol) was treated in the same manner as described for compound **7a** to afford **9a** (0.2 g, 50%) as a yellow brown powder (Table 5).

7-Methoxyisoflavone quinone (**9b**): Compound **8b** (0.3 g, 1.25 mmol) was treated in the same manner as described for compound **7a** to afford **9b** (0.2 g, 45%) as a yellow brown powder (Table 5).

3′-Methoxyflavone quinone (**9c**): Compound **8c** (0.34 g, 1.25 mmol) was treated in the same manner as described for compound **7a** to afford **9c** (0.19 g, 53%) as a yellow brown powder (Table 5).

Evaluation of Antiplatelet Activity. Material Collagen (type 1, bovine achilles tendon), obtained from Sigma Chemical Co., was homogenized in 25 ml acetic acid and then stored at −70 °C. AA, EDTA (disodium salt), sodium citrate, DMSO, and PAF were purchased from Sigma Chemical Co. Thrombin (bovine) was obtained from Park Davis Co. and dissolved in 50% (v/v) glycerol to give a stock solution of 100 NIH units/ml.

Methods Platelet Suspension Preparation: Blood, collected from the marginal ear vein of rabbits was mixed with EDTA to a final concentration of 6 mM and centrifuged at 90 ***g*** for 10 min at room temperature to obtain platelet-rich plasma. The latter was further centrifuged at 500 ***g*** for 10 min and the platelets were washed with Tyrode's solution without EDTA. After centrifugation at the same conditions, the platelets were suspended in Tyrode's solution with the following compositions (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (1.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2). Platelet numbers were determinated with a Coulter Counter (Model ZM) and adjusted to 4.5×10^8 platelets/ml.

Platelet Aggregation: Aggregation was measured by the turbidimetric method with a dual-channel Lumi aggregometer (Model 1020, Payton, Canada).[5] All glassware was siliconized. Test compounds were added one minute before the addition of the aggregation inducer and the platelet suspension was stirred at 900 rpm. The percentage of aggregation was calculated as previously described.[6]

Evaluation of Anti-inflammatory Activity. Material Sodium pentobarbital, bovine serum albumin (BSA), ferricytochrome *c*, superoxide dismutase (SOD), fMLP, phenolphthalein-β-D-glucuronide, *Micrococcus lysodeikticus* and Triton X-100 were purchased from Sigma Chemical Co.

Methods Isolation of Neutrophils: EDTA-mixed fresh blood was obtained from the abdominal aorta of pentobarbital (60 mg/kg, ip)-anesthetized rats (Sprague-Dawley, 300—350 g). Neutrophils were separated from other blood cells by dextran sedimentation and centrifugation on Ficoll-hypaque.[7] Erythrocytes in the pellets were lysed by suspending the cells in 0.05% NaCl for 15 s, followed by washing with 1.75% NaCl containing 0.25% BSA. Cells were resuspended in Hanks' balanced salt solution containing 4 mM $NaHCO_3$ and 10 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), pH 7.4 Hank's balanced salt solution (HBSS) to a final concentration of 2×10^6 cells/ml. The cell preparations consisted of 90—95% neutrophils (viability approximately 95% by trypan blue exclusion).

Measurement of β-Glucuronidase and Lysozyme Release: The neutrophil suspension was preincubated at 37 °C with DMSO or test compound for 3 min in the presence of cytochalasin B (5 μg/ml), and the release reaction was triggered by the addition of 1 μM fMLP. The reaction was stopped 45 min later by the addition of ice-cold Tyrode's solution and the mixture was centrifuged for 10 min at 1000 ***g***. β-Glucuronidase activity in the supernatant was determined by spectrophotometry at 550 nm after reaction with phenolphthalein-β-glucuronide as substrate.[8] Lysozyme activity in the supernatant was measured, with *Micrococcus lysodeikticus* as substrate by spectrophotometry at 450 nm.[9] The release of β-glucuronidase and lysozyme was expressed as percentage release=[(release elicited by secretagogue−spontaneous release)/total content]×100. The total content was measured after treatment of the cell suspension with Triton X-100. Spontaneous release was less than 10%.

Measurement of Superoxide Anion Production: The production of superoxide anion (O_2^-) was determined by SOD-inhibitable ferricytochrome *c* reduction as previously described[10] with modifications. Assay mixtures contained 0.2 ml cell suspension (5×10^6 cells/ml) and 0.9 mg/ml of ferricytochrome *c* in a final volume of 0.4 ml. The reference tube also received 12.5 μg/ml of SOD. Both reference and sample tubes were incubated at 37 °C for 3 min. The reactions were then started by the addition of 0.3 μM fMLP and incubated at 37 °C for 30 min with occasional agitation. After centrifugation, the supernatant was transferred to a 96-well plate, and the absorbance at 550 nm was recorded with a microplate reader. The amount of O_2^- in the reaction mixture was calculated from the formula:[11] O_2^- (nmol)=19.08× absorbance.

Evaluation of Antiallergic Activity. Materials Heparin (grade I-A; from porcine intestinal mucosa), compound 48/80 and *o*-phthaldialdehyde were purchased from Sigma Chemical Co.

Methods Rat Peritoneal Mast Cell Preparation: Rat peritoneal mast cells were isolated as previously described.[12] Briefly, heparinized Tyrode's solution was injected into the peritoneal cavity of exsanguinated rats (Sprague-Dawley, 250—300 g). After abdominal massage, the cells in the peritoneal fluid were harvested and separated in 38% BSA in glucose-free Tyrode's solution. The cell pellet was washed and suspended in Tyrode's solution of the following composition (mM): NaCl (137), KCl (2.7), $NaHCO_3$ (12), $MgCl_2$ (1.0), NaH_2PO_4 (0.3), $CaCl_2$ (1.0), glucose (5.6) and 0.1% BSA. The mast cell count was adjusted to $1—1.5\times10^6$ cells/ml. Cell viability (>95%) was assessed by the trypan blue exclusion test.

Measurement of Histamine and β-Glucuronidase Release: The mast cell suspension was preincubated at 37 °C with DMSO or test compound for 3 min, and the release reaction was triggered by the addition of 10 μg/ml of compound 48/80. The reaction was stopped 15 min later by the addition of ice-cold Tyrode's solution and the mixture was centrifuged for 10 min at 1000 ***g***. Histamine in the supernatant was determined by fluorescence spectrophotometry at 350/450 nm after condensation with *o*-phthaldialdehyde.[13] β-Glucuronidase activity in the supernatant was measured as described above. The release of histamine and β-glucuronidase was expressed as percentage release=[(release elicited by secretagogue−spontaneous release)/total content]×100. The total content was measured after treatment of cell suspensions with Triton X-100. Spontaneous release was less than 10%.

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