Effect of Structure on Potency and Selectivity in 2,6-Disubstituted 4-(2-Arylethenyl)phenol Lipoxygenase Inhibitors

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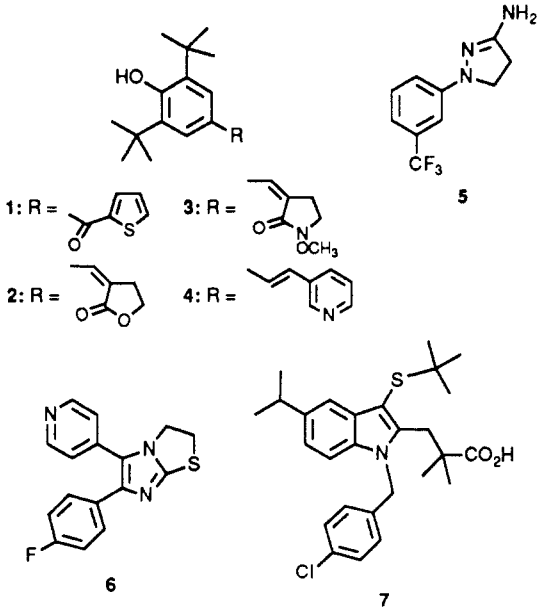
A series of 2,6-disubstituted 4-(2-arylethenyl)phenols with potent human neutrophil 5-lipoxygenase (5-LO) inhibiting activity (IC_{50}s in the 10^{-7} M range) and weaker human platelet cyclooxygenase (CO) inhibiting activity (IC_{50}s in the 10^{-6} M range) is described. This series evolved from the chemical modification of an antiinflammatory dual CO/5-LO inhibitor, 2,6-di-*tert*-butyl-4-[2-(3-pyridyl)ethenyl]phenol (BI-L-93 BS). The potency and selectivity for 5-LO inhibition is greatly influenced by the nature of the substituents in the 2- and 6-positions. Other structure–activity relationships that determine relative 5-LO and CO potency are discussed. In vivo activity against antigen-induced leukotriene-mediated bronchoconstriction and cell influx in guinea pigs is presented. Representatives of the series are active when administered at 30 mg/kg ip.

Leukotrienes (LTs) are a class of potent inflammatory mediators that result from the metabolism of arachidonic acid by 5-lipoxygenase (5-LO). The peptidoleukotrienes LTC_4, LTD_4, and LTE_4 are potent constrictors of airway smooth muscle and promote changes in vascular permeability, increased mucus production in the lung, and vasodilation or vasoconstriction.[1-3] Leukotriene B_4 (LTB_4, 5,12-diHETE) is a potent chemoattractant for neutrophils.[4] Products of 5-LO metabolism also stimulate neutrophil activation and degranulation.[5] These properties, and the detection of LTs in effected tissues suggest that LTs play a significant role in a number of allergic and inflammatory diseases including asthma, psoriasis, inflammatory bowel disease, and arthritis.[2] For these reasons inhibition of 5-LO has been an attractive target for many investigators.

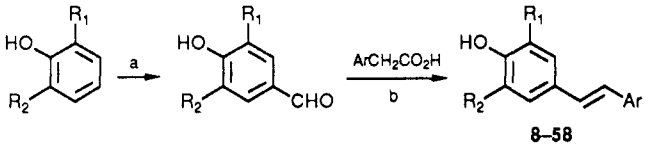
In general, inhibitors of 5-LO that have been reported can be classified as either dual inhibitors of cyclooxygenase (CO) and 5-LO or selective inhibitors of 5-LO with much less potent or no demonstrable CO inhibitory activity. Dual inhibitors include phenolic compounds 1 (R-830),[6]



2 (KME-4),[7] 3 (E-5110),[8] and 4 (BI-L-93 BS).[9] There are also nonphenolic dual inhibitors such as 5 (BW 755C)[10] and 6 (SK&F 86002).[11] On the basis of the biological activity reported for these compounds, they are generally targeted against inflammatory disease, particularly arthritis.

Selective 5-LO inhibitors include AA-861,[12] Rev-5901,[13] and a group of compounds containing a hydroxamic acid functionality.[14,15] Compound 7 (MK-886), a specific 5-LO inhibitor, is reported to act by the novel mechanism of inhibition of translocation of the enzyme from the cytosol to the membrane.[16]

Scheme I[a]



[a] (a) $C_6H_{12}N_4$, H^+; (b) piperidine.

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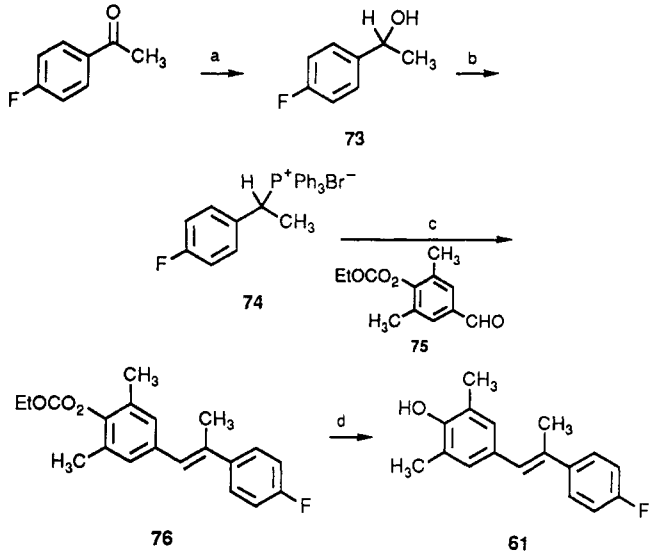
Scheme II[a]



[a] (a) $NaBH_4$; (b) HBr, Ph_3P; (c) *n*-BuLi; (d) NaOH.

In vivo activity reported for selective 5-LO inhibitors often includes an animal pulmonary function model or direct measurement of inhibition of LT production in the pleural or peritoneal cavities. These compounds are often targeted toward treatment of asthma.

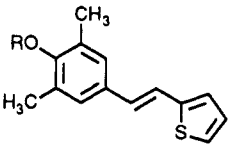
The dual inhibitor **4** is a more potent inhibitor of CO (IC_{50} 0.26 μM) than 5-LO (IC_{50} 1.7 μM) and it shows potent in vivo activity in antiinflammatory models.[9] However, inhibition of 5-LO could not be demonstrated in vivo with this compound. In this paper we report on the chemical modification of **4** that led to a series of selective 5-LO inhibitors with demonstrable in vivo inhibition of LT-mediated bronchoconstriction and cell influx.

Chemistry

(Arylethenyl)phenols (**8–58**) were prepared by reaction of a 3,5-disubstituted 4-hydroxybenzaldehyde with an arylacetic acid in the presence of piperidine. As with the earlier 2,6-di-*tert*-butylphenol series,[9] coupling constants were in the 16-Hz range, consistent with the *E* configuration. The benzaldehyde derivatives were prepared by formylation of corresponding phenols with hexamethylenetetramine, either in HOAc[17] or ethylene glycol/boric acid followed by hydrolysis with H_2SO_4[18] (Scheme I).

2-Ethyl(and -propyl)-6-methoxyphenols were prepared as described for related compounds,[19] by Grignard reaction of the benzyl ether of *o*-vanillin, followed by catalytic reduction and debenzylation with Pd/C in EtOAc with a catalytic amount of H_2SO_4. 2-Chloro(and -fluoro)-6-methylphenol were synthesized by a Reimer–Tiemann reaction with the 2-halophenols[20] followed by catalytic reduction as above. 2,6-Diethylphenol was made by diazotization and hydrolysis of 2,6-diethylaniline.[21] 2-Fluoro-6-methoxyphenol was prepared from *m*-fluoroanisole as described in the literature.[22]

Scheme III



70: R = CH_3
71: R = CH_2CO_2H
72: R = CH_2CH_2OH

Compounds with an alkyl substituent on the olefin (**61** and **62**) were prepared via a Wittig reaction as outlined in Scheme II for compound **61**. Reduction of 4-fluoroacetophenone followed by reaction with HBr and triphenylphosphene in HOAc gave the required phosphonium salt (**74**). Wittig reaction using *n*-BuLi with **75** followed by hydrolysis gave **61**.

Compounds **63** and **64** were prepared by catalytic reduction of the corresponding olefins using tris(triphenylphosphine)rhodium chloride and Pd/C, respectively. Catalytic reduction of **66** using Pd/C gave **67**. Condensation of 3,5-dimethyl-4-hydroxybenzaldehyde with the appropriate methyl aryl ketone in ethanolic HCl gave **65** and **66**. The product with the reverse propenone chain (**68**) was prepared by condensation of 4-acetyl-2,6-dimethylphenol with 2-thiophenecarboxaldehyde.

Compound **69** was prepared by reaction of 3-lithiopyridine with 4-(benzyloxy)-2,6-dimethylbenzaldehyde, followed by catalytic reduction and debenzylation of the resulting alcohol, as described in the literature[23] for a similar compound. Alkylation of **14** gave the O-substituted compounds (**70–72**) shown in Scheme III.

Results and Discussion

Earlier structure–activity studies on 2,6-di-*tert*-butyl-4-(2-arylethenyl)phenols showed that replacing the t-Bu groups of **4** with isopropyl (**8**) resulted in a loss of in vivo antiinflammatory activity.[9] However an enhancement of in vitro 5-LO inhibition was also observed. This prompted us to continue an expanded study on the effect of alkyl group modification on 5-LO inhibition (Table I).

Compounds **4** and **8–24** illustrate the relationship between the size of the alkyl group and the potency of 5-LO inhibition. In general, we have found that for 2,6-dialkyl-4-(2-arylethenyl)phenols, reducing the size of the alkyl groups from *tert*-butyl to methyl results in a marked increase in 5-LO inhibitory potency while CO inhibition is unchanged or slightly diminished. This results in a significant alteration of the selectivity for 5-LO vs CO inhibition. For example, with compounds bearing a 2-thienyl aryl substituent, di-*tert*-butyl analogue **11** is a 35-fold more potent inhibitor of CO than 5-LO, while dimethyl analogue **14** is 14-fold more potent as an inhibitor of 5-LO compared to CO.

The effects of further modification of the alkyl groups are illustrated by compounds **25–32**. In these examples, the 2-thienyl aryl substituent is kept constant and R_1 and R_2 are varied. Compounds bearing 2-alkyl-6-methoxy substituents (**25–27**) have IC_{50} values in the 0.1 μM range and are at least 10-fold less potent as CO inhibitors. The 2-methyl-6-allyl (**30**) and 2,6-dimethoxy (**32**) analogues have similar profiles. Replacement of a methyl group with chlorine or fluorine (**28** and **29**) weakened the 5-LO inhibitory potency and resulted in a more potent CO inhibitor. The 2-fluoro-6-methoxy (**31**) analogue was also

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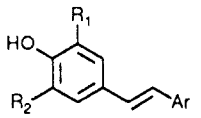
Table I. Activity of 2,6-Disubstituted 4-[2-Arylethenyl]phenols



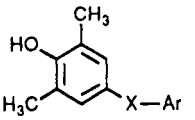
no.	R_1	R_2	Ar[a]	formula	anal.	mp, C	% inhibn 1 μM	% inhibn 0.1 μM	LO[b] IC_{50}, μM	% inhibn 10 μM	% inhibn 1 μM	CO[b] IC_{50}, μM
4	*t*-Bu	*t*-Bu	3P	$C_{21}H_{27}NO$	C,H,N	104–105[c]			1.7 (1.4–2.0)			0.26[d] (0.18–0.33)
8	*i*-Pr	*i*-Pr	3P	$C_{19}H_{23}NO$	C,H,N	136–138[c]			0.20 (0.18–0.23)	81	28	<10
9	Et	Et	3P	$C_{17}H_{19}NO$	C,H,N	130–131	86	36	<1	82	20	<10
10	Me	Me	3P	$C_{15}H_{15}NO$	C,H,N	146–147			0.37(0.10–0.64)			11 (5–17)
11	*t*-Bu	*t*-Bu	2T	$C_{20}H_{26}OS$	C,H,S	80–82[e]			1.2 (L–3.1)			0.034[d] (0.03–0.038)
12	*i*-Pr	*i*-Pr	2T	$C_{18}H_{22}OS$	C,H,S	45.5–48.5[c]	94	14	<1	76	64	<1
13	Et	Et	2T	$C_{16}H_{18}OS$	C,H,S	113–115	89	52	~0.1	96	39	<10
14	Me	Me	2T	$C_{14}H_{14}OS$	C,H,S	133–134			0.054 (0.046–0.063)			0.78 (0.45–1.1)
15	*t*-Bu	*t*-Bu	4-FC_6H_4	$C_{22}H_{27}FO$	C,H,F	87–88[e]	18	24	>1	71	50	0.61[d] (0.30–0.92)
16	*i*-Pr	*i*-Pr	4-FC_6H_4	$C_{20}H_{23}FO$	C,H,F	51–53	40		>1	74	12	<10
17	Me	Me	4-FC_6H_4	$C_{16}H_{15}FO$	C,H,F	145–146			0.12 (0.097–0.14)			1.3 (0.31–2.3)
18	*t*-Bu	*t*-Bu	4-$MeOC_6H_4$	$C_{23}H_{30}O_2$	C,H	115–116[e]	0		>1	5[f]	0	>10
19	*i*-Pr	*i*-Pr	4-$MeOC_6H_4$	$C_{21}H_{26}O_2$	C,H[g]	83–85	71	13[f]	<1	84	18	<10
20	Et	Et	4-$MeOC_6H_4$	$C_{19}H_{22}O_2$	C,H	122.5–123.5	90	15	<1	80	33	<10
21	Me	Me	4-$MeOC_6H_4$	$C_{17}H_{18}O_2$	C,H	139–141			0.091 (0.078–0.10)			1.5 (0.61–2.4)
22	*t*-Bu	*t*-Bu	4-MeC_6H_4	$C_{23}H_{30}O$	C,H	107–108[e]	0		>1	52	12	~10
23	Et	Et	4-MeC_6H_4	$C_{19}H_{22}O$	C,H	113–114	91	21	<1	73	35	<10
24	Me	Me	4-MeC_6H_4	$C_{17}H_{18}O$	C,H	116–117			0.29 (0.16–0.41)			3.2 (1.7–4.6)
25	Me	OMe	2T	$C_{14}H_{14}O_2S$	C,H,S	113–114			0.086 (0.078–0.094)			3.1 (L–11)
26	Et	OMe	2T	$C_{15}H_{16}O_2S$	C,H,S	85.5–86.5			0.090 (0.079–0.10)			1.4[d] (1.0–1.7)
27	*n*-Pr	OMe	2T	$C_{16}H_{18}O_2S$	C,H,S	99–100			0.086 (0.073–0.10)			1.3[d] (0.85–1.8)
28	Me	Cl	2T	$C_{13}H_{11}ClOS$	C,H,Cl,S[h]	109.5–110.5	93	29	<1			0.23 (0.17–0.29)
29	Me	F	2T	$C_{13}H_{11}FOS$	C,H,F,S	86–88	90	18	<1	95	72	<1
30	Me	allyl	2T	$C_{16}H_{16}OS$	C,H,S	79–80			0.065 (0.059–0.070)			0.73 (0.27–1.2)
31	F	OMe	2T	$C_{13}H_{11}FO_2S$	C,H,F,S	82–83	89	31	<1	97	42	<10
32	OMe	OMe	2T	$C_{14}H_{14}O_3S$	C,H,S	95–96.5	93	52	~0.1	86	1	<10
33	Me	Me	4-EtC_6H_4	$C_{18}H_{20}O$	C,H	108–109			0.59 (0.44–0.74)			0.51[d] (0.22–0.80)
34	Me	Me	4-*i*-PrC_6H_4	$C_{19}H_{22}O$	C,H	109–110	30	11[f]	>1			1.4 (0.81–2.1)
35	Me	Me	4-*n*-$PrOC_6H_4$	$C_{19}H_{22}O_2$	C,H	127–128	90	27	<1			0.19[d] (L–0.39)
36	Me	Me	4-*n*-$BuOC_6H_4$	$C_{20}H_{24}O_2$	C,H	138–139	53	7	~1	67	32	<10
37	Me	Me	C_6H_5	$C_{16}H_{16}O$	C,H	139–141			0.10 (0.085–0.12)			4.2 (0.99–7.5)
38	Me	Me	4-ClC_6H_4	$C_{16}H_{15}ClO$	C,H,Cl[i]	146–148			0.17 (0.12–0.23)			0.75[d] (L–1.6)
39	Me	Me	3-MeC_6H_4	$C_{17}H_{18}O$	C,H	116–117			0.13 (0.10–0.17)			2.5 (L–4.9)
40	Me	Me	2-BrC_6H_4	$C_{16}H_{15}BrO$	C,H,Br	104–106	94	26	<1	91	84	<1
41	Me	Me	2-$CF_3C_6H_4$	$C_{17}H_{15}F_3O$	C,H,F	81–82	94	14[f]	<1	72	76	<1
42	Me	Me	2-$BzOC_6H_4$	$C_{23}H_{22}O_2$	C,H	99–101	95	18	<1	92	45	~1
43	Me	Me	2-$MeOC_6H_4$	$C_{17}H_{18}O_2$	C,H	120–121			0.10 (0.087–0.11)			1.1 (0.60–1.6)
44	Me	Me	2,4-$F_2C_6H_3$	$C_{16}H_{14}F_2O$	C,H,F	147–149	100	25	<1			0.71 (0.056–1.4)
45	Me	Me	3,5-$F_2C_6H_3$	$C_{16}H_{14}F_2O$	C,H,F	151–152	81	6[f]	<1	74	50	~1
46	Me	Me	2,6-$F_2C_6H_3$	$C_{16}H_{14}F_2O$	C,H,F	118–119	96	12[f]	<1	94	73	<1
47	Me	Me	2,5-$Me_2C_6H_3$	$C_{18}H_{20}O$	C,H	150–151			0.24 (0.15–0.33)			1.3[d] (0.93–1.7)
48	Me	Me	2-$CO_2HC_6H_4$	$C_{17}H_{16}O_3$	C,H	164–165	12		>1	25	16[f]	>10
49	Me	Me	3-$CO_2HC_6H_4$	$C_{17}H_{16}O_3$	C,H	235–237	0		>1	0	0	>10
50	Me	Me	4-$CO_2HC_6H_4$	$C_{17}H_{16}O_3$	C,H[j]	241–243	0		>1	0	0	>10
51	Me	Me	5-Cl2T	$C_{14}H_{13}ClOS$	C,H,Cl,S	125–126	92	32	<1			1.3 (0.73–1.8)
52	Me	Me	5-Me2T	$C_{15}H_{16}OS$	C,H	104–105	87	26	<1			0.78 (0.59–0.96)
53	Me	Me	3T	$C_{14}H_{14}OS$	C,H,S	144–145			0.045 (0.039–0.050)	96	38	<10
54	Me	Me	2P	$C_{15}H_{15}NO$	C,H,N	163–164	82	29	<1			2.5[d] (L–5.3)
55	Me	Me	4P	$C_{15}H_{15}NO$	C,H,N	208–209	51	1[f]	~1	17	0	>10
56	Me	Me	1-naphthyl	$C_{20}H_{18}O$	C,H	131–132	94	27	<1			0.59 (0.043–1.1)
57	Me	Me	2-naphthyl	$C_{20}H_{18}O$	C,H	166–168	61	16[f]	<1	66	37	<10
58	Me	Me	3TN	$C_{18}H_{16}OS$	C,H,S	153–154	87	13	<1			0.4 (0.23–0.7)

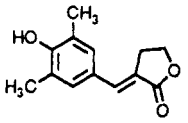
[a] 3P = 3-pyridyl, 2T = 2-thienyl, 3TN = 3-thianaphthenyl. [b] In examples where an IC_{50} value is given, $n = 2$ or more. IC_{50} values were determined by using a method of curve fitting by means of nonlinear regression analysis. A Hill type equation (the sigmoid E_{max} model) served as the model for analysis.[27,28] Numbers in parentheses are 95% confidence limits, L indicates lower limit is 0 or a negative number. I_{max} values were in the range of 90–110% except where noted (*d*). If $n = 1$ the IC_{50} is only estimated (indicated by <, >, or ~) based upon the percent inhibition which is shown for 1 and 0.1 μM (LO) or 10 and 1 μM (CO). Percent inhibition is statistically significant compared to that of the control by analysis of variance with Tukey's multiple comparisons test, $p < 0.05$ except where noted (*f*). IC_{50} values for standard compounds: LO-BW 755C, 16 μM (10–22); CO-indomethacin, 0.0026 μM (0.0023–0.0030). These IC_{50} values and those for **4** vary slightly from values reported in ref 9 due to the use of nonlinear (as opposed to linear) regression analysis in this manuscript. [c] Reported previously in ref 9. [d] I_{max} 70–89% due to solubility limitation of test compound. [e] Reported previously in ref 9, and the synthesis was also reported previously in ref 29. [f] Percent inhibition not statistically different from control. [g] C: calcd, 81.25; found, 80.60. [h] Cl: calcd, 14.14; found, 12.97. [i] Cl: calcd, 13.70; found, 13.25. [j] C: calcd, 76.10; found, 75.63.

a weaker 5-LO inhibitor than dimethyl analogue **14**.

The activities resulting from further modification of the aryl substituent are illustrated by compounds **33**–**58** (Table I). In these examples the 2,6-dimethyl substituents in the phenol ring are kept constant. Comparing compounds **33** and **34** with **24** shows that increasing the size of the 4-alkyl

Table II. Modification of Olefin Connecting Chain



no.	X	Ar[a]	formula	anal.	mp, C	% inhibn 1 μM	% inhibn 0.1 μM	LO[b] IC_{50}, μM	% inhibn 10 μM	% inhibn 1 μM	CO[b] IC_{50}, μM
1			$C_{19}H_{24}O_2S$	C,H,S	129–130[c]			4.8 (0.78–8.8)			0.0057 (0.0041–0.0074)
59	C(O)	2T	$C_{13}H_{12}O_2S$	C,H,S	95–96	1[d]		>1	90	60	<1
2			$C_{19}H_{26}O_3$	C,H	149–150[e]			1.1 (L–6.6)			0.11 (0.016–0.20)
60			$C_{13}H_{14}O_3$	C,H	180–181[f]	1[d]		>1	0	0	>10
61	—CH=C(Me)—	4-FC_6H_4	$C_{17}H_{17}FO$	C,H,F	88–90			0.43 (0.32–0.53)			0.63 (0.43–0.82)
62	—CH=C(n-Bu)—	4-FC_6H_4	$C_{20}H_{23}FO$	C,H,F	85–86	93	17	<1	86	52	~1
63	—CH_2CH_2—	2T	$C_{14}H_{16}OS$	C,H,S[g]	62–64	67	11[d]	<1			0.11[h] (0.077–0.16)
64	—CH_2CH_2—	4-FC_6H_4	$C_{16}H_{17}FO$	C,H,F	90–91	44		>1			0.3 (0.094–0.51)
65	—CH=CHC(O)—	2T	$C_{15}H_{14}O_2S$	C,H,S	162–163	22		>1	82	37	<10
66	—CH=CHC(O)—	4-FC_6H_4	$C_{17}H_{15}FO_2$	C,H,F	170–172	33		>1	71	43	<10
67	—$CH_2CH_2C(O)$—	4-FC_6H_4	$C_{17}H_{17}FO_2$	C,H,F	90–92	44		>1	80	29	<10
68	—C(O)CH=CH—	2T	$C_{15}H_{14}O_2S$	C,H,S	124.5–126.5	0		>1	82	25	<10
69	—CH_2—	3P	$C_{14}H_{15}NO$	C,H,N	159–161	5		>1	7	0	>10

[a] 2T = 2-thienyl, 3P = 3-pyridyl. [b] See note *b*, Table I. [c] Literature[6] mp 127–130 °C. [d] Percent inhibition not statistically different from that of the control. [e] Literature[24] mp 155–156 °C. [f] Literature[24] mp 180–182 °C. [g] C: calcd, 72.36; found, 73.05. [h] I_{max} 70–89% due to solubility limitation of test compound.

group on the phenyl substituent results in loss of potency and selectivity of 5-LO inhibition. The same trend is also seen upon increasing the size of 4-alkyloxy groups (compounds **21**, **35**, and **36**). Compounds **37–47** illustrate the variety of substituents tolerated at the 2-, 3-, or 4-position of the phenyl ring. While all compounds have IC_{50} values in the 0.1–1.0 μM range for 5-LO, selectivity among the compounds varies. A bulky group in the 2-position also leads to reduced 5-LO inhibitory potency (**42** vs **43**). A carboxylic acid substituent in the 2-, 3-, or 4-position destroys both 5-LO and CO inhibitory activity (**48–50**).

Other aryl substituents are shown in compounds **51–57**. Substitution on the thiophene ring with chlorine (**51**) or methyl (**52**) results in diminished 5-LO inhibitory activity, while CO inhibition was unchanged compared to the unsubstituted thiophene analogue (**14**). 2-Pyridyl isomer **54** had activity similar to that of 3-isomer **10**, while 4-isomer **55** appeared to be weaker. 1-Naphthyl (**56**), 2-naphthyl (**57**), and 3-thianaphthenyl (**58**) aryl substituents yield compounds with little selectivity.

The effects of modifying the olefin that connects the phenol and aryl rings are illustrated in Table II. As the examples show, the unsubstituted olefin provides the most potent and selective 5-LO inhibitors. The enhancement in 5-LO inhibitory activity observed between **4** and **10** is not seen following analogous modification of the alkyl groups of **1** or **2** (see **59** and **60**). This is in agreement with structure–activity work published for **2**[24] and compounds related to **1**.[25]

The effect of alkyl substitution on the olefin is illustrated by compounds **61** and **62**. 5-LO inhibitory potency and selectivity are reduced compared to the unsubstituted compound **17**. Other modifications or replacements of the olefin result in a more drastic loss of activity. Analogues with ethyl chains (**63** and **64**) are on the order of 10-fold less potent 5-LO inhibitors as the corresponding olefins. CO inhibition, however, appears relatively unchanged. Other connecting chains (**65–69**) produced compounds with less than 50% 5-LO inhibition at 1 μM.

Alkylation of the phenol results in a loss of in vitro activity. Compounds **70–72** (Scheme III) showed no significant inhibition of LO or CO at 1 μM. This is consistent with the requirement of the phenol for 5-LO inhibition with compounds related to **1**[25] and for CO inhibition with analogues of **2**.[24] The phenol requirement in all of these

Table III. Inhibition of Antigen-Induced Leukotriene-Mediated Bronchoconstriction and Cell Influx in Conscious Guinea Pigs

no.	dose, mg/kg	route	% inhibn of bronchoconstriction[a]	% inhibn of cell influx: total cells	% inhibn of cell influx: PMN
4	10	po		0	0
4	30	po	−24[b]		
4	100	po	−2.0 ± 24.2 (n = 4)	0	0
4	300	po	7.7 ± 4.2 (n = 2)		
4	100	ip	10	0	0
14	10	ip	34.5 ± 7.5 (n = 2)		
14	30	ip	70.3 ± 13.6 (n = 3)	78	95
17	30	ip	43		
21	30	ip	28		
24	30	ip	59		
25	30	ip	41		
26	30	ip	51		
27	30	ip	57	77	54
39	30	ip	49		
53	30	ip	26		

[a] Percent inhibition of the decrease in Cdyn seen in antigen challenged animals. Five to 10 animals were used for each dose level; n = 1 unless stated, in which case the percent inhibition is an average ± SEM. Results were analyzed using Donnet's T test. Greater than 40% inhibition was typically required for significance; $p < 0.05$. [b] A negative number indicates increased bronchoconstriction.

(24) Katsumi, I.; Kondo, H.; Yamashita, K.; Hidaka, T.; Hosoe, K.; Yamashita, T.; Watanabe, K. *Chem. Pharm. Bull.* **1986**, *34*, 121.

(25) Swingle, K. F.; Bell, R. L.; Moore, G. G. I.; *Anti-Inflammatory and Antirheumatic Drugs*; Rainsford, K. D., Ed.; CRC: Boca Raton, FL, 1985; Vol. III, Chapter 4.

cases suggests enzyme inhibition occurs by a redox-based mechanism of action.

The series of compounds described in Table I evolved from the antiinflammatory dual CO/5-LO inhibitor 4, which was inactive in our models used to detect inhibition of leukotriene production in vivo. These models include inhibition of antigen-induced leukotriene-mediated bronchoconstriction and cell influx in guinea pigs. As seen in Table III, compound 4 was inactive in these models at doses up to 100 mg/kg ip or 300 mg/kg po.

We have shown that by altering the structure of 4 the in vitro profile can be markedly altered from a more potent CO inhibitor to a more potent and selective inhibitor of 5-LO. This change is also reflected in vivo, as shown in Table III. Several representatives from Table I inhibit antigen-induced leukotriene-mediated bronchoconstriction in the guinea pig at 30 mg/kg ip. Compounds 14 and 27 were also found to be active against leukotriene B_4-mediated cell influx. Compound 14 was tested orally in the bronchoconstriction model but gave variable results. This is presumably due to poor absorption or extensive first-pass metabolism.

Experimental Section

Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. ^1H NMR were all consistent with molecular structures and were recorded on a Bruker 250 WM spectrometer. Elemental analyses were performed at Micro-Tech Laboratories, Inc., Skokie, IL, and were within 0.4% of the calculated values unless otherwise indicated.

(*E*)-2,6-Dimethyl-4-[2-(2-thienyl)ethenyl]phenol (14). A stirred mixture of 30 g of 3,5-dimethyl-4-hydroxybenzaldehyde (0.2 mol), 34 g of 2-thiopheneacetic acid (0.239 mol), and 43 g of piperidine (0.505 mol) in 75 mL of CH_2Cl_2 was heated gradually to 130 °C on an oil bath. After 7 h, the mixture was cooled to room temperature and eluted through a silica gel column with CH_2Cl_2. The fractions containing product were concentrated, and the resulting solid was recrystallized from toluene, giving 21.5 of 14 (0.093 mol, 46.5%): mp 132–133 °C; NMR ($CDCl_3$) δ 7.1 (m, 6 H, aromatic plus one olefin), 6.8 (d, 1 H, J = 16.2 Hz, olefin), 4.7 (s, 1 H, OH), 2.25 (s, 6 H, CH_3). Anal. ($C_{14}H_{14}OS$) C, H, S.

2,6-Dimethyl-4-(2-thenoyl)phenol (59). Thiophenecarbonyl chloride (11 g, 75 mmol) in 40 mL of CH_2Cl_2 was added dropwise, under N_2, to a suspension of $AlCl_3$ (10 g, 75 mmol) in 75 mL of CH_2Cl_2, cooled on ice. After stirring 30 min, 2,6-dimethylphenol (8.1 g, 66.2 mmol) in 40 mL of CH_2Cl_2 was added dropwise. The reaction was stirred for 4.5 h at room temperature and then poured into 400 mL of cold 5 N HCl. The organic phase was separated and the aqueous phase was extracted with 100 mL of CH_2Cl_2. The combined organic extracts were washed with 2 N HCl (200 mL) and H_2O (200 mL), dried (Na_2SO_4), and concentrated.

The residue was refluxed with 75 mL of MeOH and 50 mL of 2 N NaOH to hydrolyze that portion of the product with acylated phenol. After 3 h the reaction mixture was concentrated to remove the MeOH, and acidified with 2 N HCl. The product was extracted into EtOAc (2 × 100 mL), and the EtOAc extracts were washed with saturated solutions of $NaHCO_3$ (1 × 100 mL) and NaCl solution (1 × 50 mL), dried (Na_2SO_4), and concentrated. The product was recrystallized from EtOH, giving 4.77 g of 59 (20.5 mmol, 31%): mp 95–96 °C; NMR ($CDCl_3$) δ 7.7 (m, 2 H, thiophene), 7.6 (s, 2 H, Ph), 7.15 (m, 1 H, thiophene), 5.7 (s, 1 H, OH), 2.3 (s, 6 H, CH_3). Anal. ($C_{13}H_{12}O_2S$) C, H, N.

[1-(4-Fluorophenyl)ethyl]triphenylphosphonium Bromide (74). A solution of 27.4 g of 4-fluoroacetophenone (0.198 mol) in 150 mL of 2-PrOH was added dropwise to a suspension of 5.6 g of $NaBH_4$ (0.2 mol) in 150 mL of 2-PrOH. The reaction was stirred at room temperature for 3.5 h and then poured slowly onto 1000 mL of ice-cold 2 N HCl. The product was extracted into CH_2Cl_2 (1 × 300 mL, 1 × 200 mL). The organic extracts were washed with saturated NaCl solution (100 mL), dried (Na_2SO_4), and concentrated to give 25.5 g of 73 as an oil (0.182 mol, 92%): NMR ($CDCl_3$) δ 7.1 (m, 4 H, Ph), 4.7 (q, 1 H, CH), 3.1 (s, 1 H, OH), 1.3 (d, 3 H, CH_3). The product was used directly in the next step without further purification.

Triphenylphosphine (47.7 g, 0.181 mol), 25.5 g of 73, and 49 g of 30% HBr in HOAc (14.7 g of HBr, 0.182 mol) were combined in 36 mL of HOAc and heated on an oil bath at 80–90 °C for 21.5 h. The reaction mixture was poured into 600 mL of ether and stirred for 1 h. The ether was decanted and replaced with 600 mL of fresh ether. The product was cooled on ice and triturated. This process was repeated until the product became a white powder. The product was filtered, rinsed with ether, and dried, giving 77.1 g of 74 (0.166 mol, 91.5%): mp 238–239 °C; NMR (DMSO-d_6) δ 7.95 (m, 3 H, Ph), 7.7 (m, 12 H, Ph), 7.2 (m, 2 H, Ph), 7.0 (m, 2 H, Ph), 5.8 (q, 1 H, CH), 1.7 (m, 3 H, CH_3). Anal. ($C_{26}H_{23}BrFP$) C, H, Br, F, P.

2,6-Dimethyl-4-formylphenyl Ethyl Carbonate (75). A solution of 17.9 g of ethyl chloroformate (0.165 mol) in 50 mL of CH_2Cl_2 was added dropwise to a solution of 22.5 g of 2,6-dimethyl-4-hydroxybenzaldehyde (0.15 mol) and 16.7 g of Et_3N (0.165 mol) in 450 mL of CH_2Cl_2. The reaction mixture was refluxed for 3.5 h, washed with H_2O (2 × 200 mL) and saturated NaCl solution (100 mL), dried (Na_2SO_4), and concentrated. The product was recrystallized from EtOH, giving 24.9 g (0.112 mol, 75%) of 75: mp 45–47 °C; NMR ($CDCl_3$) δ 9.9 (s, 1 H, CHO), 7.6 (s, 2 H, Ph), 4.45 (q, 2 H, CH_2), 2.3 (s, 6 H, CH_3), 1.45 (t, 3 H, CH_3). Anal. ($C_{12}H_{14}O_4$) C, H.

2,6-Dimethyl-4-[2-(4-fluorophenyl)-2-methylethenyl]-phenyl Ethyl Carbonate (76). A solution of 32.5 mL of 1.6 N *n*-BuLi in hexane (52 mmol) was added to a suspension of 23.3 g of 74 (50 mmol) in 500 mL of anhydrous ether, under N_2, at room temperature. After 3 h, a solution of 10.7 g of 75 (48 mmol) in 100 mL of anhydrous ether was added dropwise. The reaction was stirred for 24 h, filtered through Celite, and concentrated. The resulting solid was recrystallized from EtOH giving 7.8 g of 76 (24 mmol, 50%): mp 90–91 °C; NMR (DMSO-d_6) δ 7.6 (m, 2 H, Ph), 7.2 (m, 2 H, Ph), 6.8 (s, 1 H, olefin), 4.3 (q, 2 H, CH_2), 2.2 (s, 3 H, CH_3), 2.1 (s, 6 H, CH_3), 1.3 (t, 3 H, CH_3). Anal. ($C_{20}H_{21}FO_3$) C, H, N.

2,6-Dimethyl-4-[2-(4-fluorophenyl)-2-methylethenyl]-phenol (61). A mixture of 76 (7.65 g, 23.3 mmol) in 150 mL of EtOH and 50 mL of 2 N NaOH was refluxed for 5 h. The reaction mixture was cooled, poured into ice and water (400 mL), and acidified with 2 N HCl. The resulting solid was filtered, rinsed with H_2O, and recrystallized from EtOH, giving 2.66 g of 61 (10.4 mmol 45%): mp 88–90 °C; NMR ($CDCl_3$) δ 7.5 (m, 2 H, Ph), 7.0 (m, 4 H, Ph), 6.65 (s, 1 H, olefin), 4.65 (s, 1 H, OH), 2.3 (s, 6 H, CH_3), 2.25 (s, 3 H, CH_3). Anal. ($C_{17}H_{17}FO$) C, H, F.

2,6-Dimethyl-4-[2-(2-thienyl)ethyl]phenol (63). Tris(triphenylphosphine) rhodium chloride (0.6 g) was combined with 14 (3 g, 13 mmol) in 75 mL of EtOH and hydrogenated at an initial pressure of 50 psi. After 67 h the mixture was filtered; the filtrate was concentrated and eluted through a silica gel column with CH_2Cl_2. The product coeluted with some remaining starting material. This mixture (2.6 g) was recrystallized from EtOH–H_2O. Two crops containing most of the starting material were obtained. The filtrate was concentrated to give 1.1 g, which was recrystallized from ligroine. The second crop yielded 0.11 g (0.4 mmol, 3.6%) of 63: mp 62–64 °C; NMR ($CDCl_3$) δ 7.1 (d, 1 H, thiophene), 6.95 (t, 1 H, thiophene), 6.85 (s, 2 H, Ph), 6.8 (m, 1 H, thiophene), 4.45 (s, 1 H, OH), 3.05 (m, 2 H, CH_2), 2.85 (m, 2 H, CH_2), 2.2 (s, 3 H, CH_3). Anal. ($C_{14}H_{16}OS$) C, H, S.

2,6-Dimethyl-4-[2-(4-fluorophenyl)ethyl]phenol (64). A solution of 1.5 g of 17 (6.2 mmol) in 75 mL of EtOH was combined with 300 mg of 10% Pd/C (1:1 w/w H_2O) and hydrogenated at an initial pressure of 34 psi. After 1.5 h, hydrogen uptake had stopped. The reaction mixture was filtered through Celite and concentrated. The residue was recrystallized from EtOH–H_2O, giving 0.76 g of 64 (3.1 mmol, 50%): mp 90–91 °C; NMR ($CDCl_3$) δ 7.1 (m, 2 H, Ph), 7.0 (m, 2 H, Ph), 6.76 (s, 2 H, Ph), 4.5 (s, 1 H, OH), 2.8 (m, 4 H, CH_2), 2.25 (s, 6 H, CH_3). Anal. ($C_{16}H_{17}FO$) C, H, F.

(*E*)-3-(3,5-Dimethyl-4-hydroxyphenyl)-1-(2-thienyl)-2-propen-1-one (65). 3,5-Dimethyl-4-hydroxybenzaldehyde (4.0 g, 26.6 mmol) and 2-acetylthiophene (3.78 g, 30 mmol) were combined in 200 mL of EtOH saturated with HCl and stirred at room temperature for 18 h. The mixture was poured onto 600 mL of ice and water. The precipitated product was filtrated and recrystallized from EtOH, giving 5.02 g of 65 (19.4 mmol, 73%): mp 162–163 °C; NMR (DMSO-d_6) δ 9.0 (br s, 1 H, OH), 8.3 (d,

1 H, thiophene), 8.05 (d, 1 H, thiophene), 7.65 (d, 1 H, J = 15.8 Hz, olefin), 7.6 (d, 1 H, thiophene), 7.5 (2, 2 H, phenyl), 7.3 (d, 1 H, J = 15.8 Hz, olefin), 2.25 (s, 6 H, CH_3). Anal. ($C_{15}H_{14}O_2S$) C, H, S.

3-(3,5-Dimethyl-4-hydroxyphenyl)-1-(4-fluorophenyl)-propan-1-one (67). A solution of 3 g of **66** (11.1 mmol) in 110 mL of EtOH was combined with 370 mg of 10% Pd/C (1:1 w/w H_2O) and hydrogenated at an initial pressure of 40 psi. After 2 h the reaction mixture was filtered through Celite and concentrated, giving a yellow oil. The crude product was eluted through a silica gel column, giving 1.9 g of yellow solid. After recrystallization from EtOH–H_2O, 0.65 g of **67** (2.4 mmol, 21.5%) was obtained as a white solid: mp 90–92 °C: NMR ($CDCl_3$) δ 7.95 (m, 2 H, Ph), 7.1 (m, 2 H, Ph), 6.85 (s, 2 H, Ph), 5.6 (s, 1 H, OH), 3.2 (m, 2 H, CH_2), 2.9 (m, 2 H, CH_2), 2.3 (s, 6 H, CH_3).

2,6-Dimethyl-4-[2-(2-thienyl)ethenyl]anisole (70). A mixture of 1 g of **14** (4.3 mmol), 3.2 g of CH_3I (22.7 mmol), and 4.2 g of K_2CO_3 (30.4 mmol) in 25 mL of acetone was stirred at room temperature for 19 h. The solids were filtered off and rinsed with acetone. The filtrates were concentrated, and the residue was partitioned between EtOAc (100 mL) and H_2O (50 mL). The organic phase was washed with saturated NaCl sslution (50 mL), dried (Na_2SO_4), and concentrated. The residue was recrystallized from EtOH, giving 0.63 g of **70** (2.6 mmol, 60%): mp 47–48 °C; NMR ($CDCl_3$) δ 7.15 (m, 1 H, thiophene), 7.1 (s, 2 H, Ph), 7.0 (m, 1 H, thiophene), 6.8 (d, 1 H, thiophene), 3.7 (s, 3 H, CH_3), 2.25 (s, 3 H, CH_3). Anal. ($C_{15}H_{16}OS$) C, H, S.

2,6-Dimethyl-4-[2-(2-thienyl)ethenyl]phenoxyacetic Acid (71). A mixture of 0.9 g of **14** (3.9 mmol), 1.38 g of K_2CO_3 (10 mmol), and 0.7 g of ethyl bromoacetate (4.2 mmol) in 25 mL of acetone was refluxed for 2 h, then stirred at room temperature for 17 h. The reaction mixture was filtered, and the filtrate was concentrated. The resulting oil was combined with 15 mL of MeOH and 10 mL of 2 N NaOH and refluxed for 4 h. The reaction mixture was poured into 150 mL of ice and water, acidified with 2 N HCl, and extracted with CH_2Cl_2 (1 × 100 mL, 1 × 50 mL). The organic extracts were washed with saturated NaCl solution (50 mL), dried (Na_2SO_4), and concentrated. The resulting solid was recrystallized from 2-PrOH, giving 0.7 g of **71** (2.4 mmol, 62%): mp 173.5–175.5 °C; NMR ($CDCl_3$) δ 10.7 (br s, 1 H, COOH), 7.2 (d, 1 H, olefin), 7.1 (m, 5 H, aromatic), 6.8 (d, 1 H, olefin), 5.5 (s, 2 H, CH_2), 2.3 (s, 6 H, CH_3). Anal. ($C_{16}H_{16}O_3S$) C, H, S.

2-[2,6-Dimethyl-4-[2-(2-thienyl)ethenyl]phenoxy]ethanol (72). A mixture of 0.8 g of **14** (3.5 mmol), 0.48 g of 2-bromoethanol (3.8 mmol), and 1.38 g of K_2CO_3 (10 mmol) in 25 mL of acetone was refluxed for 12 h. During the course of the reaction, additional 2-bromoethanol and K_2CO_3 were added at 2-h intervals. The reaction mixture was filtered and concentrated; the residue was partitioned between EtOAc and H_2O and then dried (Na_2SO_4) and concentrated. The residue was eluted through a silica gel column with CH_2Cl_2–MeOH (97.5:2.5). This removed the starting material (higher R_f). The impure product was passed through a second column, eluting with CH_2Cl_2–MeOH (99:1). Fractions containing pure product were combined and concentrated. The resulting oil crystallized from EtOH–H_2O, giving 40 mg of **72** (0.15 mmol, 4%): mp 65–67 °C; NMR ($CDCl_3$) δ 7.2 (d, 1 H, olefin), 7.1 (m, 5 H, aromatic), 6.8 (d, 1 H, olefin), 3.95 (m, 4 H, CH_2), 2.3 (s, 6 H, CH_3), 2.2 (t, 1 H, OH). Anal. ($C_{16}H_{18}O_2S$) C, H, N.

Biological Methods. Cyclooxygenase-Inhibition Assay. Cyclooxygenase inhibition was determined with human platelets as described in ref 9.

5-Lipoxygenase-Inhibition Assay. 5-Lipoxygenase inhibition was determined with human peripheral blood leukocytes as described as ref 9.

Inhibition of Antigen-Induced, Leukotriene-Mediated Bronchoconstriction. Male outbred Hartley strain albino guinea pigs (250–300 g) were actively sensitized (ovalbumin), fasted 18 h, and anesthetized. A catheter-tip pressure transducer was inserted into the pleural space of each animal. The guinea pigs were pretreated 60 min before antigen challenge with pyrilamine (10 mg/kg ip), indomethacin (10 mg/kg ip), and test compound. All compounds administered ip were given as a suspension in 5% Tween 80.

The guinea pigs were placed in a "head out" plethysmograph and allowed to recover from anesthesia. Pleural pressure and airflow were measured, and tidal volume, dynamic pulmonary compliance (C_{dyn}), and breathing frequency were calculated according to a published technique.[26] After base-line values for pulmonary function were obtained, the animals were challenged by aerosolized ovalbumin. The overall changes in pulmonary function parameters were determined by calculating the mean changes from base line during the period 5–15 min following antigen challenge. Drug efficacy was determined by comparing the mean of the percent decrease in C_{dyn} from the base line in drug-treated animals to that of control animals (N = 10–12).

Inhibition of Antigen-Induced, Late-Phase Inflammatory Cell Influx. Male outbred Hartley strain albino guinea pigs (250–300 g) were sensitized and pretreated as described in the previous section. Four hours after challenge with aerosolized ovalbumin, the animals were anesthetized and then exsanguinated by severing of the abdominal artery and inferior vena cava. Whole lung lavage was performed with three 5-mL aliquots of normal saline buffered with sodium bicarbonate.

Total cell counts were performed on a Coulter counter, and differential cell counts were performed. Compounds were evaluated for their ability to alter the antigen-induced total cell influx and, more specifically, neutrophil (PMN) influx.

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Registry No. **1**, 69425-13-4; **2**, 83677-24-1; **4**, 116376-62-6; **8**, 116376-77-3; **8** (aldehyde), 10537-86-7; **9**, 127035-55-6; **9** (aldehyde), 69574-07-8; **10**, 127035-56-7; **10** (aldehyde), 2233-18-3; **11**, 116405-78-8; **11** (aldehyde), 1620-98-0; **12**, 116376-78-4; **13**, 127035-57-8; **14**, 127035-58-9; **15**, 116376-70-6; **16**, 127035-59-0; **17**, 127035-60-3; **18**, 116376-66-0; **19**, 127035-61-4; **20**, 127035-62-5; **21**, 127035-63-6; **22**, 116376-65-9; **23**, 127035-64-7; **24**, 127035-65-8; **25**, 127035-66-9; **25** (aldehyde), 32263-14-2; **26**, 127035-67-0; **26** (aldehyde), 125722-35-2; **27**, 127035-68-1; **27** (aldehyde), 25006-17-1; **28**, 127063-62-1; **28** (aldehyde), 107356-10-5; **29**, 127035-69-2; **29** (aldehyde), 127036-07-1; **30**, 127035-70-5; **30** (aldehyde), 127036-08-2; **31**, 127035-71-6; **31** (aldehyde), 79418-78-3; **32**, 127035-72-7; **32** (aldehyde), 134-96-3; **33**, 127035-73-8; **34**, 127035-74-9; **35**, 127035-75-0; **36**, 127035-76-1; **37**, 122917-30-0; **38**, 127063-63-2; **39**, 127035-77-2; **40**, 127035-78-3; **41**, 127035-79-4; **42**, 127035-80-7; **43**, 127035-63-6; **44**, 127035-81-8; **45**, 127035-82-9; **46**, 127035-83-0; **47**, 127035-84-1; **48**, 127035-85-2; **49**, 127035-86-3; **50**, 127035-87-4; **51**, 127035-88-5; **52**, 127035-89-6; **53**, 127035-90-9; **54**, 127063-64-3; **55**, 127035-91-0; **56**, 127035-92-1; **57**, 127035-93-2; **58**, 127035-94-3; **59**, 66203-38-1; **60**, 87411-49-2; **61**, 127063-65-4; **62**, 127035-95-4; **63**, 127035-96-5; **64**, 127035-97-6; **65**, 127035-98-7; **66**, 127063-66-5; **67**, 127035-99-8; **68**, 127036-00-4; **69**, 127036-01-5; **70**, 127036-02-6; **71**, 127036-03-7; **72**, 127036-04-8; **73**, 403-41-8; **74**, 127036-05-9; **75**, 127036-06-0; **76**, 127063-67-6; 2TC(O)Cl, 5271-67-0; 4-$FC_6H_4C(O)Bu$, 64436-59-5; 3P-CH_2CO_2H, 501-81-5; 2T-CH_2CO_2H, 1918-77-0; 4-$FC_6H_4CH_2CO_2H$, 405-50-5; 4-$MeOC_6H_4CH_2CO_2H$, 104-01-8; 4-$MeC_6H_4CH_2CO_2H$, 622-47-9; 4-$EtC_6H_4CH_2CO_2H$, 14387-10-1; 4-*i*-$PrC_6H_4CH_2CO_2H$, 4476-28-2; 4-$PrOC_6H_4CH_2CO_2H$, 26118-57-0; 4-$BuOC_6H_4CH_2CO_2H$, 4547-57-3; $C_6H_5CH_2CO_2H$, 103-82-2; 4-$ClC_6H_4CH_2CO_2H$, 1878-66-6; 3-$MeC_6H_4CH_2CO_2H$, 621-36-3; 2-$BrC_6H_4CH_2CO_2H$, 18698-97-0; 2-$CF_3C_6H_4CH_2CO_2H$, 3038-48-0; 2-$BzOC_6H_4CH_2CO_2H$, 22047-88-7; 2-$MeOC_6H_4CH_2CO_2H$, 93-25-4; 2,4-$F_2C_6H_3CH_2CO_2H$, 81228-09-3; 3,5-$F_2C_6H_3CH_2CO_2H$, 105184-38-1; 2,6-$F_2C_6H_3CH_2CO_2H$, 85068-28-6; 2,5-$Me_2C_6H_3CH_2CO_2H$, 13612-34-5; 2-$CO_2HC_6H_4CH_2CO_2H$, 89-51-0; 3-$CO_2HC_6H_4CH_2CO_2H$, 2084-13-1; 4-

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Synthesis and Ca^{2+} Antagonistic Activity of 2-[2-[(Aminoalkyl)oxy]-5-methoxyphenyl]-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzothiazines

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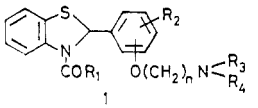
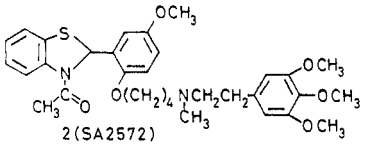
As an extension of the previous investigation (*J. Med. Chem.* **1988**, *31*, 919), we synthesized a series of 2-[2-[(aminoalkyl)oxy]-5-methoxyphenyl]-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzothiazines (**3**) and evaluated their Ca^{2+} antagonistic activities. Ca^{2+} antagonistic activity was measured with isolated depolarized guinea pig taenia cecum. On the basis of their potent Ca^{2+} antagonistic activity, six benzothiazines were selected and further evaluated for their vasocardioselectivity. Among these six compounds, the key compound **15** [3,4-dihydro-2-[5-methoxy-2-[3-[*N*-methyl-*N*-[2-[3,4-(methylenedioxy)phenoxy]ethyl]amino]propoxy]phenyl]-4-methyl-3-oxo-2*H*-1,4-benzothiazine hydrogen fumarate] was recognized as having the lowest cardioselectivity. Following optical resolution, the absolute configuration of the compound's optically active enantiomer was determined by means of X-ray crystallography of a synthetic precursor (+)-**4a**. The Ca^{2+} antagonistic activity of **15** was found to reside primarily in (+)-**15** (which was about 7 times more potent than (−)-**15**). The in vitro study showed that (+)-**15** had a low cardioselectivity compared to verapamil and diltiazem. This result suggests that (+)-**15** would exhibit less adverse effects due to cardiac inhibition than diltiazem and verapamil in therapeutic use.

Ca^{2+} antagonists, useful in the treatment of angina pectoris, hypertension, and certain cardiac arrhythmias, are classified structurally into two large groups: the non-dihydropyridines, represented by verapamil and diltiazem, and the dihydropyridines, represented by nifedipine and nicardipine.[1-3] We were interested in both the chemical structures and therapeutic benefit of the former. However, in some patients with impaired ventricular function and/or those undergoing β-adrenergic blocker therapy, non-dihydropyridine type Ca^{2+} antagonists caused several adverse effects (cardiac failure, bradycardia, and/or asystole) due to myocardial suppression and conduction disturbances.[4-7] Accordingly, we anticipate that a novel non-dihydropyridine type Ca^{2+} antagonist having weak cardiac suppression would be safer than conventional ones such as verapamil and diltiazem in clinical use.

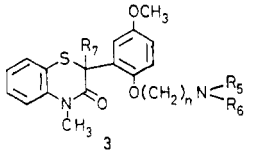
In our previous paper,[8] on our study of the structure–activity relationship of benzothiazoline derivatives **1**, we reported that 3-acetyl-2-[5-methoxy-2-[4-[*N*-methyl-*N*-(3,4,5-trimethoxyphenethyl)amino]butoxy]phenyl]benzothiazoline (**2**: SA2572) was a potent Ca^{2+} antagonist, possessing the same level of activity as diltiazem. Here, we report further developments in our research on new Ca^{2+} antagonists. Anticipating a bioisosteric effect, we changed the benzothiazoline nucleus into benzothiazine's and synthesized 2-[2-[(aminoalkyl)oxy]-5-methoxyphenyl]-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzothiazine (**3**).



1



2 (SA2572)



3

Compound **15**, which was selected from the series of synthesized benzothiazines, was a racemic compound possessing an asymmetric center at the 2-position in the benzothiazine ring. Because differences in the biological activity of enantiomers are often recognized,[9] we resolved **15** by fractional crystallization to produce (+)-**15** and (−)-**15**. A biological evaluation of both enantiomers in vitro suggests that the potent Ca^{2+} antagonistic activity of **15** residues stereoselectively in (+)-**15**.

Chemistry. In the previous publication,[10] we reported the synthetic method for 3,4-dihydro-2-(2-hydroxy-5-methoxyphenyl)-4-methyl-3-oxo-2*H*-1,4-benzothiazines

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