determined as described above. Eight mice were used for each dose group.

Chronic Gastrointestinal Erosive Activity. This assay was effected as described by Rooks et al.[15,16] Thus, male rats weighing 190-220 g (Cox/SD obtained from Laboratory Supply Co., Indianapolis, IN) were acclimated for ca. 1 week. The animals (groups of 5 rats/dose) were given the test material po daily in phosphate-buffered saline (1 mL/100 g of body weight) for 7 consecutive days. One day after the last dose, the rats were sacrificed. Body weights were obtained on the first day of dosing and at sacrifice. Food, but not water, was removed from the cages in the last day of dosing. At necropsy, the stomach and small intestine were removed from each rat and examined blindly for lesions, which were scored as follows:

focal		diffuse
0	no gastric changes	
1	minimal, rare	2
3	slight, low, few	4
5	moderate, medium, several	6
7	marked, severe, many	8

In addition, a score of 10 was assigned to those rats that died during the test from complications due to gastrointestinal erosion. The scores for each rat ranged from 0 to 10; the scores for each dosage group ranged from 0 to 50. Arbitrarily, the dose giving a score of 5 (1/rat) was assigned as the minimum effective dose and that dose giving a score of 25 (5/rat) was the median effective erosive dose.

Acknowledgment. We thank Dr. Peter H. Nelson for many useful discussions and Janis Nelson for her help in the NMR studies.

Registry No. **1a** (X = O), 90843-31-5; **1b** (X = S), 7019-66-1; **2a** (X = O), 97483-11-9; **2b** (X = S), 34104-91-1; **3a** (X = O, R = H), 69999-16-2; **3a** (X = O, R = CH_3CH_2), 69999-18-4; **3b** (X = S, R = H), 34104-86-4; **3b** (X = S, R = CH_3CH_2), 104172-39-6; **4a**, 97483-15-3; **4b**, 97483-16-4; **4c**, 97483-14-2; **4c** (ethyl ester), 97483-12-0; **4d**, 104172-31-8; **4e**, 97483-28-8; **5a**, 104172-32-9; **5b**, 97483-27-7; **5c**, 97483-18-6; **5d**, 97483-23-3; **5e**, 97483-19-7; **5f**, 104172-33-0; **5g**, 97483-17-5; **5g** (ethyl ester), 104172-37-4; **5h**, 104172-34-1; (±)-**6a**, 97483-21-1; (±)-**6b**, 97483-22-2; (±)-**6c**, 104172-38-5; (±)-**6d**, 97509-14-3; (±)-**6d** (ethyl ester), 97483-13-1; (±)-**7a**, 97483-24-4; (±)-**7b**, 104172-35-2; (±)-**7c**, 104172-36-3; (±)-**7d**, 97483-25-5; 2,3-dihydrobenzofuran, 496-16-2; 4-(methylthio)benzoyl chloride, 1442-06-4; 4-chlorobenzoyl chloride, 122-01-0.

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Synthesis and Antiallergic Activity of a Novel Series of 5-Lipoxygenase Inhibitors

Kenneth L. Kees,* John H. Musser,* Joseph Chang, Maureen Skowronek, and Alan J. Lewis

Wyeth Laboratories, Inc., Philadelphia, Pennsylvania 19101. *Received March 28, 1986*

A series of novel substituted [[(phenoxymethyl)phenyl]amino]oxoalkanoic acid esters have been synthesized. These compounds were tested in vitro for their ability to inhibit the synthesis of 5-hydroxyeicosatetraenoic acid and leukotriene (LT) B_4 from rat polymorphonuclear leukocytes (PMN) and in vivo as inhibitors ovalbumin- (OA) and LTD_4-induced bronchospasm in the guinea pig. Compounds **5**-**12** and **25** had IC_{50}'s between 1 and 5.6 μM in the rat PMN 5-lipoxygenase assay. Compounds **1**, **3**, and **16** inhibited OA-induced bronchoconstriction (61%, 64%, and 57%, respectively), but only **1** showed activity against LTD_4-induced bronchoconstriction. When tested against LTD_4-induced contraction of isolated guinea pig tracheal spiral strips, **1** was a competitive inhibitor with a pK_B of 4.94.

Slow reacting substance of anaphylaxis (SRS-A) has long been known as an important mediator of anaphylactic and other immediate hypersensitivity reactions.[1] It is now believed that leukotriene (LT) C_4, LTD_4, and LTE_4 account for the biological properties of SRS-A.[2] LTs are derived from arachidonic acid via the 5-lipoxygenase (LO) biosynthetic pathway and are classified as the cysteine-containing LTs (LTC_4, LTD_4, LTE_4, and LTF_4) and the nonpeptidic LTs of which LTB_4 has stimulated the most interest. In vitro LTB_4 is a potent chemotactic agent and aggregating substance for migrating cells and stimulates cell accumulation and increases vascular permeability in vivo.[3] LTC_4 and LTD_4 induce smooth muscle contraction and constriction of small airways, promote secretion of mucus, enhance leakage from postacpillary venules, and cause vasoconstriction and edema formation.[4] The ubiquitous nature of LTs coupled with the variety and potency of their actions justifies the search for agents that block their actions and/or their synthesis.

The first reported antagonist of SRS-A was FPL-55712[5] (Figure 1). Since its discovery in 1973 FPL-55712 has been used extensively to delineate the role of cysteine-containing LTs in allergic responses of animals and man.[6] Over the last 12 years many analogues of FPL-55712 have been prepared for which LT antagonism is claimed.[7,8] With few exceptions, these analogues were obtained through substantial modification of the chromone side of the bisaryl system of FPL-55712 while retaining the hydroxyacetophenone moiety. For example, Wy-44,329 (Figure 1), which inhibits bronchoconstriction in the guinea pig induced by either LTC_4, LTD_4, or ovalbumin (OA), contains the hydroxyacetophenone fragment but employs a trisubstituted benzene ring in place of the chromone ring.[9]

In 1981 Kadin revealed that certain meta-substituted (phenylamino)-4-oxobutanoic acid derivatives (e.g., Pfizer, Figure 1) antagonized the effects of SRS-A,[10] although no

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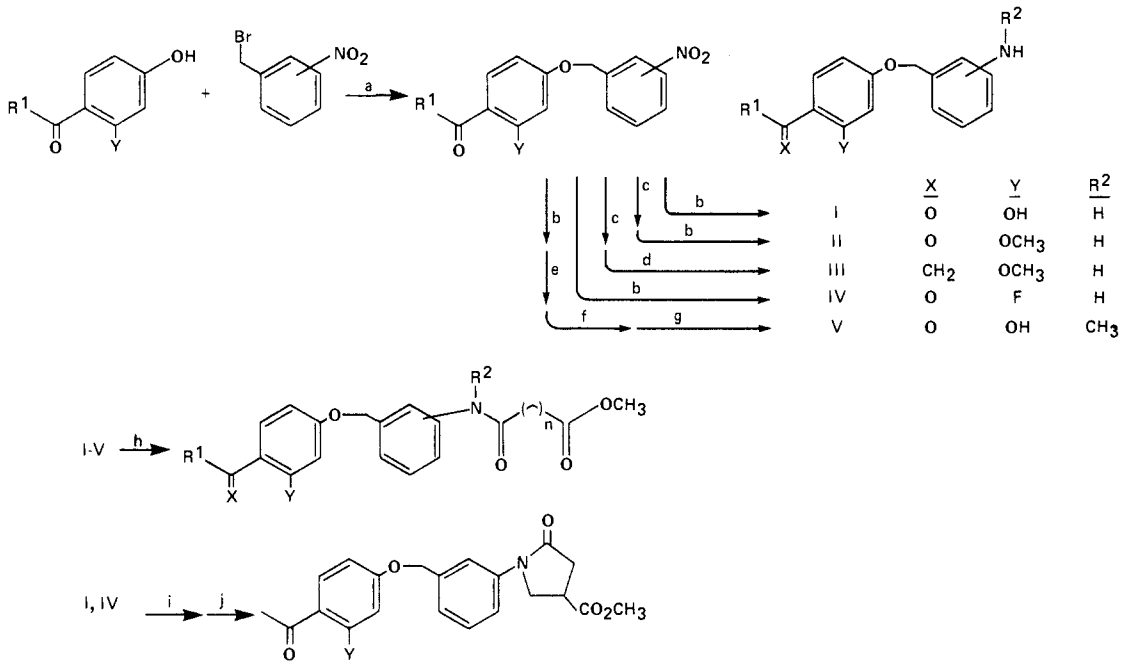
Scheme I



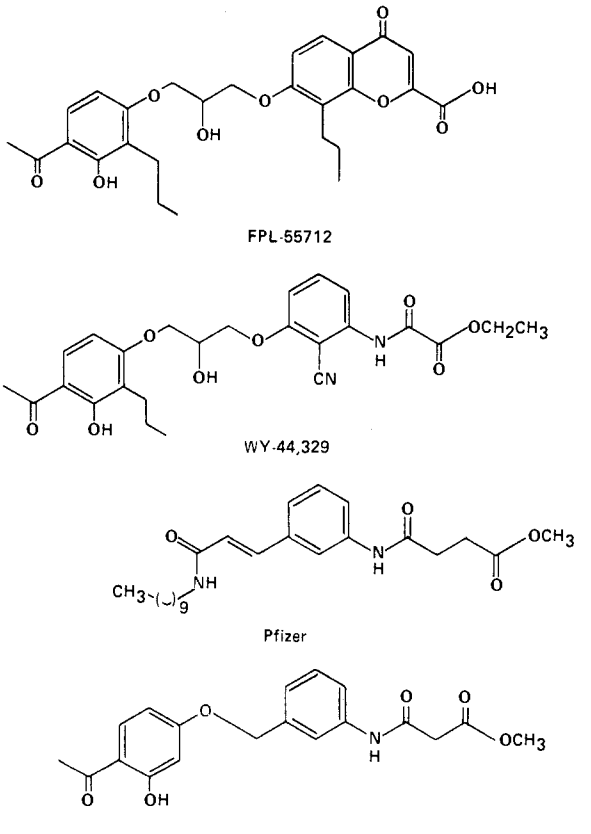
(a) K_2CO_3, Cs_2CO_3 (cat.); (b) Fe/HCl; (c) NaH, MeI; (d) Zn(Hg)/HCl; (e) $(CF_3CO)_2O$; (f) K_2CO_3, MeI; (g) NaOH; (h) n = 1, $(CH_3OC-O)_2CH_2$, 150 °C; n = 0, 2, or 3, $ClCO(CH_2)_nCO_2CH_3$·Et_3N; (i) itaconic acid, fuse; (j) MeOH, PTSA.

biological data were presented. Based on these studies, we have employed the (phenylamino)-4-oxobutanoic acid group as a starting point in the synthesis of LT antagonists of novel structure.[11]

We were also interested in synthesizing compounds that inhibit 5-LO. Agents that inhibit 5-LO and antagonize the effects of LTs have precedent since both FPL-55712 and Wy-44,329 are also 5-LO inhibitors.[12,13] The list of various compounds that inhibit 5-LO is rapidly expanding.[8] Many of these 5-LO inhibitors are related by the presence of a bis-aryl system, a feature also common to many of the known cyclooxygenase inhibitors.[14,15]

We anticipated that a hybrid of the hydroxyacetophenone fragment with the (phenylamino)-ω-oxoalkanoic acid group of Pfizer to form a new bis-aryl system (represented by structure 1, Figure 1) would possess the mixed LT antagonism/5-LO inhibitory profile we sought.

Chemistry. Scheme I illustrates the general synthetic route for most of the compounds in Table I. Alkylation of the appropriate 2,4-dihydroxyphenone with either 2- or 3-nitrobenzyl bromide and potassium carbonate/cesium carbonate mixture in acetone gave the bis-aryl system. This intermediate was either alkylated (sodium hydride, methyl iodide, THF, reflux) and reduced to give anilines II or reduced directly to give anilines I. In either case, reduction of the nitro group was carried out with iron powder in ethanol saturated with anhydrous HCl. Alternatively, anilines of type III were prepared from the methylated intermediates using Clemmenson conditions. The fluorinated bis-aryl anilines (type IV) were prepared analogously from the corresponding 2-fluoro-4-hydroxyacetophenone. The starting material was obtained from acylation of 3-fluorophenol using anhydrous aluminum chloride (1 equiv) in refluxing ethylene dichloride. The N-methylaniline V was prepared by the sequence of trifluoroacetylation, methylation, and hydrolysis. The (phenylamino)-3-oxopropionic acid esters were then ob-



1

Figure 1. Compound structures (see text for additional information).

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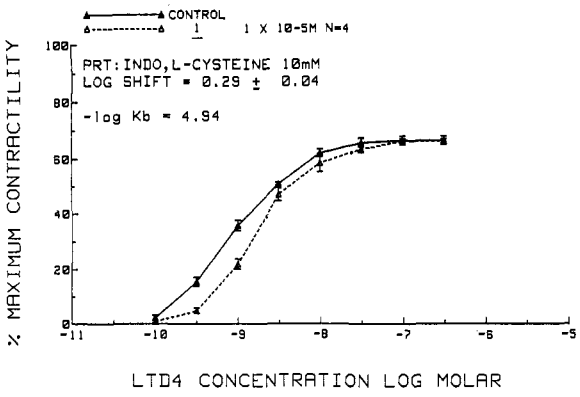

Figure 2. Dose-response curves, for 1 and control, to LTD_4 on isolated guinea pig trachea.

tained by heating the aniline in dimethyl malonate at 150 °C for 2 h followed by removal of excess malonate in vacuo and recrystallization of the product. The remaining amide esters were prepared by treatment of the aniline with the appropriate chloroacylcarboxylate in the presence of triethylamine at low temperature (−78 °C to room termperature).

The 4-carbomethoxypyrrolidinones **24** and **25** were prepared in a one-pot procedure from itaconic acid and the corresponding aniline at 180 °C (1 h neat), followed by dilution with methanol and reflux overnight in the presence of *p*-TSA.

Biological Evaluation and Discussion

The first member of this series (1, Table I) showed promising acitivity both in vitro and in vivo. Compound 1 inhibited both 5-HETE and LTB_4 production from isolated rat polymorphonuclear leukocytes (PMNs) (IC_{50} = 14.20 μM and 11.70 μM, respectively) and antagonized LTD_4- (48% inhibition) and OA- (61%) induced bronchoconstriction in the guinea pig by intraduodenal (id) administration (50 mg/kg doses). Since both FPL-55712 andd Wy-44,329 were not active when administered id, compound 1 represented a significant improvement.[16]

The cumulative dose-response curve to LTD_4 was obtained for compound 1 on isolated guinea pig trachea (Figure 2). Although the shift to the right was significant, the pK_B value of 4.94 indicates this compound is not as potent a LTD_4 antagonist as FPL-55712 (pK_B = 6.57) or Wy-44,329 (pK_B = 9.4).

We thus sought to increase the LT antagonist potency in vivo. Based on the precedent set by the FPL-55712 series,[17] we felt the incorporation of an *n*-propyl group would boost LT antagonist potency; however, compound **20** was virtually inactive. Furthermore, all of the analogues summarized in Table I failed as inhibitors of LTD_4-induced bronchospasm. In contrast, several other members of this series (**2**, **3**, **15**, **16**, **20**, and **22**) besides 1 showed activity against OA-induced bronchospasm. In both screens, the administration of indomethacin prior to challenge was used to shunt arachidonate metabolism away from cyclooxygenase (CO) components. In the antigen screen, chlorpheniramine was also administered (vide infra) in order to attenuate the histaminic component of guinea pig anaphylaxis. These results suggest that in vivo activity in this series might be due to either mediator release inhibition, LTC_4 antagonism, or 5-LO inhibition.

Theoretically, one would expeect that more potent 5-LO inhibitors would show greater activity in vivo against antigen. As Table I shows, we were able to improve 5-LO inhibition. In general, changes on compound 1 that markedly enhanced lipophilic character (e.g., **5**–**12**) increased the potency of 5-LO inhibition. However, this group of compounds was devoid of in vivo activity in either screen. Within the range of 5-LO inhibitors in Table I (IC_{50} = 1.08–76 μM), we see no correlation between 5-LO inhibitory potency and activity against OA-induced bronchospasm in the guinea pig. This result may be due to problems associated with absorption, distribution, or metabolism. Alternatively, 5-LO inhibitors with much greater potency (e.g., nanomolar IC_{50}) may be required before correlations can be drawn to in vivo activity.

Compound 1 and the best 5-LO inhibitor **11** were screened in vitro for activity against 15-LO (soybean) and 12-LO (rabbit platelet). Neither compound possessed any inhibitory activity against 15- and 12-LO at 100 μM. Thus, among the lipoxygenases studied, compounds 1 and **11** are specific 5-LO inhibitors. Additionally, compounds **1**, **11**, and several others in this series were screened for cyclooxygenase activity (CO/rat PMN, data not shown). While all the compounds tested were found to lack any significant CO inhibitory activity, some members were selective for thromboxane (Tx) synthetase inhibition.[18]

In summary, we have found that 1 displays our desired biological profile: a 5-LO inhibitor with id activity against both LTD_4- and OA-induced bronchoconstriction in the guinea pig. Due to the modest pK_B value relative to FPL-55712 and Wy-44,329 in vivo, as well as mutagenic activity in the Ames test,[19] further development is not anticipated with **1**. However, we are using information gained in this study to develop more effective compounds with a similar profile.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were recorded on a Varian XL-300 at 300 MHz, a Varian XL-100 at 100 MHz, or a Varian FT-80A at 80 MHz. Mass spectra were recorded on a Kratos MS-25 or MS-50. IR spectra were recorded with a Perkin-Elmer 229 infrared spectrophotometer. Elemental analysis were recorded with a Perkin-Elmer 240C elemental analyzer, and all compounds were within 0.4% of theoretical values unless otherwise noted.

Representative Procedures for Scheme I. 3-[[3-[(4-Acetyl-3-(hydroxyphenoxy)methyl]phenyl]amino]-3-oxopropanoic Acid, Methyl Ester (1). **3-[(4-Acetyl-3-hydroxyphenoxy)methyl]nitrobenzene.** A mixture of potassium carbonate (anhydrous, 31.7 g, 0.23 mol) and cesium carbonate (catalytic amount) was pulverized with mortar and pestle, then added to a solution of 2,4-dihydroxyacetophenone (35 g, 0.23 mol) in acetone (250 mL), and the mixture was refluxed for 30 min. A solution of *m*-nitrobenzyl bromide (50 g, 0.23 mol) in acetone (100 mL) was added in one portion and the reaction refluxed for 3 days. After cooling to room temperature, water was added until a precipitate formed. The product was collected on a Buchner funnel and washed with water and ether. Recrystallization from acetone provides 36.9 g (0.13 mol, 56% yield) of needles: mp 117–118 °C; IR (KBr) 3400 (br), 1620 (br), 1520, 1345, 1255 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.57 (s, 3 H), 5.2 (s, 2 H), 6.5–6.6

(16) For comparison, the recently introduced Ly-171,883 (ref 20) produced 92.6% inhibition at 50 mg/kg dose id. However, since this compound is also a potent PDE inhibitor and bronchodilator, it is not clear to what extent this observed result is due to LTD_4 antagonism.

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(18) For example, **16**: IC_{50} (PGE_2) >100 μM, IC_{50} (Tx) = 46.4 μM. **20**: IC_{50} (PGE_2) >50 μM, IC_{50} (Tx) = 7.63 μM.

(19) Dr. Stephen Dizio, Wyeth internal correspondence.

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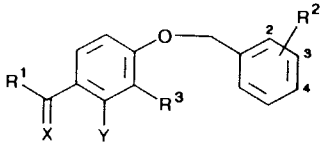
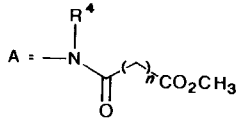
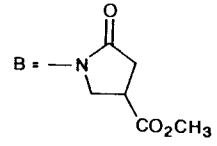
Table I. Inhibition of Rat PMN 5-LO and GP Bronchospasm







compd	X	Y	R^1	R^2	R^3	R^4	n	mp, °C	formula[a]	synth[b] method	% yield	in vitro PMN (IC_{50}, μM)[c] 5-HETE	in vitro PMN (IC_{50}, μM)[c] LTB_4	in vivo[d] OA % inhibn	N[e]
1	O	OH	CH_3	3A	H	H	1	130–132	$C_{19}H_{19}NO_6$	1	88	14.20	11.70	61.4	6
2	O	OH	CH_3	3A	H	H	2	121–123	$C_{20}H_{21}NO_6$	2	53	20.80	27.80	37.9	2
3	O	OCH_3	CH_3	3A	H	H	1	93.5–96.5	$C_{20}H_{21}NO_6$	1	37	12.10	19.90	64.3[f]	6
4	O	OCH_3	CH_3	3A	H	H	2	123.5–124.5	$C_{21}H_{23}NO_6 \cdot 0.25H_2O$	2	26	36.30	34.70	0.11	3
5	O	OH	$CH_3(CH_2)_4$	3A	H	H	1	90–91	$C_{23}H_{27}NO_6$	1	72	3.44	4.14	−0.5	3
6	O	OH	$CH_3(CH_2)_4$	3A	H	H	2	69–70	$C_{24}H_{29}NO_6$	2	37	2.01	2.24	0.0	3
7	O	OCH_3	$CH_3(CH_2)_4$	3A	H	H	2	103–105	$C_{25}H_{31}NO_6 \cdot 0.25H_2O$	2	60	3.71	3.87	0.0	3
8	O	OCH_3	$CH_3(CH_2)_4$	3A	H	H	1	79–81	$C_{24}H_{29}NO_6$	1	47	2.73	3.14	0.0	3
9	H_2	OCH_3	CH_3	3A	H	H	2	69–70	$C_{21}H_{25}NO_5$	2	25	5.60	7.09	0.2	3
10	H_2	OCH_3	$CH_3(CH_2)_4$	3A	H	H	1	73.5–75.5	$C_{24}H_{31}NO_5$	1	38	1.59	1.70	−0.2	3
11	H_2	OCH_3	$CH_3(CH_2)_4$	3A	H	H	2	63–65	$C_{25}H_{33}NO_5$	2	38	1.08	1.33	0.2	3
12	H_2	OCH_3	CH_3	3A	H	H	1	83–85	$C_{20}H_{23}NO_5$	1	34	4.54	5.50	0.2	3
13	O	OH	CH_3	3A	H	H	0	155–157	$C_{18}H_{17}NO_6$	2	28	25.1	39.5	0.1	3
14	O	OH	CH_3	3A	H	H	3	110–112	$C_{21}H_{23}NO_6$	2	17	32.67	39.34	−0.1	3
15	O	F	CH_3	3A	H	H	2	123–126	$C_{20}H_{20}FNO_5$	2	60	22		20.8	3
16	O	F	CH_3	3A	H	H	0	141–142	$C_{18}H_{16}FNO_5$	2	78	28.5		57.3	2
17	O	F	CH_3	3A	H	H	1	102–104	$C_{19}H_{18}FNO_5 \cdot 0.25H_2O$	1	36	25.53	22.52	0.0	3
18	O	F	CH_3	3A	H	H	3	104–106	$C_{21}H_{22}FNO_5$	2	42	38.56	62.07	0.0	2
19	O	OH	CH_3	3A	H	CH_3	1	83–86	$C_{20}H_{21}NO_6$	1	50	>50			
20	O	OH	CH_3	3A	$CH_3(CH_2)_2$	H	1	159.5–161	$C_{22}H_{25}NO_6$	1	40	1.1		24.8	2
21	O	OH	CH_3	2A	H	H	1	129–130	$C_{19}H_{19}NO_6$	1	46	7.74			
22	O	H	CH_3	3A	H	H	1	118.5–120	$C_{19}H_{19}NO_5$	1	75	*g*		37	2
23	H_2	OCH_3	H	3A	H	H	1	65–69	$C_{18}H_{19}NO_5$	1	58	*g*			
24	O	OH	CH_3	3B	H			111–114	$C_{21}H_{21}NO_6$	24	29	76.00	70		
25	O	F	CH_3	3B	H			88.5–91.5	$C_{21}H_{20}FNO_5$	24	22	5.35	20.36		
Ly-171,883												41.0	21.6[h]	78.8	8

[a] Analyses (C, H, N) were within ±0.4% of theoretical values. [b] Compound number, see Experimental Section. [c] All IC_{50}'s were calculated by nonlinear regression analysis and were significant at $p < 0.05$ level. [d] Dose of 50 mg/kg administered id 10 min prior to challenge. [e] N = number of animals. [f] Statistically significant using Students t test ($p < 0.05$). [g] <50% inhibition at 50 μM. [h] This result from radioimmunoassay.

(m, 2 H), 7.5–7.8 (m, 3 H), 8.18–8.32 (m, 2 H), 12.93 (s, 1 H); MS (CI), m/z 228 (MH^+).

3-[(4-Acetyl-3-hydroxyphenoxy)methyl]aniline (I, R^1 = Me). A mixture of 3-[(4-acetyl-3-hydroxyphenoxy)methyl]-nitrobenzene (10.2 g, 35.5 mmol) and iron powder (10.4 g, 186 mmol) in ethanol (150 mL saturated with anhydrous HCl) was stirred at room temperature for 15 h. After removal of excess iron and concentration in vacuo, the residue was partitioned between dichloromethane and saturated sodium bicarbonate solution. The organic phase was filtered through a Florisil column with the aid of dichloromethane. Concentration gave 7.4 g of product (81% yield): mp 153–154 °C; IR (KBr) 3455, 3350, 1620–1580 (br), 1360, 1250 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.55 (s, 3H), 2.8–3.8 (br, 2 H), 5.02 (s, 2 H), 6.4–6.84 (m, 4 H), 7.16 (t, 1 H), 7.62 (d, 1 H, J = 10 Hz), 12.7 (s, 1 H); MS (EI), m/z 257 (M^+).

3-[[3-[(4-Acetyl-3-hydroxyphenoxy)methyl]phenyl]-amino]-3-oxopropanoic Acid, Methyl Ester (1). A mixture of 3-[(4-acetyl-3-hydroxyphenoxy)methyl]aniline (3.3 g) and dimethyl malonate (50 mL) was heated in an oil bath at 140 °C for 2 h. Excess malonate was removed in vacuo, and the residue was dissolved in acetone. Storage at 0 °C gave crystalline product (4.1 g, 88%): mp 130–132 °C; IR (KBr) 3230, 3075, 1735, 1635 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.55 (s, 3 H), 3.51 (s, 2 H), 3.82 (s, 3 H), 5.09 (s, 2 H), 6.68–6.78 (m, 2 H), 7.2 (d, 1 H, J = 3 Hz), 7.38 (t, 1 H), 7.55 (d, 1 H, J = 3 Hz), 7.68 (m, 2 H), 9.28 (br, 1 H), 12.76 (s, 1 H); MS (EI), m/z 357 (M^+). Anal. ($C_{19}H_{19}NO_6$) C, H, N.

In like manner, the following compounds were prepared from dimethyl malonate and the corresponding aniline: **3, 5, 8, 10, 12, 17,** and **19–23** (See Table I).

4-[[3-[(4-Acetyl-3-hydroxyphenoxy)methyl]phenyl]-amino]-4-oxobutanoic Acid, Methyl Ester (2). A solution of 3-[(4-acetyl-3-hydroxyphenoxy)methyl]aniline (3.4 g, 13.2 mmol, prepared as above) and triethylamine (1.62 g, 16 mmol in 40 mL of tetrahydrofuran) was cooled to −78 °C under nitrogen. To this solution was added methyl 3-(chloroformyl)propionate (2 g, neat, 13.5 mmol) dropwise. The reaction was left to warm to room temperature overnight, then poured onto 10% HCl and extracted with ether. The extracts were washed with saturated brine and then dried over $MgSO_4$ and concentrated on a rotary evaporator. The residue was dissolved in a minimum amount of acetone, to which several drops of ether was added and then stored at 0 °C. Filtration gave 2.6 g (53% yield) of product: mp 121–123 °C; IR (KBr) 3330, 1710, 1620, 1600, 1440, 1370, 1250 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.55 (s, 3 H), 2.73 (m, 4 H), 3.72 (s, 3 H), 5.08 (s, 2 H), 6.5 (m, 2 H), 7.05–7.75 (m, 5 H), 12.72 (s, 1 H); MS (CI), m/z 372 (MH^+). Anal. ($C_{20}H_{21}NO_6$) C, H, N.

In like manner, the following compounds were synthesized from the corresponding aniline and either methoxalyl chloride, methyl 3-(chloroformyl)propionate, or methyl 4-(chloroformyl)butyrate: **4, 6, 7, 9,** 11, and **13–18.**

3-[(4-Acetyl-3-methoxyphenoxy)methyl]nitrobenzene (II, R^1 = Me). To a degreased suspension of sodium hydride (23 g, 60% suspension, 57.5 mmol) in tetrahydrofuran at ice temperature was slowly added (under nitrogen) a solution of 3-[(4-acetyl-3-hydroxyphenoxy)methyl]nitrobenzene (13.6 g, 47.4 mmol, prepared as above) in tetrahydrofuran (100 mL). When hydrogen evolution ceased, the mixture was allowed to warm to room temperature and was stirred for 2 h. To this slurry was added iodomethane (6.7 g, neat, 47.2 mmol), and the mixture was stirred for 15 h at room temperature. Another 6.7 g of iodomethane was then added, and the mixture was refluxed for 6 h. After cooling to room temperature, the reaction mixture was poured into 10% HCl and extracted with ether. The extracts were washed with saturated brine then dried over $MgSO_4$ and concentrated. Recrystallization from acetone gave 9.1 g (64% yield) of product: mp 111–114 °C; IR (KBr) 1665, 1605, 1530, 1360 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.57 (s, 3 H), 3.82 (s, 3 H), 5.22 (s, 2 H), 6.6 (m, 2 H), 7.48–7.9 (overlapping multiplets, 3 H), 8.1–8.4 (overlapping multiplets, 2 H); MS (EI), m/z 301 (M^+).

3-[(4-Ethyl-3-methoxyphenoxy)methyl]aniline (III, R^1 = Me). Zinc dust (8.8 g) and mercuric chloride (0.7 g) were stirred in 25 mL of water for 10 min. The water was then decanted and the amalgam cooled in an ice bath as a solution of 3-[(4-acetyl-3-methoxyphenoxy)methyl]nitrobenzene (4 g, 13.3 mmol, prepared as above) in ethanol (saturated with anhydrous HCl) was added cautiously. After addition was complete, the reaction mixture was refluxed overnight. After cooling to room temperature, the reaction mixture was poured into saturated sodium bicaronate and extracted with dichloromethane. The extracts were dried over $MgSO_4$ and filtered through a column of Florisil. Concentration gave 2.9 g of product as a red-brown oil. The product was taken on to the next step without further purification: ^1H NMR (80 MHz, $CDCl_3$) δ 1.15 (t, 3 H, J = 8 Hz), 2.55 (q, 2 H, J = 8 Hz), 3.57 (s, 2 H, exch), 3.77 (s, 3 H), 4.93 (s, 2 H), 6.35–7.25 (overlapping multiplets, 7 H); MS (CI), m/z 258 (MH^+).

Representative Procedures for Aniline IV. 3-[(4-Acetyl-3-fluorophenoxy)methyl]nitrobenzene. To a solution of anhydrous aluminum chloride (208 g, 1.56 mol) in ethylene dichloride (500 mL) cooled in ice under nitrogen was added dropwise 3-fluorophenol (155 g, 1.38 mol, neat). When addition was complete, acetyl chloride (120 g, 1.53 mol) was added (neat) dropwise. The ice bath was then removed and the reaction mixture refluxed for 4 days. After cooling to room temperature, the reaction mixture was then poured onto ice water and extracted with ether. The extracts were washed with saturated aqueous sodium chloride solution and stored over $MgSO_4$. After filtration and concentration at the rotary evaporator, the crude product was washed with petroleum ether and dried under vacuum: IR (KBr) 3090 (br), 1650, 1600 (br), 1560 (br) cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.62 (d, 3 H, J = 4 Hz), 6.7 (triplet of doublets, 2 H, J_{obs} = 3 Hz), 7.84 (t, 1 H, J_{obs} = 8 Hz); MS (EI), m/z 154 (M^+).

To a solution of 4-acetyl-3-fluorophenol (22.4 g, 145 mmol) in acetone (100 mL) was added powdered potassium carbonate (anhydrous, 20 g, 145 mmol) and cesium carbonate (catalytic amount). After 30 min of reflux, a solution of m-nitrobenzyl bromide (32.7 g, 151 mmol) in acetone (100 mL) was added in one portion, and the mixture was refluxed for 15 h. Water was then added to dissolve all solids, followed by ether and saturated brine solution. The ether layer was dried over $MgSO_4$ and concentrated to an orange-red oil. Addition of petroleum ether gave orange-yellow crystals (31 g, 74% yield): mp 92–96 °C; IR (KBr) 3440 (br), 3075, 1670, 1625, 1525, 1360, 1260 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.6 (d, 3 H, J = 5 Hz), 5.23 (s, 2 H), 6.74 (dd, 1 H, J = 12 Hz, 2 Hz), 6.85 (dd, 1 H, J = 12 Hz, 2 Hz), 7.63 (t, 1 H), 7.8 (d, 1 H), 7.95 (t, 1 H), 8.25 (br d, 1 H), 8.24 (br s, 1 H); MS (CI), m/z 290 (MH^+).

3-[(4-Acetyl-3-fluorophenoxy)methyl]aniline (IV). The title compound is prepared by use of the procedure described above. From 15 g (52 mmol) of 3-[(4-acetyl-3-fluorophenoxy)-methyl]nitrobenzene, 9.09 g (67% yield) of recrystallized (ether) product is obtained: mp 85–87 °C; IR (KBr) 3440, 3390, 3340, 1650, 1600, 1260 cm^{-1}; ^{1} NMR (100 MHz, $CDCl_3$) δ 2.59 (d, 3 H, J = 4 Hz), 3.69 (br s, 2 H), 5.2 (s, 2 H), 6.6–6.88 (m, 5 H), 7.18 (triplet of doublets, 1 H, J = 8 Hz, 2 Hz), 7.87 (triplet of doublets, 1 H, J = 8 Hz, 2 Hz); MS (CI), m/z 260 (MH^+). Anal. ($C_{15}H_{14}NO_2F$) C, H, N.

1-[3-[(4-Acetyl-3-hydroxyphenoxy)methyl]phenyl]-2-oxopyrrolidine-4-carboxylic Acid, Methyl Ester (24). A mixture of itaconic acid (2.06 g, 15.7 mmol) and 3-[(4-acetyl-3-hydroxyphenoxy)methyl]aniline (3.67 g, 14.3 mmol) was heated (neat) in a 170 °C oil bath under nitrogen for 1 h. The mixture was cooled to room temperature; methanol (20 cm^3) and p-toluenesulfonic acid (trace) were added, and the mixture was refluxed for 15 h. The methanol was removed in vacuo, and the residue was chromatographed on silica gel (elution with 1:1 hexane–ethyl acetate) to give 1.6 g (29% yield) of product: mp 111–114 °C; IR (KBr) 3440 (br), 1735, 1700, 1600 (br) cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 2.55 (s, 3 H), 2.82–2.95 (m, 2 H), 3.2–3.52 (m, 1 H), 3.78 (s, 3 H), 4.08 (m, 2 H), 5.08 (s, 2 H), 6.52 (m, 2 H), 7.14–7.74 (m, 5 H), 12.7 (s, 1 H); MS (CI), m/z 383 (M^+). Anal. ($C_{21}H_{21}NO_6$) C, H, N.

In like manner, **25** was prepared from the corresponding aniline.

Rat PMN 5-LO. Peritoneal PMNs were obtained from female Wistar rats (150–250 g) that received an intraperitoneal (ip) injection of 6% glycogen (10 mL). After 24 h, rats were killed by CO_2 asphyxiation, and peritoneal cells were harvested by peritoneal lavage using Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution (HBSS). The peritoneal exudate was centrifuged at 400g for 10 min. The lavaged fluid was removed, and the cell pellet

was resuspended in HBSS containing Ca^{2+} and Mg^{2+} and 10 mM L-cysteine at a concentration of 2×10^7 cells/mL. To 1-mL portions of cell suspension, test drugs or vehicle was added and incubated at 37 °C for 10 min. Following the preincubation, the calcium ionophore A23187 (10 μM) was added together with 0.5 μCi of [^{14}C]arachidonic acid and further incubated for 10 min. The reaction was stopped by adding ice-cold water (3 mL) and acidifying to pH 3.5. LO and CO products were then extracted twice into diethyl ether. The pooled ether extracts were evaporated to dryness under nitrogen, and the residue was redissolved in a small volume of methanol and spotted on aluminum-backed precoated thin-layer chromatographic plates. The samples were then cochromatographed with authentic reference 5-HETE, 12-HETE, and LTB_4 in the solvent system hexane–ether–acetic acid (50:50:3). For assessing CO activity, the methanolic extracts were chromatographed in the solvent system ethyl acetate–formic acid (80:1) with authentic PGE_2 and TxB_2. After chromatography, the areas associated with authentic 5-HETE, LTB_4, or PGE_2 and TxB_2 were identified by autoradiography, cut out, and quantitated by liquid scintillation. IC_{50} values for each metabolite and test compound were calculated from the line generated by nonlinear regression analysis of raw dose–response data.

The results are summarized in Table I.

LTD_4-Induced Bronchospasm in the Guinea Pig (GP). Male Hartley strain GPs (350–600 g) were anesthetized with pentobarbital sodium (50 mg/kg, ip). The jugular vein was cannulated for injection of drugs and the carotid artery for monitoring blood pressure. The trachea was cannulated for artificial ventilation by a miniature Starling pump and for indirect measurement of respiratory volume changes as described below. Additional pentobarbital sodium (15 mg/kg) was administered intravenously (iv) to arrest spontaneous respiration. A CO inhibitor, indomethacin (10 mg/kg in Tris buffer, iv) was administered 9 min before LTD_4 challenge to shunt arachidonate metabolism to the LO pathways. Submaximal bronchoconstrictor responses were established in control animals by varying the dose levels of LT. Intravenous dose level range for LTD_4 was from 0.3 to 1 μg/kg. Test drugs were administered by the intraduodenal route (except where noted) 10 min before induction of bronchospasm by administration of LTD_4 at the predetermined dose levels. Control animals received saline in place of drug. Respiratory volume changes were determined by a calibrated piston whose travel was recorded, via a linear transducer, on a Beckman Dynograph recorder. Maximal bronchoconstrictor volume was determined by clamping off the trachea at the end of the experiment. Overflow volumes at 1, 3, and 5 min were obtained from the recorded charts. The overflow volume at 1, 3, and 5 min was expressed as a percentage of maximal bronchoconstriction. Combined group values were used from each of these time intervals to determine the inhibitory effect of drugs.

$$\% \text{ inhibition} = [(\% \text{ bronchoconstriction (bc) in control group} - \% \text{ bc in drug-treated groups}) / \% \text{ bc in control group}] \times 100$$

A Students t test unpaired data was used to determine statistical significance. Dose–response curves were generated, and ED_{50} doses were interpolated from the regression lines.

Results for compounds are summarized in Table I.

OA-Induced Bronchospasm in the Guinea Pig. Male Hartley strain GPs weighing 350–600 g were sentitized to OA (5 mg intraparentally (ip) and 5 mg subcutaneous) on day 0. Three to five weeks later, the animals were anesthetized with pentobarbital sodium (50 mg/kg, ip). The jugular vein was cannulated for injection of drugs and the carotid artery for monitoring blood pressure. The trachea was cannulated for artificial ventilation by miniature Starling pump and for indirect measurement of respiratory volume changes as described below. Additional pentobarbital sodium (15 mg/kg, iv) was administered to arrest spontaneous respiration. A CO inhibitor, indomethacin (10 mg/kg in Tris buffer, iv) was administered 9 min before OA challenge to shunt arachidonate metabolism to the LO pathways. One minute later, chlorpheniramine (1.0 mg/kg in saline, iv) was given to attenuate the histaminic component of anaphylactic bronchoconstriction. Test drugs were administered id (except where noted) at 10 min before antigen challenge. Anaphylactic bronchoconstriction was induced by administration of aerosolized OA (1%). Control animals received saline in place of drug. Respiratory volume changes and determinations of the inhibitory effects of drugs were determined as described in the preceding section.

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Registry No. 1, 98618-87-2; 1 (R^2 = 3-NH_2), 98618-89-4; 1 (R^2 = 3-NO_2), 98618-88-3; **2**, 98618-90-7; **3**, 98618-92-9; **3** (R^2 = 3-NH_2), 98618-94-1; **3** (R^2 = 3-NO_2), 98618-93-0; 4, 98618-95-2; **5**, 98618-96-3; **5** (R^2 = 3-NH_2), 103981-23-3; **5** (R^2 = 3-NO_2), 103981-28-8; **6**, 98618-97-4; **7**, 98618-98-5; **7** (R^2 = 3-NH_2), 103981-24-4; **7** (R^2 = 3-NO_2), 103981-30-2; **8**, 98618-99-6; **9**, 98619-00-2; **9** (R^2 = 3-NH_2), 98619-01-3; **10**, 98619-03-5; **10** (R^2 = 3-NH_2), 103981-25-5; 11, 98619-04-6; **12**, 98619-02-4; **13**, 98633-94-4; 14, 98618-91-8; **15**, 98619-06-8; **15** (R^2 = 3-NH_2), 98619-08-0; **15** (R^2 = 3-NO_2), 98646-39-0; **16**, 98619-09-1; **17**, 98619-12-6; **18**, 98619-10-4; **19**, 103981-18-6; **19** (R^2 = 3-$NHCH_3$), 103981-26-6; **20**, 103981-19-7; **20** (R^2 = 3-NH_2), 104013-60-7; **21**, 103981-20-0; **21** (R^2 = 2-NH_2), 103981-31-3; **21** (R^2 = 2-NO_2), 103981-33-5; **22**, 103981-21-1; **22** (R^2 = 3-NH_2), 103981-32-4; **22** (R^2 = 3-NO_2), 103981-34-6; **23**, 103981-22-2; **23** (R^2 = 3-NH_2), 103981-27-7; **23** (R^2 = 3-NO_2), 103981-29-9; **24**, 98619-05-7; **25**, 98619-11-5; $ClCOCO_2CH_3$, 5781-53-3; $ClCO(CH_2)_2CO_2Me$, 1490-25-1; $ClCO(CH_2)_3CO_2Me$, 1501-26-4; 4-$CH_3COC_6H_4OH$, 99-93-4; 2-$O_2NC_6H_4CH_2Br$, 3958-60-9; 2,4-dihydroacetophenone, 89-84-9; *m*-nitrobenzyl bromide, 3958-57-4; dimethyl malonate, 108-59-8; itaconic acid, 97-65-4; 1-(4-resorcinyl)caproaldehyde, 3144-54-5; 4-acetyl-3-fluorophenol, 98619-07-9; 4-formylresorcinol, 95-01-2; 3-fluorophenol, 372-20-3; acetyl chloride, 75-36-5.