3,4-Dihydroxychalcones as Potent 5-Lipoxygenase and Cyclooxygenase Inhibitors

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Received April 22, 1993•

A novel series of 3,4-dihydroxychalcones was synthesized to evaluate their effects against 5-lipoxygenase and cyclooxygenase. Almost all compounds exhibited potent inhibitory effects on 5-lipoxygenase with antioxidative effects, and some also inhibited cyclooxygenase. The 2′,5′-disubstituted 3,4-dihydroxychalcones with hydroxy or alkoxy groups exhibited optimal inhibition of cyclooxygenase. We found that 2′,5′-dimethoxy-3,4-dihydroxychalcone (**37**; HX-0836) inhibited cyclooxygenase to the same degree as flufenamic acid and 5-lipoxygenase, more than quercetin. Finally, these active inhibitors of 5-lipoxygenase inhibited arachidonic acid-induced mouse ear edema more than phenidone.

Introduction

The leukotrienes are important mediators of smooth muscle constriction,[1] increased vascular permeability,[2] and leukocyte chemotaxis.[3] The enzyme 5-lipoxygenase catalyzes the initial step in the metabolism of arachidonic acid leading to leukotrienes. Inhibition of the enzyme activity has provided a new therapeutic approach to treating a variety of inflammatory and allergic diseases. Especially, 5-lipoxygenase inhibitors have emerged as an attractive approach to treatment of inflammatory skin diseases, such as psoriasis and contact dermatitis, since these inflammatory dermatoses are not significantly improved by nonsteroidal antiinflammatory drugs. Leukotrienes are elevated in the skin of patients with psoriasis and atopic eczema, whereas prostaglandins are not significantly elevated.[4]

Four mechanisms can be considered for 5-lipoxygenase inhibition:[5] (1) antioxidant and/or free radical scavenging, (2) iron chelation, (3) the inhibition of 5-lipoxygenase translocation, and (4) substrate mimicking. In particular, several antioxidant 5-lipoxygenase inhibitors (e.g., DuP 654,[6] lonapalene,[7] and R-68,151[8]) have been shown to be clinically effective against topical inflammatory conditions.

Numerous studies have been undertaken on the 5-lipoxygenase inhibitors. The current status of the research has been reviewed in *Journal of Medicinal Chemistry* and *Progress in Medicinal Chemistry*.[5,9] Some reports have pointed out toxicological problems associated with nonspecific antioxidants, such as methemoglobinemia.[10] However, clinical trials of lonapalene have demonstrated no side effects to preclude its administration.[7] It is possible that the efficacy of the antioxidant 5-lipoxygenase inhibitor is independent of its toxicity. For example, 5-lipoxygenase inhibitors readily metabolized by systemic administration would be suitable topical antiinflammatory agents.

Nakadate *et al.* have reported that known hydroxychalcones inhibit 12-lipoxygenase and cyclooxygenase in the mouse epidermis.[11] Some chalcone derivatives have been reported as antiinflammatory or antiallergic agents.[12] Futhermore, we found that chalcones with a 3,4-dihydroxycinnamoyl structure strongly inhibited lipid peroxidation in rat liver microsomes.[13] The 3,4-dihydroxychalcones are rapidly and extensively metabolized after systemic administration. These findings suggest that some chalcones may be promising nontoxic topical antiinflammatory agents. In this paper, we describe the biological activity of various hydroxychalcones against 5-lipoxygenase and cyclooxygenase using *in vitro* and *in vivo* topical inflammatory models and discuss their structure–activity relationships.

Results

Chemistry. We synthesized 3,4-dihydroxychalcone (**8**) using the Claisen–Schmidt condensation of acetophenone with a unprotected 3,4-dihydroxybenzaldehyde. However, this procedure afforded compound **8** in a low yield (below 10%). The 3,4-dihydroxybenzaldehyde protected as the bis(tetrahydropyranyl) ether was condensed with acetophenone to give compound **8** in a good yield (45%). Previously, the benzyl and methoxymethyl ethers of the phenolic hydroxy group were used in the Claisen–Schmidt condensation,[14] although these ethers required purification before the condensation step. However, the purification procedure was not necessary when using tetrahydropyranyl ethers to yield the desired hydroxychalcones. The chemical structures and yields of the entitled chalcones (**1–53**) are listed in Tables I–III. Known chalcones (**1–9, 11–13, 16, 19,** and **41**) are presented with supplementary reference numbers in Tables I and II.[12b,15]

Biological Evaluation. We investigated the effects of hydroxychalcones (**1–13**) and related compounds (**14–19**) on 5-lipoxygenase, cyclooxygenase, and lipid peroxidation. Those which had a 3,4-dihydroxycinnamoyl moiety inhibited 5-lipoxygenase and lipid peroxidation (Table I). We found that 3,4-dihydroxychalcone **8** and 2′,3,4-trihydroxychalcone **9** also inhibited cyclooxygenase. However, these activities were less effective than those against 5-lipoxygenase (cf. IC_{50} values of compound **8**: 34 and 0.043 μM against cyclooxygenase and 5-lipoxygenase, respectively). We selected compound **8** for the lead compound as a dual inhibitor against cyclooxygenase and 5-lipoxygenase. Therefore we synthesized monosubstituted 3,4-dihydroxychalcones (**20–31**) which bear a substitution group at the 2′-3′-, or 4′-position (Table II). Among these monosubstituted 3,4-dihydroxychalcones (**20–31**), 4′-chloro, 3′-methoxy, or 3′- or 4′-(dimethylamino) groups were effective against 5-lipoxygenase, whereas 2′- or 3′-

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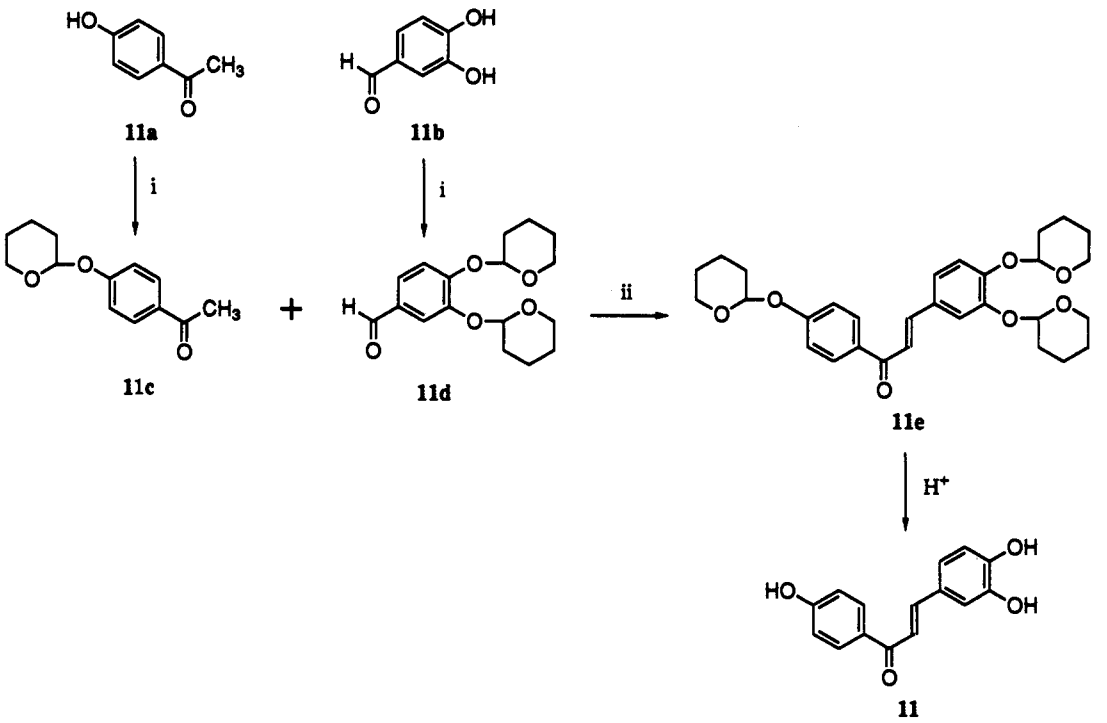
• Abstract published in *Advance ACS Abstracts*, October 15, 1993.

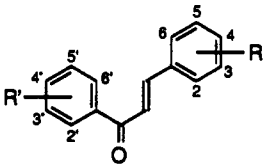
0022-2623/93/1836-3904$04.00/0

Scheme I. Synthesis of 4′,3,4-Trihydroxychalcone (11)[a]



[a](i) 3,4-Dihydro-2*H*-pyran + pyridinium *p*-toluenesulfonate; (ii) $Ba(OH)_2 \cdot H_2O$.

Table I. Inhibition of 5-Lipoxygenase, Cyclooxygenase, and Lipid Peroxidation by Hydroxychalcones and Related Compounds



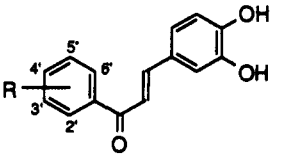
no.	R′	R	yield (%)	mp (°C)	crystn solvent	formula	IC_{50} (μM)[d] RBL-15-LO[a]	IC_{50} (μM)[d] SSV CO[b]	RLM LPOX[c] % inhibn at 1 μM
1[15a]	2′-OH		65	82–85	MeOH/H_2O	$C_{15}H_{12}O_2$	(19% at 100)	(13% at 100)	6
2[15b]	4′-OH		86	171–174	MeOH/H_2O	$C_{15}H_{12}O_2$	230	(20% at 100)	5
3[15c]	2′,4′-OH		74	145–146	MeOH/H_2O	$C_{15}H_{12}O_3$	400	280	5
4[15d]	2′,4′,6′-OH		78	178–180	MeOH/H_2O	$C_{15}H_{12}O_4$	140	(6.9% at 100)	9
5[15e]	2′-OH	4-OH	75	158–160	MeOH/H_2O	$C_{15}H_{12}O_3$	42	390	6
6[15e]	2′,4′-OH	4-OH	76	215–219	MeOH/H_2O	$C_{15}H_{12}O_4$	35	130	9
7[15d]	2′,4′,6′-OH	4-OH	70	200–201	MeOH/H_2O	$C_{15}H_{12}O_5$	100	(3.1% at 100)	8
8[15f]		3,4-OH	45	202–204	MeOH/H_2O	$C_{15}H_{12}O_3$	0.043	34	44
9[15g]	2′-OH	3,4-OH	66	178–180	MeOH/H_2O	$C_{15}H_{12}O_4$	0.023	44	45
10	3′-OH	3,4-OH	48	191–192	MeOH/H_2O	$C_{15}H_{12}O_4$	0.0042	140	24
11[15b]	4′-OH	3,4-OH	67	218–219	MeOH/H_2O	$C_{15}H_{12}O_4$	0.0040	320	36
12[15h]	2′,4′-OH	3,4-OH	64	250–253	MeOH/H_2O	$C_{15}H_{12}O_5$	0.0046	120	40
13[15i]	2′,4′,6′-OH	3,4-OH	63	249–252	MeOH/H_2O	$C_{15}H_{12}O_6$	0.14	(11% at 100)	18
14	2-thienyl	3,4-OH	24	183–185	benzene	$C_{13}H_{10}O_3S$	0.022	34	56
15	3-pyridyl	3,4-OH	57	242–250	EtOH/H_2O	$C_{14}H_{11}O_3N$	0.21	(7.7% at 100)	10
16[15j]	2′-OH	3-OCH_3, 4-OH	37	126–127	MeOH/H_2O	$C_{16}H_{14}O_4$	17	63	14
17	4′-Cl	3-OCH_3, 4-OH	57	99–101	MeOH/H_2O	$C_{16}H_{13}O_3Cl$	8.9	41	not tested
18	4′-OCH_3	3-OCH_3, 4-OH	49	155–156	MeOH/H_2O	$C_{17}H_{16}O_4$	12	(−7.3% at 100)	2.3
19[15k]	2′-OH	3-OH, 4-OCH_3	39	153–156	MeOH	$C_{16}H_{14}O_4$	(−4.5% at 10)	(8.6% at 100)	11

[a] 5-Lipoxygenase from RBL-1 cells. [b] Sheep seminal vesicle cyclooxygenase. [c] Lipid peroxidation in rat liver microsomes. [d] IC_{50} based on duplicate three-point titration. Values in parentheses are percent inhibition at the concentration shown (μM) where IC_{50} values were not determined.

methoxy groups had better anti-cyclooxygenase activity. The anti-cyclooxygenase activity was reduced by 4′-substitution.

We investigated disubstituted chalcones (**32–39**) which bear hydroxy, methyl, and/or methoxy groups at the 2′,4′-, 2′,5′-, 2′,6′-, or 3′,4′-positions. Additionally, we assessed the biological evaluations of trisubstituted chalcones (**40** and **41**). We found 2′,5′-dimethoxy-3,4-dihydroxychalcone (**37**) was as effective a dual inhibitor as DuP 654 (IC_{50} values of 7.8 nM and 9.2 μM, against 5-lipoxygenase and cyclooxygenase, respectively). Furthermore, we assayed the 3,4-dihydroxychalcones (**42–53**) bearing disubstitution groups at the 2′,5′-position *in vitro* and *in vivo*. These chalcones (**42–53**), except for compound **47**, exhibited potent inhibitory effect on 5-lipoxygenase. Especially, compounds **44**, **45**, **50**, and **53** inhibited 5-lipoxygenase to a greater degree than compound **37** (IC_{50} values of compounds **44**, **45**, **50**, and **53**: 5.3, 4.0, 3.8, and 2.4 nM against 5-lipoxygenase, respectively). In the series of these anti-cyclooxygenase activities, compound **52** was the most

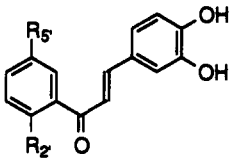
Table II. Inhibition of 5-Lipoxygenase and Cyclooxygenase by 3,4-Dihydroxychalcones



no.	R	yield (%)	mp (°C)	crystn solvent	formula	RBL-1 5-LO IC_{50} (nM)[a]	SSV CO IC_{50} (μM)[a]
8	H	45	202–204	MeOH/H_2O	$C_{15}H_{11}O_3$	43	34
20	2′-Cl	58	174–178	benzene	$C_{15}H_{11}O_3Cl$	92	71
21	4′-Cl	58	228–230	acetone/H_2O	$C_{15}H_{11}O_3Cl$	8.5	1400
22	4′-NO_2	16	268–269	benzene/EtOAc	$C_{15}H_{11}O_5N$	23	5%[b]
23	2′-CF_3	63	178–180	MeOH/H_2O	$C_{16}H_{11}O_3F_3$	58	230
24	3′-CH_3	43	163–164	benzene	$C_{16}H_{14}O_3$	27	71
25	4′-CH_3	35	201–202	MeOH/H_2O	$C_{16}H_{14}O_3$	76	210
26	2′-OCH_3	33	145–147	benzene	$C_{15}H_{14}O_4$	27	13
27	3′-OCH_3	52	152–153	benzene/acetone	$C_{16}H_{14}O_4$	6.5	15
28	4′-OCH_3	43	172–178	MeOH/H_2O	$C_{15}H_{14}O_4$	20	490
29	3′-$N(CH_3)_2$	49	145–149	benzene	$C_{17}H_{17}O_3N$	9.8	41
30	4′-$N(CH_3)_2$	39	211–213	acetone/H_2O	$C_{17}H_{17}O_3N$	4.7	810
31	4′-$OCH(CH_3)_2$	42	160–166	benzene/acetone	$C_{18}H_{18}O_4$	41	11%[b]
32	2′-OH, 4′-OCH_3	46	170–173	benzene	$C_{16}H_{14}O_5$	15	65
33	2′-OH, 5′-OCH_3	63	149–151	MeOH/H_2O	$C_{16}H_{14}O_5$	41	41
34	4′-OH, 3′-OCH_3	44	174–177	benzene	$C_{15}H_{14}O_5$	9.0	14%[b]
35	2′-CH_3, 4′-CH_3	33	131–134	benzene/*n*-hexane	$C_{17}H_{16}O_3$	17	41
36	2′-OCH_3, 4′-OCH_3	51	160–161	EtOH/H_2O	$C_{17}H_{16}O_5$	10	160
37	2′-OCH_3, 5′-OCH_3	53	158–160	benzene/acetone	$C_{17}H_{16}O_5$	7.8	9.2
38	2′-OCH_3, 6′-OCH_3	22	192–194	benzene/*n*-hexane	$C_{17}H_{16}O_5$	370	15%[b]
39	3′-OCH_3, 4′-OCH_3	55	132–137	benzene/acetone	$C_{17}H_{16}O_5$	18	140
40	2′-CH_3, 4′-CH_3, 6′-CH_3	27	175–176	benzene/*n*-hexane	$C_{18}H_{16}O_4$	400	280
41[12b]	3′-OCH_3, 4′-OCH_3, 5′-OCH_3	48	153–154	benzene	$C_{18}H_{18}O_6$	16	13%[b]

[a] IC_{50} based on duplicate three-point titration. [b] Values are percent inhibition versus control at 100 μM; average of two determinations.

Table III. Inhibition of 5-Lipoxygenase, Cyclooxygenase, and Arachidonic Acid-Induced Mouse Ear Edema by 2′,5′-Disubstituted 3,4-Dihydroxychalcones



no.	$R_{2'}$	$R_{5'}$	yield (%)	mp (°C)	crystn solvent	formula	RBL-1 5-LO IC_{50} (nM)[a]	SSV CO IC_{50} (μM)[a]	AA ear edema % inhibition[b]
37	OCH_3	OCH_3	53	158–160	benzene/acetone	$C_{17}H_{16}O_5$	7.8 (4.5–14)	9.2 (6.8–13)	77 ± 9.6**
42	OH	OH	61	204–205	benzene/acetone	$C_{15}H_{12}O_5$	64 (39–110)	170 (100–280)	39 ± 3.4*
43	OH	CH_3	44	177–183	MeOH/H_2O	$C_{16}H_{14}O_4$	39 (24–61)	120 (69–230)	52 ± 7.6**
33	OH	OCH_3	63	149–151	MeOH/H_2O	$C_{16}H_{14}O_5$	41 (21–79)	41 (34–51)	38 ± 3.8*
44	OH	OC_2H_5	47	167–169	benzene/acetone	$C_{17}H_{16}O_5$	5.3 (3.1–9.2)	130 (100–160)	42 ± 6.8*
45	OH	$CH(CH_3)_2$	37	157–158	benzene	$C_{18}H_{18}O_4$	4.0 (0.90–17)	37 (31–45)	16 ± 3.6
46	OH	$OCH(CH_3)_2$	33	177–178	benzene	$C_{18}H_{18}O_5$	11 (6.2–21)	140 (80–230)	67 ± 8.5**
47	OH	OC_4H_9	47	162–163	benzene/acetone	$C_{19}H_{20}O_5$	1000 (300–3600)	4.2%[c]	33 ± 4.6
48	CH_3	CH_3	52	154–155	benzene	$C_{17}H_{16}O_3$	16 (7.1–37)	44 (36–54)	58 ± 7.7**
49	OCH_3	CH_3	21	150–151	benzene/EtOAc	$C_{17}H_{16}O_4$	24 (11–49)	30 (24–37)	47 ± 11.9*
50	OCH_3	OC_2H_5	49	115–122	benzene	$C_{18}H_{18}O_5$	3.8 (2.2–6.7)	26 (21–32)	13 ± 3.6
51	OCH_3	$OCH(CH_3)_2$	29	42–43	not recrystallized	$C_{18}H_{20}O_5$	14 (8.7–21)	60 (48–75)	34 ± 9.2*
52	OC_2H_5	OCH_3	60	122–125	benzene/acetone	$C_{18}H_{18}O_5$	27 (13–53)	2.0 (1.4–2.9)	7.4 ± 2.2
53	OC_2H_5	OC_2H_5	53	153–155	benzene	$C_{18}H_{20}O_5$	2.4 (1.2–4.6)	24 (20–29)	−18 ± 19.4

[a] IC_{50} based on duplicate three-point titration; 95% confidence limits are in parentheses. [b] Mean ± SE value (n = 4–8) at 30 μg/ear. Significantly different from control, (*) $p < 0.05$, (**) $p < 0.01$. [c] Value is percent inhibition versus control at 100 μM; average of two determinations.

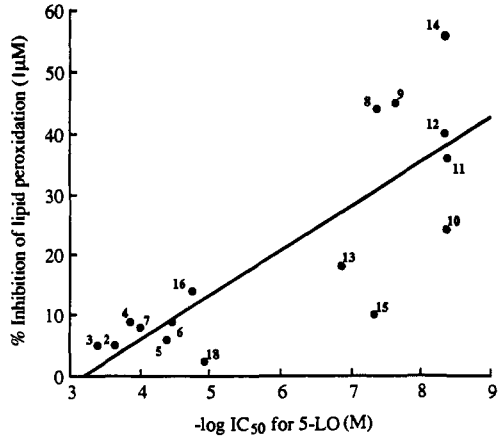
potent inhibitor (IC_{50} value of compound **52**: 2.0 μM against cyclooxygenase).

The antiinflammatory effects of the chalcones (**33**, **37**, and **42–53**) *in vivo* were determined using arachidonic acid-induced mouse ear edema. All chalcones studied (**33**, **37**, and **43–53**), except for compounds **52** and **53**, inhibited topical inflammation. Compounds **37**, **43**, **46**, and **48** were potently antiinflammatory (Table III).

Discussion

We found, while screening the effects of various chalcones on lipid peroxidation and 5-lipoxygenase, that those bearing a 3,4-dihydroxy group were especially potent inhibitors. The anti-5-lipoxygenase activities of hydroxychalcones was related to their antilipid peroxidative activities (Figure 1). Thody *et al.* reported that the anti-5-lipoxygenase activities of NDGA and BW-755c were dependent on their antioxidative activities.[16] Also, 3,4-dihydroxychalcones should inhibit 5-lipoxygenase by means of their antioxidative activities. However, a relationship between anti-cyclooxygenase activity and antilipid peroxidative activity was not recognized.

We synthesized various 3,4-dihydroxychalcones bearing some electron-donating groups based on preliminary



%Inhibition of lipid peroxidation = - 23.375 + 7.3407 (-log IC_{50} for 5-LO) R= 0.673

Figure 1. Inhibition of lipid peroxidation versus inhibition of 5-lipoxygenase (compounds **2**-**16** and **18**).

screening (Table I) with which to study the structure–activity relationships to anti-5-lipoxygenase and cyclooxygenase activities (Tables II and III). Their anti-cyclooxygenase activities were increased by substituting alkoxy groups at the 2′,3′- and 2′,5′-positions, although they were remarkably reduced by 4′-substitution. On the other hand, chalcone **38** bearing a group at the 2′,6′-position had not only decreased anti-cyclooxygenase activity but also anti-5-lipoxygenase activity. Finally, 2′,5′-dimethoxy-3,4-dihydroxychalcone (**37**) and 2′-ethoxy-5′-methoxy-3,4-dihydroxychalcone (**52**) possesed the most potent anti-5-lipoxygenase and anti-cyclooxygenase activities in the series.

Many catechol derivatives, such as nordihydroguaiaretic acid (NDGA), quercetin, and caffeic acid, have been reported as 5-lipoxygenase inhibitors.[9] The 3,4-dihydroxychalcones (**37** and **52**), selected here, were stronger inhibitors than these catechol derivatives (Table IV). Additionally, these chalcones bearing catechol moiety obviously possessed anti-cyclooxygenase activity, although the known catechol derivatives were very weak.

Milton *et al.* reported that the ability of a series of 2,3-dihydro-5-benzofuranols to inhibit leukotriene biosynthesis *in vitro* is directly related to the lipophilicity represented by the logarithm of the octanol/water partition coefficients (log *P*).[17] They suggested that the inhibitory activity of benzofuranols was increased by an increase in lipophilicity to increase the concentration of the inhibitor in the cell membranes. We investigated the relationship between the hydrophobicity (*k*′)[18] values of 3,4-dihydroxychalcones which were calculated from HPLC analysis using 60% MeOH as the eluent, as well as by their anti-5-lipoxygenase activities *in vitro*. The anti-5-lipoxygenase activity of 3,4-dihydroxychalcones did not correlate with their hydrophobicity (Figure 2). The structure–activity relationships of 3,4-dihydroxychalcones to 5-lipoxygenase would be further complicated by their hydrophobicity, steric effects, and the other factors. However, electron-attracting groups were not suitable because anti-5-lipoxygenase activity is based upon the antioxidative activity.

The 3,4-dihydroxychalcones that exhibited potent anti-5-lipoxygenase activities, except for compounds **45**, **50**, and **53**, potently inhibited arachidonic acid-induced mouse ear edema. A comparison indicated that the potency of anti-5-lipoxygenase activity relatively contributed to antiinflammatory effects rather than their anti-cyclooxygenase activities. This result agreed with the inhibitory effects of 2-substituted-1-naphthols which are potent 5-lipoxygenase inhibitors with anti-cyclooxygenase activity in arachidonic acid-induced mouse ear edema.[6] There was a correlation in arachidonic acid-induced mouse ear edema model between the reduction of eicosanoids and edema.[19] The present results suggested that the anti-5-lipoxygenase activities were more predominant in arachidonic acid-induced inflammation. However, the vascular permeability was more increased by coexisting leukotrienes and prostaglandins.[20] Therefore, the most potent topical antiinflammatory activity of compound **37** was thought to combine the anti-5-lipoxygenase and anti-cyclooxygenase activities.

After oral or intravenous administration of 3,4-dihydroxychalcones (**37** and **52**), unchanged forms were undetectable in rat plasma using HPLC analysis.[21] The 3,4-dihydroxychalcones were rapidly and extensively metabolized after systemic administration. Thus, 2′,5′-dimethoxy-3,4-dihydroxychalcone (HX-0836, **37**) was selected and further pharmacologically and toxicologically evaluated since it has an attractive profile as a topical antiinflammatory agent.

Experimental Section

Chemistry. Melting points were determined with a micro melting point apparatus (Yanagimoto) and were not corrected. Chromatography was performed on a Kieselgel 60 column (70–230 mesh, Merck). NMR spectra (all compounds and intermediates) were recorded on a Varian Unity 200 spectrometer (200 MHz), using Me_4Si as the internal standard. All elemental analyses were within ±0.4% of the calculated values. The purity of chalcone derivatives was verified by HPLC ($MeOH/H_2O$/AcOH, 600:400:5), using a Cosmosil $10C_8$ column (Nakalai Tesque). The UV spectra of chalcone derivatives were recorded on a Hitachi 50–20 spectrophotometer.

General Procedure for Obtaining Chalcones 1–53. 4′,3,4-Trihydroxychalcone (11). 4-Hydroxyacetophenone (**11a**, 2.72 g, 20 mmol) and pyridinium *p*-toluenesulfonate (0.12 g, 0.48 mmol) were stirred in methylene chloride (80 mL), and then 3,4-dihydro-α-pyran in methylene chloride (5.05 g, 60 mmol, 20 mL) was added dropwise. The reaction mixture was stirred at room temperature until the components dissolved. The reaction mixture was washed twice with water, dried, and evaporated *in vacuo*. The residue yielded crude 4-(tetrahydropyran-2-yloxy)-acetophenone (**11c**).

3,4-Dihydroxybenzaldehyde (**11b**, 2.76 g, 20 mmol) and pyridinium *p*-toluenesulfonate (0.12 g, 0.48 mmol) were stirred in methylene chloride (80 mL), and then 3,4-dihydro-α-pyran in methylene chloride (10.09 g, 120 mmol, 20 mL) was added dropwise. The reaction mixture was stirred at room temperature until all the components dissolved. The reaction mixture was washed twice with water, dried, and evaporated *in vacuo*. The residue yielded crude 3,4-bis(tetrahydropyran-2-yloxy)benzaldehyde (**11d**).

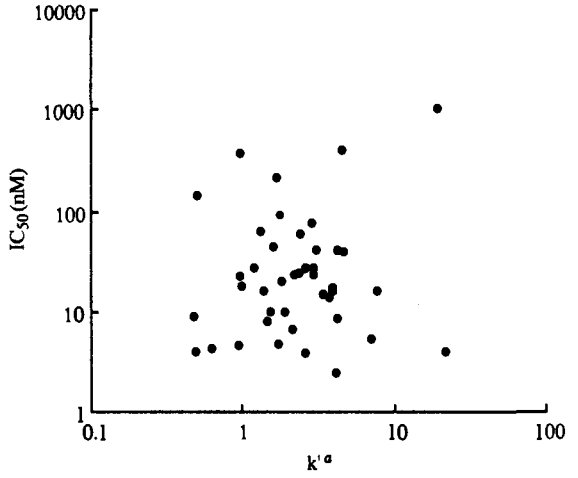
Crude **11c**, **11d**, and barium hydroxide octahydrate (6.52 g, 20 mmol) were dissolved in MeOH (100 mL). The reaction mixture was stirred for 12 h at 40 °C and then evaporated *in vacuo*. Water (100 mL) was added to the mixture, neutralized with 1 M HCl, and extracted with EtOAc. The organic layer was separated, washed with water, dried, and evaporated *in vacuo*. This residue yielded crude 4′,3,4-tris(tetrahydropyran-2-yloxy)chalcone (**11e**).

Crude **11e** and *p*-toluenesulfonic acid (0.091 g, 0.48 mmol) were dissolved in MeOH (100 mL). The reaction mixture was stirred for 3 h at room temperature, and then evaporated *in vacuo*. Water (100 mL) was added to the mixture, neutralized with 5% $NaHCO_3$, 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 EtOAc/benzene to give 2.5 g of compound **11**: yield 48% ($C_{15}H_{12}O_4$); mp 218–219 °C (recrystallized from $MeOH/H_2O$); NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ 7.93 (d, 2 H, *J* = 9 Hz), 7.66 (d, 1 H, *J* =

Table IV. Biological Data of Standard Drugs and Selected 3,4-Dihydroxychalcones

compound	IC_{50} (M)[a] RBL-1 5-LO	IC_{50} (M)[a] SSV CO	AA ear edema % inhibition[b]
phenidone	1.0×10^{-6} (7.0×10^{-7}–1.8×10^{-6})	8.0×10^{-5} (6.0×10^{-5}–1.0×10^{-4})	24.6 ± 9.7*
caffeic acid	4.3×10^{-5} (2.3×10^{-5}–8.0×10^{-5})	16%[c]	not tested
quercetin	2.0×10^{-7} (2.0×10^{-7}–2.7×10^{-7})	13%[c]	not tested
NDGA[d]	1.0×10^{-7} (7.0×10^{-8}–1.4×10^{-7})	2.1×10^{-4} (1.3×10^{-4}–3.5×10^{-4})	not tested
indomethacin	3.7×10^{-3} (1.0×10^{-3}–1.3×10^{-2})	8.0×10^{-7} (4.0×10^{-7}–1.5×10^{-6})	4.8 ± 8.5
flufenamic acid	−0.1%[c]	1.9×10^{-5} (1.2×10^{-5}–2.7×10^{-5})	not tested
piroxicam	19%[c]	4.6×10^{-4} (3.3×10^{-4}–6.5×10^{-4})	not tested
DuP 654[e]	6.9×10^{-9} (3.6×10^{-9}–1.3×10^{-8})	2.4×10^{-5} (1.7×10^{-5}–3.4×10^{-5})	67 ± 4.0**
36	1.0×10^{-8} (5.8×10^{-9}–1.7×10^{-8})	1.6×10^{-4} (9.4×10^{-5}–2.6×10^{-4})	56 ± 5.6**
37	7.8×10^{-9} (4.5×10^{-9}–1.4×10^{-8})	9.2×10^{-6} (6.8×10^{-6}–1.3×10^{-5})	77 ± 9.6**
52	2.7×10^{-6} (1.3×10^{-6}–5.3×10^{-6})	2.0×10^{-5} (1.4×10^{-6}–2.9×10^{-5})	7.4 ± 2.2

[a] IC_{50} based on duplicate three-point titration; 95% confidence limits are in parentheses. [b] Mean ± SE value (n = 4–8) at 30 μg/ear. Significantly different from control, (*) $p < 0.05$, (**) $p < 0.01$. [c] Values are percent inhibition versus control at 10^{-4} M; average of two determinations. [d] Nordihydroguaiaretic acid. [e] 2-Benzyl-1-naphthol.



[a] k'= tR/t0-1; tR is the retention time of 60% MeOH in the eluent and t0 is the column dead-time.

Figure 2. The IC_{50} for 5-lipoxygenase inhibition versus the hydrophobicity (k') of 3,4-dihydroxychalcones and related compounds (**8–15** and **20–53**).

16 Hz), 7.36 (d, 1H, J = 16 Hz), 7.20 (d, 1 H, J = 2 Hz), 7.07–7.02 (m, 1 H), 6.92 (d, 2 H, J = 9 Hz), 6.88 (d, 1 H, J = 8 Hz).

Compounds **1**, **5**, **8**, **9**, **14–41**, and **43–53** were condensed without protection to acetophenone (compounds **14** and **15** were condensed using 2-acetylthiophene and 3-acetylpyridine, respectively). The acetophenones (**40a** and **44a–53a**) corresponding to the compounds (**40** and **44–53**) were synthesized in the usual way. The other acetophenones were commercial reagents. Tables I–III show yields, mp, recrystallization solvents, and formulas of compounds **1–53**.

Biological Evaluation. Measurement of Lipid Peroxidation. The modified method of Ohkawa *et al.*[22] was used. The assay system (1 mL) consisted of 83.5 mM KCl and 37.2 mM Tris-HCl buffer (pH 7.4), the test compound in DMF (0.01 mL), 1 mM ADP, 10 μM $FeCl_3$, the microsomal fraction (1.0 mg of protein), and 0.2 mM NADPH. Reaction mixtures were incubated at 37 °C for 20 min and then cooled on ice to terminate the reaction. Thereafter, 8.1% sodium dodecyl sulfate (0.2 mL), 20% AcOH containing 0.27 M HCl adjusted to pH 3.5 with NaOH (1.5 mL), and 0.8% thiobarbituric acid (1.5 mL) were added to the reaction mixture. The mixture was then heated at 95 °C for 20 min, and the reaction was stopped by cooling on ice. Thereafter, n-BuOH/pyridine (15:1, 4.0 mL) was added with vigorous mixing. After the reaction mixture was centrifuged (800g, 10 min), the organic layer was separated and the absorbance was measured at 532 nm.

Measurement of RBL-1 5-Lipoxygenase Activity. The modified method of Blackham *et al.*[23] was used. RBL-1 cells were grown in RPMI-1640 medium containing 10% heat-inactivated newborn calf serum, sodium bicarbonate 2.0 g/L, glutamine 0.3 g/L, penicillin 1000 unit/mL, and streptomycin 1 mg/mL. Cells were cultured at 37 °C in 5% CO_2/air. Cells in the growth phase (5×10^5 to 1×10^6 cells/mL) were collected by centrifugation and suspended at a density of 3×10^7 cells/mL in 50 mM phosphate buffer (0.25 M sucrose, 1 mM EDTA, 2 mM glutathione, pH 7.4). The RBL-1 cells containing 5-lipoxygenase were stored at −70 °C. The assay system (0.5 mL) consisted of 50 mM phosphate buffer (0.25 M sucrose, 1 mM EDTA, 2 mM glutathione, pH 7.4), the test compound in 10% DMF (0.01 mL), 2 mM $CaCl_2$, 0.66 mM arachidonic acid, and RBL-1 cell homogenate (5×10^6 cells). Reaction mixtures were incubated at 37 °C for 3 min, and then MeOH (0.5 mL) was added to terminate the reaction. The mixture was centrifuged (2000g, 15 min) to remove the precipitated protein, 5-HETE in the supernatant was analyzed by HPLC. The mixture was eluted through a Cosmosil $5C_{18}$ column (4.6 × 150 mm) at room temperature with 85% acetonitrile containing 0.1% AcOH. Absorbance was monitored at 235 nm.

Measurement of Sheep Seminal Vesicle Cyclooxygenase Activity. The modified method of Yanagi *et al.*[24] was used. The assay system (0.1 mL) consisted of 50 mM phosphate buffer (2 mM glutathione, 0.6 mM epinephrine, 83 μM EDTA-2Na, pH 7.4), the test compound in 10% DMF (0.01 mL), [^{14}C]arachidonic acid (0.1 μCi/mL), and sheep seminal vesicle microsomes (32 μg of protein). Reaction mixtures were incubated at 37 °C for 10 min, and then n-hexane/EtOAc (2:1, 0.3 mL) was added to terminate the reaction. After the mixture was mixed vigorously (30 s) and centrifuged (2000g, 1 min), the organic layer was removed. This procedure was repeated. Ethanol was added to the aqueous phase to remove precipitated protein. After the aqueous phase was mixed and centrifuged (2000g, 1 min), the amount of radioactivity in the supernatant (100 μL) was measured using a scintillation counter.

Effect upon Arachidonic Acid-Induced Ear Edema. The procedure of Young *et al.*[25] was used. Arachidonic acid (1 mg/mL in acetone) was prepared fresh daily. Test compounds were dissolved in acetone and then applied to the ears of groups of four to eight male ICR mice (25–28 g). Thereafter, the mice was challenged with 1 mg of arachidonic acid, applied to the inner surface of one ear. The unchallenged ear served as the negative control. One hour postchallenge, animals were killed and the ears were quickly removed and weighed. Swelling was measured as the difference in weight between challenged and unchallenged ears. The percent inhibition was calculated using $[(C - T)/C] \times 100\%$, where C is the positive control swelling and T is the drug-tested swelling. Statistical significance was determined by Student's t test.

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