Synthesis and Anti-inflammatory Effect of Chalcones and Related Compounds

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Purpose. Mast cell and neutrophil degranulations are the important players in inflammatory disorders. Combined with potent inhibition of chemical mediators released from mast cells and neutrophil degranulations, it could be a promising anti-inflammatory agent. 2′,5′-Dihydroxychalcone has been reported as a potent chemical mediator and cyclooxygenase inhibitor. In an effort to continually develop potent anti-inflammatory agents, a novel series of chalcone, 2′- and 3′-hydroxychalcones, 2′,5′-dihydroxychalcones and flavanones were continually synthesized to evaluate their inhibitory effects on the activation of mast cells and neutrophils and the inhibitory effect on phlogist-induced hind-paw edema in mice.

Methods. A series of chalcones and related compounds were prepared by Claisen-Schmidt condensation of appropriate acetophenones with appropriate aromatic aldehyde and the anti-inflammatory activities of these synthetic compounds were studied on inhibitory effects on the activation of mast cells and neutrophils.

Results. Some chalcones showed strong inhibitory effects on the release of β-glucuronidase and histamine from rat peritoneal mast cells stimulated with compound 48/80. Almost all chalcones and 4′-hydroxyflavanone exhibited potent inhibitory effects on the release of β-glucuronidase and lysozyme from rat neutrophils stimulated with formyl-Met-Leu-Phe (fMLP). Some chalcones showed potent inhibitory effects on superoxide formation of rat neutrophils stimulated with fMLP/cytochalasin B (CB) or phorbol myristate acetate (PMA). 2′,3-Dihydroxy-, 2′,5′-dihydroxy-4-chloro-, and 2′,5′-dihydroxychalcone showed remarkable inhibitory effects on hind-paw edema induced by polymyxin B in normal as well as in adrenalectomized mice.

Conclusions. These results indicated that the anti-inflammatory effects of these compounds were mediated, at least partly, through the suppression of chemical mediators released from mast cells and neutrophils.

KEY WORDS: chalcone; flavanone; chemical mediator.

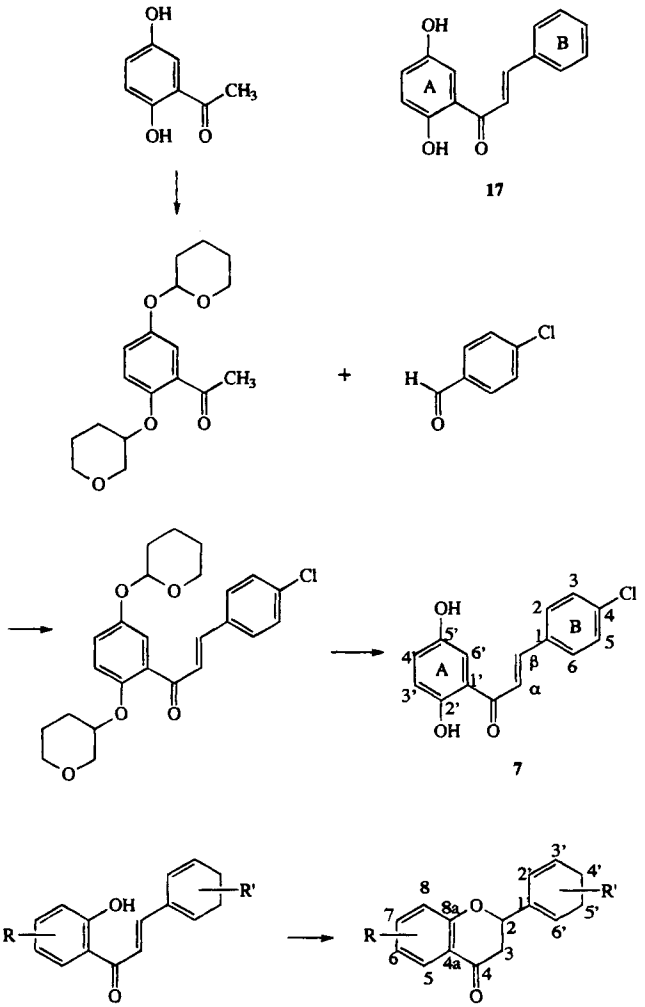
INTRODUCTION

Mast cells play a central role in the pathogenesis of diseases such as allergic asthma, rhinoconjunctivitis, urticaria, anaphylaxis and systemic mastocytosis and may well be important players in other chronic inflammatory disorders (1). 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 microbicidal and inflammatory effects (2,3). Hence, inhibition of chemical mediators released from mast cells and neutrophils is a rational therapeutic approach to treat a variety of inflammatory and allergic diseases.

Some chalcone derivatives have been reported as anti-inflammatory or antiallergic agents and 3,4-dihydroxychalcones have been reported as 5- or 12-lipoxygenase and cyclooxygenase inhibitor (4). Furthermore, we have found 2′,5′-dihydroxychalcone as a potent chemical mediator and cyclooxygenase inhibitor (5). These findings suggested that some chalcones may be promising anti-inflammatory agents. In this paper, we further synthesized and described the anti-inflammatory effects using *in vitro* and *in vivo* inflammatory models and discuss their structure-activity relationships.

CHEMISTRY

We further prepared a number of new (**1–11** and **13–16**) and known (**12**) chalcones and flavanones using Claisen-Schmidt condensation of appropriate acetophenones or hydroxyacetophenones, protected as tetrahydropyranyl ether, with appropriate aromatic aldehyde or hydroxyaromatic aldehyde, protected as tetrahydropyranyl ether (Scheme I). This procedure afforded



Scheme I. Synthesis of 2′,5′-dihydroxy-4-chlorochalcone (**7**) and general synthesis of flavanones.

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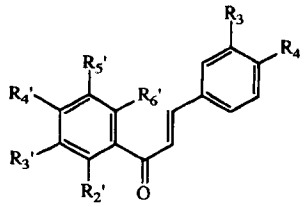
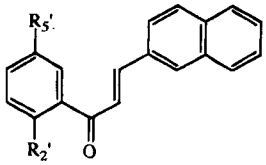
Table I. Chalcones **1–11**



1, 2, 6–11



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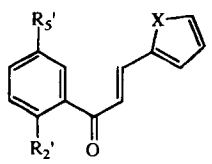


4: x = o 5: x = s

Compound	R2′	R3′	R4′	R5′	R6′	R3	R4	mp°C	Recrystn solvent	% yield	Formula	Anal
1	H	H	H	H	H	H	OH	178–180	$CHCl_3$	48	$C_{15}H_{12}O_3$	C, H
2	OH	H	H	H	H	OH	H	158–160	$CHCl_3$	52	$C_{15}H_{12}O_4$	C, H
3	OH	H	H	OH	H			235–237	CH_3OH	45	$C_{19}H_{14}O_3$	C, H
4	OH	H	H	OH	H			151–152	CH_3OH	46	$C_{13}H_{10}O_4$	C, H
5	OH	H	H	OH	H			162–163	CH_3OH	53	$C_{15}H_{12}O_3S$	C, H, S
6	OH	H	H	OH	H	H	F	220–221	CH_3OH	43	$C_{15}H_{11}FO_3$	C, H, O
7	OH	H	H	OH	H	H	Cl	208–210	CH_3OH	65	$C_{15}H_{11}ClO_3$	C, H, O
8	OH	H	H	H	H	OCH_3	OCH_3	103–104	CH_3OH	53	$C_{17}H_{16}O_4$	C, H
9	H	OH	H	H	H	OCH_3	OCH_3	115–116	CH_3OH	62	$C_{17}H_{16}O_4$	C, H
10	H	OH	H	H	H	OH	OH	191–192	CH_3OH	48	$C_{15}H_{12}O_4$	C, H
11	OH	H	H	OH	H	OCH_3	OCH_3	131–132	CH_3OH	50	$C_{17}H_{16}O_5$	C, H

various chalcone or flavanone derivatives in a good yield (Table I). Appropriate 2′- or 2′,4′-dihydroxychalcones reacted with 25% HCl / MeOH to give **12** and **13,** respectively, also in a good yield (Table II). All the ^1H and ^{13}C NMR data of compounds **1–16** were listed in Table III and IV.

MATERIALS AND METHODS

General Experimental Procedures

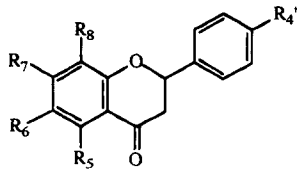
Melting points (uncorrected) were determined with a Yanaco Micro-Melting Point apparatus. IR spectra were determined with a Hitachi model 260-30 IR spectrophotometer.^1H and ^{13}C NMR spectra [δ (ppm), J (Hz)] were determined with a Varian Gemini 200 MHz FT-NMR spectrometer. Mass spectra were determined 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.

General Procedure for Obtaining Chalcones 2–11 (4) and Flavanone 12–16

2′,5′-Dihydroxy-4-chlorochalcone (7)

2,5-Dihydroxyacetophenone (3.8 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were stirred for 0.5 h in methylene chloride (100 mL), and then 3,4-dihydro-α-pyran in methylene chloride (13 mL in 20 mL) was added dropwise. The reaction mixture was stirred 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 (**7a**).

Table II. Flavanones of **12–16**



12–16

Compound	R4′	R5	R6	R7	R8	mp°C	Recrystn solvent	% yield	Formula	Anal
12	H	H	H	H	H	65–66	C_6H_{12}	45	$C_{15}H_{12}O_2$	C, H
13	H	H	H	OH	H	174–193	C_6H_{12}	63	$C_{15}H_{12}O_3$	C, H
14	OH	H	H	H	H	117–118	C_6H_{12}	32	$C_{15}H_{12}O_3$	C, H
15	OCH3	H	OH	H	H	188–190	EtOAc	35	$C_{16}H_{14}O_4$	C, H
16	CH_3	H	OH	H	H	202–203	EtOAc	38	$C_{16}H_{14}O_3$	C, H

Table III. ^{1}H NMR Data for Various Chalcone and Flavanone Derivatives

Compound	H-2′	H-6′	H-3′	H-5′	H-4′	H-2	H-6	H-3	H-5	H-4	H-α	H-β	OCH_3	
1[a]	8.02–8.07(dd)				7.49–7.64(m)				6.85(dd)		7.68(d)	7.76(d)		
2[a]		8.10(dd)	6.85–7.03(m)		7.15–7.28(m)				7.15–7.28(m)	6.85–7.03(m)				7.48–7.56(m,1H)
														7.82(s,2H)
3[b]		8.26(s)	6.84(d)	7.12(dd)										7.52–7.56(m,3H)
														7.87–8.05(m,6H)
4[a]		7.35(d)	6.83(d)		7.03(dd)			6.92(d)	7.72(m)	6.61–6.62(m)	7.54(d)	7.68(d)		
5[a]		7.33(d)	6.82(d)		7.03(dd)			7.44(d)	7.62(d)	7.14(dd)	7.48(d)	8.01(d)		
6[a]			6.83(d)		7.12(dd)		7.91(t)		7.23(t)		7.93(d)	7.94(d)		
7[a]		7.42–7.48(m)	6.84(d)		7.05(dd)		7.71–7.75(m)		7.42–7.48(m)		7.78(d)	7.85(d)		
8[c]		7.91(dd)	7.00(d)	6.87–6.96(m)	7.42–7.51(m)	7.15(d)	7.24(d)		6.89(d)		7.49(d)	7.86(d)	3.91, 3.94	
9[a]	7.40–7.44(m)	7.51–7.56(m)		7.27–7.37(m)	7.02–7.06(m)	7.32(d)	7.26(dd)		6.96(d)		7.52(d)	7.70(d)	3.85, 3.88	
10[a]	7.39–7.43(m)	7.48–7.51(m)		7.34(t)	7.02–7.04(m)	7.18(d)	7.09(dd)		6.82(d)		7.41(d)	7.65(d)		
11[a]		7.45(d)	7.00(d)		7.03(dd)	7.37(d)	7.31(dd)		6.81(d)		7.62(d)	7.83(d)	3.88, 3.91	

Compound	H-2′	H-3′	H-4′	H-5′	H-6′	H-2	Hax-3	Heq-3	H-5	H-6	H-7	H-8	OCH_3	CH_3
12[a]	7.36–7.60(m)		7.36–7.60(m)		7.36–7.60(m)	5.55(dd)	3.13(dd)	2.85(dd)	7.86(dd)	7.02–7.10(m)	7.36–7.60(m)	7.02–7.10(m)		
13[a]	7.35–7.51(m)		7.35–7.51(m)		7.35–7.51(m)	5.50(dd)	3.02(dd)	2.74(dd)	7.73(d)	6.51(dd)		6.38(d)		
14[b]	7.40–7.60(m)	6.88–6.92(m)	7.40–7.60(m)	5.52(dd)	3.17(dd)	2.78(dd)	7.81–7.86(m)	7.02–7.11(m)		7.40–7.60(m)				
15[a]	7.42(d)	6.95(d)		6.95(d)	7.42(d)	5.38(dd)	3.07(dd)	2.76(dd)	7.21(d)		7.03(dd)	6.90(d)	3.80(s)	
16[a]	7.36(d)	7.19–7.21(m)	7.36(d)	5.37(dd)	3.02(dd)	2.75(dd)	7.19–7.21(m)				7.03(dd)	6.91(d)		2.34(a)

[a]Measured in CD_3OD.
[b]Measured in $(CD_3)_2CO$.
[c]Measured in $CDCl_3$.

Table IV. ^{13}C NMR Data for Various Chalcone and Flavanone Derivatives[#]

Compound	1[a]	2[a]	4[a]	5[a]	6[a]	7[b]	8[c]	9[a]	10[a]	11[a]	Compound	12[a]	13[a]	14[b]	15[a]	16[a]
C-1′	139.7	121.7	121.2	121.2	122.1	121.3	120.0	140.9	141.1	121.6	C-2	80.8	81.0	81.0	80.6	80.7
C-2′	129.8	164.3	157.6	157.6	157.6	158.5	163.4	115.8	115.6	157.6	C-3	45.4	45.1	45.5	45.4	45.4
C-3′	129.5	119.3	119.0	119.7	119.9	120.2	118.4	159.0	159.0	119.8	C-4	194.0	193.0	192.8	194.6	194.5
C-4′	133.9	137.6	125.9	125.8	126.5	126.7	136.0	115.8	115.7	125.8	C-4a	122.0	115.0	122.7	122.2	122.2
C-5′	129.5	120.4	150.7	150.7	151.0	150.9	118.6	130.8	130.7	150.9	C-5	127.7	129.5	128.0	111.4	111.4
C-6′	129.8	131.3	118.2	115.2	116.0	116.4	129.4	121.1	121.0	115.8	C-6	122.6	111.8	122.6	153.0	153.0
C-1	127.8	137.6			132.8	135.3	127.5	129.2	128.2	129.5	C-7	137.5	166.8	1373	126.0	125.9
C-2	131.9	116.2	153.0	141.4	132.6	132.0	110.2	111.8	116.6	112.3	C-8	119.2	103.6	120.1	120.1	120.1
C-3	117.0	159.4	114.0	129.6	117.6[d]	130.6	149.2	150.7	146.9	151.2	C-8a	163.1	165.3	163.2	157.0	156.9
C-4	161.7	119.4	115.2	131.0	163.1	137.6	151.7	153.1	150.2	153.7	C-1′	140.5	140.6	131.7	132.7	137.7
C-5	117.0	131.5	132.2	133.9	117.2[d]	130.6	111.0	112.6	119.8	112.9	C-2′	127.3	127.3	129.6	128.8	127.3
C-6	131.9	121.8			132.6	132.0	123.5	124.7	123.6	125.3	C-3′	129.8	129.8	116.8	115.0	130.2
C-α	119.7	121.7	119.0	120.3	120.0	121.3	117.6	120.9	120.8	119.8	C-4′	129.6	129.6	159.3	161.4	139.4
C-β	147.0	147.0	147.2	138.8	145.2	145.0	145.5	146.5	147.3	147.0	C-5′	129.8	129.6	116.8	115.0	130.2
OCH_3							55.9	56.5		56.5	C-6′	127.3	127.3	129.6	128.2	127.3
C=O	192.7	195.9	194.5	194.3	195.1	195.2	193.4	192.5	192.7	195.5	OCH_3				55.7	
											CH_3					21.2

[#]The ^{13}C NMR data of **3** [$(CD_3)_2CO$]: δ 115.9 (C-6′), 119.9 (C-3′), 121.3 (C-1′), 122.3 (C-α), 125.3 (C-4′), 126.4, 128.2, 129.0, 130.0, 130.1, 132.5, 133.5 (C-9 or C-10), 135.8(C-2), 146.6(C-β), 150.7(C-2′), 195.0(CO).

[a] Measured in CD_3OD.

[b] Measured in $(Cd_3)_2CO$

[c] Measured in $CDCl_3$.

[d] These signals may be interchangeable in each column.

Crude **7a,** 4-chlorobenzaldehyde (3.5 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were dissolved in MeOH (100 mL). The reaction 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 (1M, 35 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried and evaporated *in vacuo.* The residue yielded crude 2′,5′-bis(tetrahydropyran-2-yloxy)-4-chlorochalcone (**7c**).

Crude **7c** and *p*-toluenesulfonic acid (0.2 g, 1.05 mmol) were dissolved in MeOH (100 mL). The reaction mixture was stirred for 4 h at room temperature, and then evaporated *in vacuo.* Water (100 mL) was added the mixture, 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 eluted through a silica gel column with cyclohexane-EtOAc (4:1) to give **7** (4.5g, 16.2mmol, 65%): mp 208–210 °C (CH_3OH); IR (KBr) 3388, 1646 cm^{-1}, MS m/z 274 (M^+,27).

7-Hydroxyflavanone (13)

A mixture of 2′,4′-dihydroxychalcone (1.0 g, 4.16 mmol) and 50 mL of 25% HCl/MeOH was refluxed for 48 h, and then evaporated in vacuo. Water (100 mL) was added the mixture, neutralized with 5% $NaHCO_3$ (50 mL), and extracted with EtOAc. The organic layer was separated, washed with water, dried with anhydrous $MgSO_4$, and evaporated *in vacuo.* The residue was eluted through a silica gel column with $CHCl_3$-EtOAc (2:1) to give **13** (0.6 g, 2.62 mmol, 63 %): mp 173–174°C (C_6H_{12}); IR (KBr) 3250, 2800,1650 cm^{-1}; MS m/z 240 (M^+, 100).

4-Dihydroxychalcone (1)

Acetophenone (3 g, 25 mmol) , 4-hydroxybenzaldehyde (3.05 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **1** (2.7g, 12mmol, 48%): mp 178–180 °C ($CHCl_3$); IR (KBr) 3223, 1650 cm^{-1}; MS m/z 224 (M^+, 76).

2′,3-Dihydroxychalcone (2)

2-Hydroxyacetophenone (3.4 g, 25 mmol) or 3-hydroxybenzaldehyde (3.05 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were treated as in **7a** to give crude 2′-(tetrahydropyran-2-yloxy)acetophenone (**2a**) or 3-(tetrahydropyran-2-yloxy)benzaldehyde (**2b**).

Crude **2a,** crude **2b**, and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **2** (3.1g, 13 mmol, 52 %): mp 158–160 °C ($CHCl_3$); IR (KBr) 3361, 1635 cm^{-1}; MS m/z 240 (M^+, 62).

2′,5′-Dihydroxy-2-naphthylchalcone (3)

2,5-Dihydroxyacetophenone (3.8 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were treated as in **7c** to give crude 2′,5′-bis(tetrahydropyran-2-yloxy)acetophenone (**3a**).

Crude **3a,** 2-naphthaldehyde (3.9 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25mmol) were treated as in **7c** to give **3** (3.3 g, 11.3 mmol, 45%): mp 235–237 °C (CH_3OH); IR (KBr) 3287, 1636 cm^{-1}; MS m/z 290 (M^+, 47).

2′,5′-Dihydroxy-2-furfurylchalcone (4)

Crude **3a,** 2-furaldehyde (2.4 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c**

to give **4** (2.6 g, 11.5 mmol, 46%): mp 151–152 °C (CH_3OH); IR (KBr) 3374, 1645 cm^{-1}; MS m/z 230 (M^+, 57).

2',5'-Dihydroxy-2-thienylchalcone (**5**)

Crude **3a,** 2-thiophenecarboxaldehyde (2.8 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **5** (3.2 g, 13.3 mmol, 53%): mp 162–163 °C (CH_3OH); IR (KBr) 3390, 1644 cm^{-1}; MS m/z 246 (M^+, 53).

2',5'-Dihydroxy-4-fluorochalcone (**6**)

Crude **3a,** 4-fluorobenzaldehyde (3.1 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **6** (2.8 g, 10.8 mmol, 43 %): mp 220–221 °C (CH_3OH); IR (KBr) 3369, 1643 cm^{-1}; MS m/z 258 (M^+, 64).

2'-Hydroxy-3,4-dimethoxychalcone (**8**)

2-Hydroxyacetophenone (3.4 g, 25 mmol), 3,4-dimethoxybenzaldehyde (4.2 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **8** (3.7 g, 13.3 mmol, 53 %): mp 103–104 °C (CH_3OH); IR (KBr) 3078, 1632 cm^{-1}; MS m/z 284 (M^+, 66).

3'-Hydroxy-3,4-dimethoxychalcone (**9**)

3-Hydroxyacetophenone (3.4 g, 25 mmol), 3,4-dimethoxybenzaldehyde (4.2 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **9** (4.4 g, 15.5 mmol, 62 %): mp 115–116 °C (CH_3OH); IR (KBr) 3441, 1661 cm^{-1}; MS m/z 284 (M^+, 100).

3',3,4-Trihydroxychalcone (**10**)

3-Hydroxyacetophenone (3.4 g, 25 mmol), or 3,4-dihydroxybenzaldehyde (3.45 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were treated, respectively, as in **7a** to give crude 4'-(tetrahydropyran-2-yloxy)acetophenone (**10a**) or 3,4-bis(tetrahydropyran-2-yloxy)benzaldehyde (**10b**).

Crude **10a,** crude **10b,** and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** and **7** to give **10** (3.1 g, 12 mmol, 48%): mp 191–192 °C (CH_3OH) IR (KBr) 3383, 1645 cm^{-1}; MS m/z 256 (M^+, 100).

2',5'-Dihydroxy-3,4-dimethoxychalcone (**11**)

2,5-Dihydroxyacetophenone (3.8 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were treated as in **7c** to give crude 2',5'-bis(tetrahydropyran-2-yloxy)acetophenone (**11a**).

Crude **11a,** 3,4-dimethoxybenzaldehyde (4.2 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were treated as in **7c** to give **11** (3.75 g, 12.5 mmol, 50 %): mp 131–132 °C (CH_3OH); IR (KBr) 3443, 1642 cm^{-1}; MS m/z 300 (M^+, 34).

Flavanone (**12**)

2'-Hydroxychalcone (1.0 g, 4.46 mmol) and 50 mL of HCl / MeOH were treated as in **13** to give **12** (0.45 g, 2.07 mmol, 45 %): mp 65–66 °C (C_6H_{12}); IR (KBr) 2900, 1690 cm^{-1}, MS m/z 224 (M^+, 63).

4'-Hydroxyflavanone (**14**)

2',4-Dihydroxychalcone (1.0 g, 4.16 mmol) and 50 mL of HCl / MeOH were treated as in **13** to give **14** (0.32 g, 1.4 mmol, 32 %): mp 117–118 °C (C_6H_{12}); IR (KBr) 3148, 1668 cm^{-1}, MS m/z 240 (M^+, 18).

6-Hydroxy-4'-methoxyflavanone (**15**)

2',5'-Dihydroxy-4-methoxychalcone (1.0 g, 3.7 mmol) and 50 mL of HCl/MeOH were treated as in **13** to give **15** (0.35 g, 1.3 mmol, 35%): mp 188–190 °C (EtOAc); IR (KBr) 3165, 1668 cm^{-1}; MS m/z 270 (M^+,45).

6-Hydroxy-4'-methylflavanone (**16**)

2',5'-Dihydroxy-4-methylchalcone (1.0 g, 3.9 mmol) and 50 mL of HCl / MeOH were treated as in **13** to give **16** (0.38 g, 1.5 mmol, 38 %): mp 202–203 °C (EtOAc); IR (KBr) 3195, 1669 cm^{-1}, MS m/z 254 (M^+, 46).

Mast Cell Degranulation

Heparinized Tyrode's solution was injected into the peritoneal cavity of exsanguinated rat (Sprague-Dawley, 250–300 g). After abdominal massage, the cells in the peritoneal fluid were harvested and then separated in 38 % bovine serum albumin in glucose-free Tyrode's solution. The cell pellets were washed and suspended in Tyrode's solution. Cell suspension was preincubated at 37 °C with DMSO or drugs (3, 10, 30, 50 or 100 μM) for 3 min. Fifteen minutes after the addition of compound 48/80 (10 μg mL^{-1}), β-glucuronidase (phenolphthalein-β-D-glucuronide as substrate, 550 nm) and histamines (*o*-phthadialdehyde condensation, 350/450 nm) in the supernatant were determined. The total content was measured after treatment of the cell suspension with Triton X-100. The percentage released was determined (6). To eliminate the effect of the solvent on the mast cell degranulation, the final concentration of DMSO was fixed at 0.5%.

Neutrophil Degranulation

Blood was withdrawn from 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 (HBSS) (7). Cell suspension was preincubated at 37 °C with DMSO or drugs (0.1, 1, 3, 5, 10, 30 or 100 μM) for 3 min, then challenged with fMLP (1 μM)/5 μg mL^{-1} of CB. Forty-five minutes later, the lysozyme (*Micrococcus lysodeikticus* as substrate, 450 nm) and β-glucuronidase in the supernatant were determined (8). To eliminate the effect of the solvent on the neutrophil degranulation, the final concentration of DMSO was fixed at 0.5%.

Superoxide Anion Formation

The neutrophil suspension was pre-incubated with DMSO (0.5%) or drugs (1, 3, 5, 10, 30 or 100 μM) at 37 °C for 3 min, then superoxide dismutase and HBSS were added into the blank and test tubes, respectively. After addition of cytochrome *c*, reaction was initiated by challenge with fMLP (0.3 μM) / CB (5 μg mL^{-1}) or PMA (3 nM). After 30 min, the reaction was terminated by centrifugation and superoxide in the superna-

tant was determined (n mole $O_2^-/10^6$ cells) by spectrophotometry at 550 nm (9). To eliminate the effect of the solvent on the superoxide anion formation, the final concentration of DMSO was fixed at 0.5%.

Phlogist-Induced Hind-Paw Edema

Mice (ICR, 20–25 g) were used. Hind-paw edema was induced with a single subplantar injection of 5 μL phlogist (0.2 % polymyxin B) in physiological saline or an equal volume of physiological saline in the right and left hind-paw, respectively (10). The volume of both hind-paws was measured with a plethysmometer. Hind-paw swelling was calculated as following:

$$\text{paw swelling (\%)} = \{[(\text{right paw volume} - \text{initial volume})/(\text{right paw initial volume})] - [(\text{left paw volume} - \text{initial volume})/(\text{left paw initial volume})]\} \times 100$$

The data were also analyzed to compare the area under the time-paw swelling curve (AUC) based on the trapezoidal rule.

Adrenalectomized Mice

Animals were adrenalectomized according to Waynforth (11), except that mice were used. Mice were anaesthetized with intraperitoneal sodium pentobarbitone (45 mg kg^{-1}), then adrenalectomized bilaterally from the dorsal region. Shamoperated mice were also prepared concurrently. Adrenalectomized mice had free access to physiological saline as drinking water. On the fourth postoperative day, animals were used for experiments.

Statistical Analysis

Data are presented as the means ± S.E.M. The statistical significance of changes was analyzed with an analysis of variance for multiple comparisons followed by Newman-Keules test. P-values < 0.05 were considered to be significant.

BIOLOGICAL RESULTS AND DISCUSSION

The anti-inflammatory activities of **1–16** (Table I and II) were studied on inhibitory effects on the activation of mast cells and neutrophils. Compounds **2, 8, 9** and **11** caused strong and dose-dependent inhibition of mast cell degranulation induced by compound 48/80 (10 μg mL^{-1}) (Table V). The B ring of 2′,5′-dihydroxychalcone (**17**) (5), substituted by various aromatic groups such as **3–6** and **7** did not enhance the inhibitory effects on mast cell degranulation caused by compound 48/80 while substituted by 3,4-dimethoxyphenyl groups, such as **11,** showed enhancement of inhibitory effects on release of histamine from mast cell degranulation caused by compound 48/80 (Table V). The hydroxylation, methoxylation and O-methylation of **1** at C-2′ or C-3′, C-3 and C-4, respectively, enhanced the inhibitory effects on mast cells caused by compound 48/80 (Table V). As shown in Table III, flavanones did not show inhibitory effect on mast cell degranulation caused by compound 48/80. Mepacrine was used in this study as a positive control and produced a dose-dependent inhibition of mast cell degranulation caused by compound 48/80 (Table V).

Table V. The Inhibitory Effects of Chalcones and Related Compounds on the Release of β-Glucuronidase and Histamine from Rat Peritoneal Mast Cells Stimulated with Compound 48/80

	$IC_{50}(\mu M)^a$	
Compound	β-glucuronidase	histamine
1	52.5 ± 7.0	39.6 ± 6.0
2	36.1 ± 1.4	24.1 ± 2.2
3	>100(32.2 ± 7.6)	>100(30.2 ± 4.7)
4	<100(34.2 ± 10.0)	>100(51.7 ± 5.8)
5	>100(8.3 ± 15.6)	>100(21.3 ± 12.5)
6	63.6 ± 4.3	79.9 ± 6.9
7	>100(28.7 ± 3.7)	>100(41.7 ± 3.0)
8	33.0 ± 6.2	23.0 ± 1.3
9	29.6 ± 3.5	25.1 ± 1.7
10	>100(17.2 ± 7.8)	66.5 ± 3.9
11	28.0 ± 3.3	23.1 ± 1.2
12	>100(23.0 ± 2.2)	>100(20.7 ± 3.9)
13	>100(8.8 ± 1.5)	>100(1.1 ± 4.5)
14	>100(26.0 ± 10.4)	>100(31.8 ± 9.2)
15	>100(−11.2 ± 12.5)	>100(−10.6 ± 7.0)
16	>100(4.4 ± 9.2)	>100(2.8 ± 0.6)
17	20.8 ± 0.84[b]	30.1 ± 2.96[b]
Mepacrine	22.3 ± 6.2	15.1 ± 2.96

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Average ± s.e.m. (n = 3–5) of at least three separate determinations.
[b] Data from Ref. 5.

FMLP (1 μM)/cytochalasin (CB) (5 μg mL^{-1}) induced the release of β-glucuronidase and lysozyme from rat neutrophils. All the chalcone derivatives and flavanone **14,** shown in Table VI, indicated potent inhibitory effects on release of β-glucuronidase and lysozyme from neutrophil degranulation caused by fMLP/CB and most of them showed strong inhibitory effects than those of trifluoperazine. It clearly indicated that the inhibitory effects on rat neutrophil degranulation induced by fMLP/CB were not dependent with the substituted partern of chalcone derivatives. Trifluoperazine was used in this study as a positive control and produced a dose-dependent inhibition of neutrophil degranulation caused by fMLP/CB.

FMLP (0.3 μM)/CB (5 μg mL^{-1}) or PMA (3 nM) also induced the superoxide anion formation from rat neutrophils. As showed in Table VII, compounds **2, 3** and **9** showed potent inhibitory effects on superoxide anion formation from rat neutrophils caused by fMLP/CB while **4** indicated potent inhibitory effects on superoxide anion formation from rat neutrophils caused by PMA. Compound **8** showed inhibitory effects on superoxide anion formation from rat neutrophils caused by fMLP/CB. The hydroxylation of **8** at C-5′ such as **11** did not enhance the inhibitory effects on superoxide anion formation from rat neutrophils caused by fMLP/CB but the O-methylation of **10** at C-3 and C-4 such as **9** enhanced the inhibitory effects on superoxide anion formation from rat neutrophils caused by fMLP/CB. The inhibitory effects of **2** on superoxide anion formation from rat neutrophils caused by fMLP/CB were greater than those of trifluoperazine (Table VII). Compounds **3, 5, 10** and **11** showed strong inhibitory effects on superoxide anion

Table VI. The Inhibitory Effects of Chalcones and Related Compounds on the Release of β-glucuronidase and Lysozyme from Rat Neutrophils Stimulated with FMLP/CB

Compound	$IC_{50}(\mu M)^a$ β-glucuronidase	lysozyme
1	11.4 ± 2.4	10.5 ± 1.7
2	2.0 ± 0.6	4.2 ± 0.7
3	5.1 ± 2.3	13.2 ± 2.0
4	17.3 ± 4.1	19.7 ± 5.0
5	9.7 ± 1.9	23.6 ± 4.9
6	3.5 ± 1.8	12.3 ± 1.2
7	0.6 ± 0.1	2.6 ± 0.2
8	9.8 ± 0.9	14.8 ± 1.6
9	9.7 ± 2.3	5.9 ± 3.5
10	12.5 ± 0.8	7.3 ± 3.9
11	6.0 ± 0.8	10.9 ± 0.2
12	>100(−6.0 ± 7.2)	>100(−5.1 ± 2.2)
13	>100(44.1 ± 1.7)	>100(21.2 ± 0.9)
14	14.6 ± 2.1	20.2 ± 6.9
15	>100(17.3 ± 9.4)	>100(19.0 ± 2.0)
16	>100(20.4 ± 2.6)	>100(2.7 ± 5.3)
17	2.3 ± 0.2[b]	3.6 ± 0.3[b]
Trifluoperazine	22.3 ± 6.2	15.1 ± 2.9

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Average ± s.e.m. (n = 3–5) of at least three separate determinations.
[b] Data from Ref. 5.

Table VII. The Inhibitory Effects of Chalcones and Related Compounds on Superoxide Anion Formation from Rat Neutrophils Stimulated with FMLP/CB or PMA

Compound	IC_{50} (μM)[a] FMLP/CB	PMA
1	77.5 ± 13.6	>30(9.8 ± 9.1)
2	4.8 ± 2.0	>100(9.5 ± 8.4)
3	8.4 ± 2.9	15.2 ± 0.7
4	>100(19.3 ± 9.1)	6.4 ± 1.9
5	>100(−10.8 ± 8.5)	24.3 ± 4.5
6	—	—
7	>100(19.5 ± 1.7)	>100(40.3 ± 14.4)
8	26.2 ± 3.9	>100(−11.0 ± 12.7)
9	9.4 ± 1.3	>100(41.6 ± 12.2)
10	15.9 ± 6.2	23.5 ± 4.2
11	>100(46.3 ± 2.1)	16.5 ± 5.4
12	>30(5.3 ± 0.24)	—
13	>30(42.6 ± 1.3)	—
14	15.8 ± 2.9	>100(15.8 ± 9.0)
15	>100(−8.1 ± 7.0)	>100(45.4 ± 9.4)
16	>100(−51.9 ± 8.5)	>100(23.1 ± 7.1)
17	2.6 ± 0.1[b]	—
Trifluoperazine	6.3 ± 0.7	5.3 ± 0.5

[a] When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses.—Not determined. Average ± s.e.m. (n = 3–5) of at least three separate determinations.
[b] Data from Ref. 5.

formation from rat neutrophils caused by PMA (Table VII). As shown in Table VII, chalcone derivatives (except for **3, 6, 7** and **10**) and flavanone derivative, **14** showed different inhibitory effects on superoxide anion formation from rat neutrophils caused by fMLP/CB and PMA. It indicated that fMLP/CB and PMA could induce the superoxide anion formation from rat neutrophils but they utilized different transduction mechanisms and were regulated differently (12,13). Trifluoperazine was used in this study as a positive control and produced a dose-dependent inhibition of superoxide anion formation from rat neutrophils stimulated with FMLP/CB and PMA, respectively. These results indicated that the inhibitory pathway of compounds **1, 2, 8, 9** and **14** on superoxide anion formation were different from those of compounds **4, 5** and **11.** They possess more selective inhibitory effects on superoxide anion formation than those of trifluoperazine.

The process of subplantar injection of polymyxin B-induced hind-paw edema. Edematous response was significantly suppressed in mice pretreated with **2, 7** and **17** (Table VIII). Polymyxin B- induced hind-paw edema was inhibited by diphenhydramine (Table VIII). The inhibitory effects of **2, 7, 17** and diphenhydramine on polymyxin B-induced edematous response were demonstrated not only in normal mice but also in adrenalectomized mice (Table VIII).

Drugs which possess inhibitory effects on the activation of mast cells and neutrophils will alleviate the inflammatory syndrome. The chalcone derivatives, previously reported (5), showed potent inhibition of mast cells and neutrophil degranulation. They showed potent anti-inflammatory effects.

Polymyxin B- induced edema was suppressed by non-steroidal anti-inflammatory drugs such as aspirin and indomethacin, and blockers of histamine and 5-hydroxytryptamine (5-HT) (14). In this study, **2, 7, 17** and diphenhydramine significantly reduced the hind paw swelling in polymyxin B-induced edema.

Compound **2, 7** and **17** retained anti-inflammatory activity in adrenalectomized mice, indicating that the action of these three compounds does not depend upon direct or indirect stimulation of the adrenal gland.

Table VIII. Effects of **2, 7, 17** and Diphenhydramine on Polymyxin B-induced Mouse Hind-paw Edema in Normal and Adrenalectomized Mice

Compound	Dose (mg/Kg)	Normal mice	Adrenalectomized mice (area under the curve)
control		190.7 ± 8.1 (22)	164.7 ± 6.7 (9)
2	10	173.3 ± 9.2 (5)	
	30	145.1 ± 7.5*(5)	115.9 ± 6.4*(6)
7	3	186.0 ± 5.5 (5)	
	10	157.6 ± 4.8*(5)	
	30	146.1 ± 9.5*(5)	112.1 ± 2.7*(5)
17	10	157.4 ± 9.9 (5)	
	30	133.0 ± 13.2*(5)	127.6 ± 6.5*(6)
Diphenhydramine	10	118.9 ± 10.3**(10)	123.7 ± 14.2*(4)

Edematous response was induced by subplantar injection of 10 μg polymyxin B in DMSO or drug-pretreated (A) normal or (B) adrenalectomized mice. Compound **2, 7, 17** or diphenhydramine was given intraperitoneally 1h before the induction of paw swelling, respectively. Values are expressed as the means ± s.e.m. (n). *$P < 0.05$, **$P < 0.01$ compared with control. To eliminate the effect of the solvent on polymyxin B-induced mouse hind-paw edema, the final concentration of DMSO was fixed at 0.5%.

The present results demonstrated that most of the chalcone derivatives have an anti-inflammatory effect. The inhibitory effects of selected compounds, **2, 7** and **17** on inflammation are not due to the release of steroid hormones from adrenal gland, but are partly mediated through the suppression of chemical mediators released from mast cells and neutrophils.

Norathyriol (1,3,6,7-tetrahydroxyxanthone) (**18**), a nature product, widely distributed in Gentianaceous plants, showed an anti-inflammatory effect (6). The inhibitory effect of norathyriol on local edema was not due to the release of steroid hormones from the adrenal gland, but was probably partly due to suppression of mast cell degranulation and hence reduction of the release of chemical mediators which increased vascular permeability, and partly, at least in higher doses, due to protection of the vasculature from challenge by various mediators (6). The anti-inflammatory effects of some xanthone derivatives were also mediated through the suppression of chemical mediators released from mast cell or neutrophil degranulation (15). The above results indicated that the anti-inflammatory effects of **2, 7** and **17** may be partially the same as that of norathyriol.

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