Differential Effects of a Series of Hydroxamic Acid Derivatives on 5-Lipoxygenase and Cyclooxygenase from Neutrophils and 12-Lipoxygenase from Platelets and Their in Vivo Effects on Inflammation and Anaphylaxis

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The synthesis of a series of novel substituted hydroxamates has been described along with their profile of inhibitory activity against 5-lipoxygenase, 12-lipoxygenase, and cyclooxygenase enzymes. The structure-activity relationship suggests that future molecules could be designed to specifically inhibit one or more of these enzymes since there were definite differences in structure-activity relationships for these different enzymes. A representative number of these compounds have been tested in vivo and found to possess potent oral activity in a systemic anaphylaxis model mediated by leukotrienes and topical activity in an arachidonic acid induced inflammation model. One of these molecules, compound **20**, demonstrated that a leukotriene antagonist pharmacophore can be modified such that it contains both antagonist activity and 5-lipoxygenase inhibitory activity.

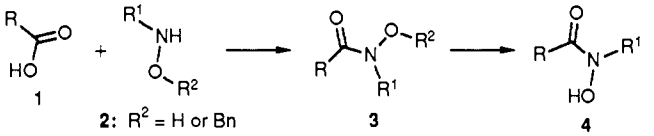
Slow-reacting substances of anaphylaxis (SRS-A) have long been implicated as important mediators of inflammation and allergic reactions.[1] It is now well established that leukotrienes C_4, D_4, and E_4 are the components of SRS-A.[2] These biologically important compounds are products derived from metabolism of arachidonic acid by the 5-lipoxygenase pathway.[3] Therefore, the inhibition of 5-lipoxygenase enzyme, the initial step in the formation of leukotrienes, could be useful as a tool for investigating the regulatory mechanism of leukotriene biosynthesis. A potent inhibitor of 5-lipoxygenase may be therapeutically useful for the treatment of disease states associated with hypersensitivity reactions and inflammation. The monohydroxyeicosatetraenoic acids (HETE) have been the focus of intense investigation due to their interesting biological activities. Particularly, 12-HETE is the major lipoxygenase product found in human platelets and has been demonstrated to be present in high levels in bronchial fluid from endotoxin-treated pigs[4] and in ischemic tissue.[5] A potent 12-lipoxygenase inhibitor would also be a useful tool to study the role of 12-HETE in certain diseases.[6]

From the known role of iron in the mechanism of soybean lipoxygenase,[7] it has been inferred that the catalytic site of the mammalian 5-lipoxygenase contains iron as well.[8] Corey and co-workers[8] hypothesized that substrate analogues containing an iron-chelating functionality, such as hydroxamic acid, would be effective inhibitors of the enzyme. Several hydroxamic acids of arachidonic acid and related compounds have indeed been shown to be potent, competitive inhibitors of 5-lipoxygenase.[8,9] Recently, several simple hydroxamates were reported to be potent 5-lipoxygenase inhibitors in in vitro studies.[10] We describe here our studies of a series of hydroxamates as potent 5- and 12-lipoxygenase inhibitors and the in vivo results for selected members of this series in two different models.

Chemistry

The hydroxamates listed in Table I were prepared readily according to Scheme I. The intermediate **3** was prepared readily by reacting the appropriate carboxylic acid **1** and the appropriate hydroxylamine **2** according to conventional methods. The hydroxylamines were prepared by reductive alkylation[11] or direct alkylation of *O*-benzylhydroxylamine with alkyl halides. The *O*-benzyl group in **3** was removed either by hydrogenolysis or boron trichloride.

Scheme I



Results and Discussion

The results obtained from testing the hydroxamates as inhibitors of 5-lipoxygenase from rat and human PMNs, 12-lipoxygenase from rat platelets, and cyclooxygenase from rat PMNs are listed in Tables I and II. Since earlier assays measured 5-lipoxygenase with intact cells, selected

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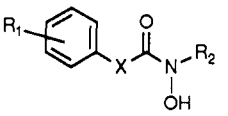
0022-2623/89/1832-1836$01.50/0

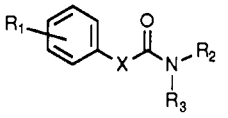
Table I. In Vitro Activities of Hydroxamates



compd no.	R_1	X	R_2	IC_{50}, μM, or % inhibition ([X], μM)				
				PMN 5-lipoxygenase			12-LOX[e]	cyclooxygenase
				g.p. supnt[d]	rat cell	human cell	rat platelets	rat PMNs
5	4-BnO[a]	CH_2	H		3% (1)		1.5	16% (30)
6	4-BnO	CH_2	CH_3	0.2	0.10[b]	0.3	0.5	9.0
7	4-BnO-3Cl	CH_2	CH_3		0.1	0.2	2.5	3.0
8	4-BnO	CH_2	$(CH_2)_2CH_3$		0.1	0.16	2.0	30
9	4-BnO	CH_2	CH_2Ph		0.1	0.12	47% (100)	32% (100)
10	4-BnO		$(CH_2)_2Ph$	0.05			<10% (100)	75
11	4-BnO	CH_2	$(CH_2)_2Ph$	0.1	0.32	0.35	1.5	<10% (30)
12	4-BnO	CH_2	$(CH_2)_3Ph$		0.5	0.6	4.5	<10% (30)
13	4-BnO	CH_2	$CH(CH_3)Ph$	0.03	0.3	0.4	65	38% (10)
14	4-BnO	CH_2	$CH(CH_2CH_3)CH_2Ph$		2.5	3.0	<10% (100)	44% (30)
15	3-BnO	$CH(CH_3)$	$(CH_2)_2Ph$		0.3	0.1	10	<10% (30)
16	4-OCH_3	CH_2	$(CH_2)_2Ph$		1	2.5	21	48% (30)
17	4-BnO	CH_2	$(CH_2)_4CO_2H$		21% (10)	4% (1)	40	50% (100)
18	4-BnO	CH=CH	$(CH_2)_2Ph$		24% (1)	<10 (10)	3% (30)	<10% (30)
19	3-(Q-CH_2O)[c]	CH_2	CH_3		0.08	1.0	3.0	30% (100)
20	4-(Q-CH_2O)[c]	CH_2	CH_3		0.35	1.0	2	<10% (30)
21	H	$(CH_2)_3$	H		11% (3)	−8% (1)	15	
22	H	$(CH_2)_3$	CH_2Ph		0.15	0.13	0.5	18% (30)
23	H	$(CH_2)_3$	$(CH_2)_2Ph$	0.13	0.3	0.18	0.4	44% (30)
24	H	$(CH_2)_3$	$(CH_2)_3CO_2H$		1% (10)		15% (100)	<10% (30)
25	H	$(CH_2)_3$	$(CH_2)_3CO_2C_2H_5$		0.3	0.5	15	44% (30)
26	4-OCH_3	$(CH_2)_3$	CH_3		0.3	1.3	1.5	<10% (30)
27	4-OCH_3	$(CH_2)_3$	$(CH_2)_2Ph$		0.12	0.25	2.5	
28	H	$(CH_2)_4$	CH_3		0.11	0.25	1.0	<10% (30)
29	H	$(CH_2)_4$	$(CH_2)Ph$		0.05	0.15	0.3	50% (30)
30	H	$(CH_2)_2$	$(CH_2)_3Ph$		0.1	0.1	0.6	15% (100)
31	H	$O(CH_2)_2$	$(CH_2)_2Ph$		0.25	0.3	3.5	18% (100)
32	H	OCH_2	$(CH_2)_2Ph$		1.0	1.2	1.5	65% (100)
33	4-$CH_2CH(CH_3)_2$	$CH(CH_3)$	CH_2Ph		1.0	2.0	5.0	20% (100)

[a] BnO = benzyloxy. [b] IC_{50}(RG 6866) = 0.10 ± 0.05 μM (N = 3). [c] Q = quinolin-2-yl. [d] Guinea pig supernatant. [e] 12-Lipoxygenase.

Table II. In Vitro Activities of Hydroxamates with Disubstituted Nitrogen



compd no.	R_1	X	R_2	R_3	IC_{50}, μM, or % inhibition ([X], μM)				
					PMN 5-lipoxygenase			12-LOX	cyclooxygenase
					g.p. supnt	rat cell	human cell	rat platelets	rat PMNs
34	$CH_2(CH_3)_2$	$CH(CH_3)$	CH_2Ph	OCH_2Ph		<10% (10)	<10% (10)	<10% (10)	39% (30)
35	4-BnO	CH_2	CH_2Ph	OCH_2Ph	<10% (10)		22% (10)	<10% (10)	24% (30)
36	H	$(CH_2)_3$	$(CH_2)_2Ph$	H	12% (10)		<10% (10)	<10% (10)	<10% (100)

compounds were also tested against 5-lipoxygenase in the 20000*g* supernatant from guinea pig PMNs to ensure that the inhibition observed in the whole cell assay was a reflection of direct enzyme inhibition. The inhibitory effects of these compounds were similar on 5-lipoxygenase measured in these three systems. On the other hand, there were definite differences in the structural requirements for inhibition of 5-lipoxygenase vs 12-lipoxygenase vs cyclooxygenase (see discussion below). The more interesting compounds were tested in vivo, and the results are summarized in Table III. The arachidonic acid induced ear inflammation model in the mouse, in which topically applied arachidonic acid produces an acute, transient inflammatory reaction with immediate erythema and edema formation, was used as a topical in vivo model. The specificity of this model for the detection of lipoxygenase inhibitors in vivo has been reviewed.[12] Antigen-induced collapse (and death) of actively immunized conscious guinea pigs, in which the leukotriene component was enhanced by pretreatment with a cyclooxygenase inhibitor, and antihistamine, and a β-adrenergic antagonist (to blunt adrenergic compensation) was used as a systemic, in vivo model for selected compounds.

All of the compounds in Tables I and II were tested for leukotriene antagonist activity in the guinea pig parenchymal strip assay. Except for compound **6** (RG 6866) and compound **20**, none of them exhibited any significant an-

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Table III. In Vivo Activity

compd no.	mouse ear inflammation: dose, mg/ear	mouse ear inflammation: % inhibition, ear weight gain[a]	g.p. systemic anaphylaxis: dose, mg/kg, po	g.p. systemic anaphylaxis: % inhibition, mortality[b]
6	2	65 ± 6** (N = 3)	60	83 ± 3** (N = 27)
7	1	65 ± 6** (N = 6)	200	17
8	2	82 ± 5**	200	0
9	2	37 ± 31		
11	2	39 ± 14 (N = 3)	200	17
12	2	12 ± 20		
13	2	47 ± 11*		
14	2	38 ± 11* (N = 3)		
16	2	51 ± 2** (N = 3)		
19	2	68 ± 7*		
20	1	48 ± 9*	60	94 ± 5** (N = 3)
21	2	28 ± 11	200	11
22	2	51 ± 11*	200	0
23	2	56 ± 9*	50 (10 iv)	17 (50*)
24	2	34 ± 14		
25	2	54 ± 7*		
26	2	67 ± 8*	200	60 ± 24* (N = 3)
27	2	64 ± 5*	200	0
28	2	77 ± 5*	200	9
29	2	52 ± 10*		
30	2	67 ± 14*	200	0

[a] When N is given, values are averaged (±SEM) from N experiments with 5 mice/group/experiment. When N is not given, the values are averaged (±SD) from 1 experiment with 5 mice/group. (*) $P < 05$; (**) $P < 0.01$. [b] When N is given, values are averaged (±SEM) from N separate experiments with 6 animals/group/experiment. Where N is not given, the values are from a single experiment with 6 animals/group. P values are as above.

tagonist activity at 30 μM vs 0.2 nM LTC_4 induced contractions (data not shown). Compound **20** had an IC_{50} of 1.2 ± 0.5 μM (N = 3) vs contractions induced by 0.2 nM LTC_4 and an IC_{50} of 0.91 μM (N = 2) vs contractions induced by 0.4 nM LTD_4. Against [^{3}H]LTD_4 binding, compound **20** had a K_i = 1.0 ± 0.1 μM (N = 2). These results are consistent with earlier studies[13] which indicated that the (2-quinolinylmethoxy)phenyl moiety is a basic LTD_4 antagonist pharmacophore. In that earlier series of compounds, RG 5901 was reported[14] to be a competitive LTD_4 antagonist with a K_i = 0.8 μM. Compound **20** illustrates that it is possible to combine the basic pharmacophores of an antagonist and a 5-lipoxygenase inhibitor.

Compound **6** inhibited LTC_4-induced contractions with an IC_{50} = 4.2 ± 2.2 μM (N = 3) and inhibited LTD_4-induced contractions with an IC_{50} = 5.0 μM (N = 2). However, against [^{3}H]LTD_4 binding, no significant inhibition was obtained up to 100 μM. Thus, compound **6** has no intrinsic affinity for the leukotriene receptor, and the activity observed with the parenchymal strip assay must be due to another mechanism of action not associated with its inhibitory effects on the lipoxygenases; other equally potent inhibitors of lipoxygenases in this series did not have this activity. This activity was not due to inhibition of cyclooxygenase as compound **7** and indomethacin (data not shown) did not inhibit these LTC_4-induced contractions.

Many of the hydroxamates showed significant 5- and 12-lipoxygenase inhibitory activity, and their activities are clearly attributed to the hydroxamic acid functionality. The corresponding amide- and oxygen-protected hydroxamates were not active as lipoxygenase inhibitors (Table II).

Compound **11** (RG 6820) was chosen to represent this series as an inhibitor of 5-lipoxygenase and 12-lipoxygenase and tested in more depth. As shown in Figure 1, compound **11** was a competitive inhibitor of both 5-lipoxygenase and 12-lipoxygenase with K_i values of 0.1 and 1.6 μM, respectively. Since the concentration–response curves for inhibition of these enzymes by all of these compounds tended to be parallel to that of **11**, it could be

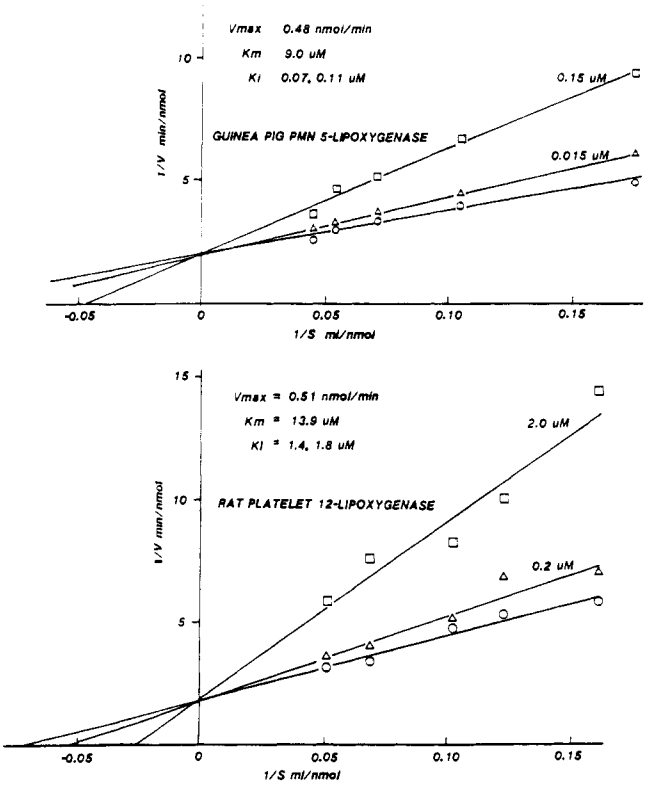

Figure 1. Kinetics of inhibition of 5-lipoxygenase from the supernatant fraction of guinea pig PMNs (top panel) and 12-lipoxygenase from rat platelets by compound 11 (RG 6820). Open circles = control in the absence of 11; other symbols = in the presence of indicated concentration of 11.

(13) RG 5901 has been previously reported as REV 5901.
(14) Van Inwegen, R. G.; Khandwala, A.; Gordon, R.; Sonnino, P.; Coutts, S. M.; Jolly, S. *J. Pharmacol. Exp. Ther.* **1987**, *241(1)*, 117.

assumed that the other compounds in this series are probably competitive inhibitors.

Within experimental error, there was generally good agreement between the measurement of 5-lipoxygenase in the intact cell and the enzyme preparation in the fractionated cell. Although some discrepencies could be a result of differences in cell penetration, it is interesting to speculate on structure–activity relationships since these compounds are closely related. There are several important structural factors that appear to affect the potency of hydroxamates as inhibitors of lipoxygenases. Substitution on the nitrogen improves potency significantly. The nitrogen-unsubstituted compounds **5** and **21** had essentially little or no inhibitory activity against 5-lipoxygenase. Although the low activity could result from the inability of these compounds to cross the cellular membrane, subsequent testing in a cell-free enzyme system indicated that they lack intrinsic inhibitory activity. The similarity in the in vitro potency for hydroxamates against 5-lipoxygenase in intact polymorphonuclear leukocytes as compared to the enzyme in broken cell preparations has been reported by others.[10] The increase in potency in the nitrogen-substituted hydroxamates may be due to increased lipophilicity of the compounds. Although the potency vs 5-lipoxygenase is relatively independent of the nature of substituent group on nitrogen, it decreases as the size of the group increases (compare **9**, **11**, and **14**).

The distance between the phenyl and the hydroxamate moieties plays little or no role in modifying 5-lipoxygenase inhibitory activity (compare **10**, **9**, **22**, **29**, and **15**). The addition of a double bond spacer (compound **18**) greatly reduced potency, although naphthylpropenohydroxamic acids have been reported to be potent 5-lipoxygenase inhibitors.[10] The addition of an ether linkage reduced activity with a simple methylene (**32** vs **30**) but had little effect with a longer alkyl (**31**). More important is the type of substitution on the nitrogen of the hydroxamate moiety. Alkyl, branched alkyl, and phenyl groups had little effect on 5-lipoxygenase inhibitory activity. Addition of 4-carboxylic acids reduced activity (**17** and **24**). As discussed below, these types of substitutions had greater effects on 12-lipoxygenase and cyclooxygenase activities.

Generally, most of the hydroxamates listed in Table I are dual inhibitors of both 5- and 12-lipoxygenases. However, there are some significant differences in the structure–activity relationships. It is interesting to note that while the N-unsubstituted hydroxamic acids **5** and **21** are not active as 5-lipoxygenase inhibitors, they are active as 12-lipoxygenase inhibitors. Inhibitory activity vs 12-lipoxygenase is more susceptible to steric influence, particularly substituents α to the nitrogen (compare **12**, **13**, and **14**). This feature allows the synthesis of a hydroxamate with high selectivity against 5- vs 12-lipoxygenases (e.g., **13**). The substitution of a free carboxylic acid moiety for the phenyl also reduced activity against 12-lipoxygenase. Compounds with the *N*-hydroxyl group missing or blocked (Table II) were also inactive against 12-lipoxygenase.

Against the PMN cyclooxygenase, only compounds **6** and **7** showed significant potency, with IC_{50} values ≤ 10 μM. These two compounds were at least 10-fold less potent than indomethacin, which had an IC_{50} of 0.1 μM in this assay. Clearly, the ability to inhibit this enzyme was affected by the substitution of bulky groups on the nitrogen.

In a guinea pig model of systemic anaphylaxis, only three of the compounds tested (**6**, **20**, and **26**) demonstrated significant oral activity (Table III). This systemic anaphylaxis model is thought to be leukotriene mediated since the animals are pharmacologically treated to inhibit effects of histamine, β-adrenergic activity, and production of lipid mediators via the cyclooxygenase pathway. Also, pure LTD_4 antagonists are effective in preventing death in this model.[15] Interestingly, each of these three compounds possessed a methyl-substituted nitrogen, and the chloro analogue of **6** (i.e., **7**) was inactive in this test after oral administration. Compound **23** was tested by iv administration and found to be active, indicating that in vivo inhibition observed in this model was a result of inhibition of 5-lipoxygenase. The poor correlation of in vitro inhibitory activity with activity in this in vivo model and the fact that only the *N*-methyl-substituted hydroxamates displayed systemic in vivo activity indicate that a combination of physical and pharmacokinetic characteristics (e.g., solubility, absorption, and metabolism), play important roles in determining in vivo activity, as does the intrinsic activity as an inhibitor of 5-lipoxygenase.

In contrast to the results in the oral model, there were several hydroxamates that caused dose-related inhibition in the arachidonic acid induced ear inflammation model when compounds were applied topically. The inhibition of swelling induced by arachidonic acid was accompanied by the reduction of associated myeloperoxidase activity (data not shown), a marker of leukocyte infiltration. The activity in this model seemed to be associated with inhibition of 5-lipoxygenase and was not dependent on the ability to inhibit 12-lipoxygenase since compounds **6** and **7** were equipotent in the ear model and as inhibitors of 5-lipoxygenase but significantly different as inhibitors of 12-lipoxygenase. Differences in the pharmacokinetic properties of these compounds probably account for the lack of correlation between the varied in vivo potencies and the equipotent in vitro inhibitory activities.

In summary, we have described a series of novel hydroxamates as potent 5- and 12-lipoxygenase inhibitors. The nature of the groups attached to the hydroxamate moiety has a great influence on the potency and selectivity as inhibitors of 5- and 12-lipoxygenase. The 5-lipoxygenase inhibitory activity was broadly elaborated after topical administration in a model of arachidonic acid mediated inflammation, but only the *N*-methyl-substituted hydroxamate displayed significant oral activity in systemic leukotriene-mediated anaphylaxis in guinea pigs.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with the assigned structure. NMR were recorded on a Varian EM-390 spectrometer at 90 MHz. IR spectra were recorded on a Perkin-Elmer Model 298 spectrophotometer. All compounds had elemental analyses for C, H, and N within ±0.4% of the theoretical value unless otherwise indicated. All hydroxamates were synthesized according the examples discussed below. Physical data for new compounds are listed in Table IV.

***N*-(3-Carbethoxypropyl)-*N*-hydroxy-4-phenylbutyramide (25).** **(a) Ethyl 4-[(Benzyloxy)amino]butyrate (25a).** A mixture of *O*-benzylhydroxylamine hydrochloride (4.8 g, 0.03 mol), ethyl 4-bromobutyrate (6 g, 0.031 mol), K_2CO_3 (10 g, 0.072 mol), and 0.8 g of KI in 50 mL of DMF was heated at 60 °C for 3 days. The reaction mixture was poured into water and extracted with EtOAc. The organic solution was washed with water, dried, and evaporated to dryness to give 8 g of crude oily product. This material was used without purification.

(b) *N*-(Benzyloxy)-*N*-(3-carbethoxypropyl)-4-phenylbutyramide (25b). A mixture of 4-phenylbutyric acid (5.8 g, 0.035

(15) (a) Huang, F.-C., et al. Submitted to *J. Med. Chem.* for publication. (b) Tomioka, K.; Yamada, T.; Mase, T.; Hara, H.; Murase, K. *Arzneim.-Forsch.* 1988, *38*, 682.

Table IV. Summary of Physical Data for Compounds Listed in Tables I and II

compd no.	mp, °C	formula[a]	yield, %
5	185–187	$C_{15}H_{15}NO_3$	75
6	94–96	$C_{16}H_{17}NO_3$	35
7	109–111	$C_{16}H_{16}ClNO_3$	21
8	94–95	$C_{18}H_{21}NO_3$[b]	58
9	142–143	$C_{22}H_{21}NO_3$	37
10	156–157	$C_{22}H_{21}NO_3$	58
11	114–115	$C_{22}H_{23}NO_3$	57
12	131–132	$C_{24}H_{25}NO_3$	47
13	140–141	$C_{23}H_{23}NO_3$	50
14	102–103	$C25H_{27}NO_3$	31
15	oil	$C_{24}H_{25}NO_3$	39
16	87–88	$C_{17}H_{19}NO_3$	37
17	128–130	$C_{17}H_{19}NO_3$	88
18	182–183	$C_{24}H_{23}NO_3 \cdot {}^1/_2H_2O$	34
19	oil	$C_{19}H_{18}N_2O_3 \cdot 2H_2O$[c]	15
20	144–145	$C_{19}H_{18}N_2O_3$	39
21	71–72	$C_{10}H_{13}NO_2$	28
22	79–80	$C_{17}H_{19}NO_2$	95
23	56–58	$C_{18}H_{21}NO_2$[d]	42
24	63–64	$C_{14}H_{19}NO_4$	56
25	oil	$C_{16}H_{23}NO_4$	74
26	74–75	$C_{12}H_{17}NO_3$	41
27	81–82	$C_{19}H_{23}NO_3$[e]	64
28	oil	$C_{12}H_{17}NO_2$	33
29	79–80	$C_{19}H_{23}NO_2$[f]	65
30	67–68	$C_{18}H_{21}NO_2$	53
31	79–80	$C_{20}H_{25}NO_2$[g]	68
32	155–156	$C_{12}H_{21}NO_3$	88
33	96–97	$C_{17}H_{19}NO_3$	75
34	oil	$C_{27}H_{31}NO_2$	85
35	oil	$C_{30}H_{29}NO_3$	67
36	81–82	$C_{18}H_{21}NO$	36

[a] All compounds were analyzed for C, H, and N and were within ±0.4% of theoretical value unless otherwise indicated. [b] N: calcd, 4.53; found, 3.88. [c] C: calcd, 63.27; found, 63.75. [d] C: calcd, 76.29; found, 75.84. [e] C: calcd, 72.82; found, 72.18. [f] C: calcd, 76.74; found, 75.71. [g] C: calcd, 71.56; found, 70.90.

mol), 6 mL of $SOCl_2$, and 5 drops of DMF was stirred at room temperature for 3 h. Excess thionyl chloride was removed in vacuo. The acyl chloride thus prepared was dissolved in 10 mL of CH_2Cl_2 and then was added dropwise to a solution of 8 g of the compound prepared above and 8 g of triethylamine in 20 mL of CH_2Cl_2 over a period of 20 min at room temperature and stirred overnight. The organic solution was washed with water, dried, and evaporated. The crude product was purified by dry column chromatography to give 4.5 g (33%) of oil: NMR ($CDCl_3$) δ 1.2 (t, 3 H), 1.9 (m, 4 H), 2.3 (m, 4 H), 2.6 (t, 2 H), 3.6 (t, 2 H), 4.1 (q, 2 H), 4.7 (s, 2 H), 7.3 (m, 10 H).

(c) Hydroxamate 25. A solution of 2.4 g (6 mmol) of **25b** and 300 mg of 5% Pd/C in 100 mL of ethanol was hydrogenated at 30 psi overnight. The reaction mixture was filtered and solvent evaporated to give 1.3 g (74%) of pure oily product: NMR ($CDCl_3$) δ 1, 2 (t, 3 H), 1.9–2.9 (m, 8 H), 3.7 (m, 2 H), 4.2 (q, 2 H), 7.3 (s, 5 H). Anal. ($C_{16}H_{23}NO_4$) C, H, N.

(d) *N*-(3-Carboxypropyl)-*N*-hydroxy-4-phenylbutyramide (24). A solution of 1 g (3.4 mmol) of the ester (**25**) obtained above in 5 mL of dioxane was treated with a solution of ethanolic KOH (460 mg in 4 mL of EtOH) and then stirred for 2 h at room temperature. The reaction mixture was acidified to pH 2 and extracted with ethyl acetate. The organic solution was washed with water, dried, and evaporated to give 0.7 g of product. Recrystallization from ethyl acetate/hexane gave 500 mg (56%) of **24**: mp 63–64 °C; NMR ($CDCl_3$) δ 1.8–2.6 (m, 10 H), 8.36 (m, 2 H), 7.1 (s, 5 H). Anal. ($C_{14}H_{19}NO_4$) C, H, N.

Compound **17** was prepared according to the procedure described above starting from ethyl 5-bromobutyrate.

***N*-Hydroxy-*N*-methyl-4-[(quinolin-2-yl)methoxy]phenylacetamide (20).** **(a) 4-[(Quinolin-2-yl)methoxy]phenylacetic Acid (20a).** A solution of 4-hydroxyphenylacetic acid (10.2 g, 0.0657 mol), 2-(chloromethyl)quinoline hydrochloride (28.1 g, 0.1312 mol), and 1 N NaOH (263 mL) in EtOH (139 mL) was stirred at 50 °C for 18 h. The mixture was then acidified to pH 5 by 1 N HCl solution and caused a precipitation. Suction filtration followed by washing with water and air drying afforded 14.4 g (75%) of the desired product as a light tan colored solid, essentially pure according to ^1H NMR, which was used without further purification: mp 154–155 °C; ^1H NMR (Me_2SO-d_6) δ 3.4 (s, 2 H), 5.28 (s, 2 H), 6.85–7.15 (m, 4 H), 7.4–8 (m, 5 H), 8.3 (s, 1 H).

(b) Hydroxamate 20. A mixture of the acid (**20a**) obtained above (14.4 g, 0.0491 mol), 1-hydroxybenzotriazole (8.3 g, 0.0614 mol), and dicyclohexylcarbodiimide (10.2 g, 0.0494 mol) in DMF (50 mL) was stirred at room temperature for 1 h and was suction filtered thereafter. To the filtrate was added *N*-methylhydroxylamine hydrochloride (4.2 g, 0.0502 mol) and triethylamine (7 mL, 0.0502 mol) in dichloromethane (75 mL). The mixture was stirred at room temperature for 18 h and was poured into water. The organic layer was extracted with 1 N NaOH (2 × 150 mL). The alkaline extracts were combined and then were acidified with 1 N HCl solution to pH 5 to cause a precipitation. Suction filtration followed by washing with water and air-drying gave an off-white solid. Recrystallization from EtOAc afforded 6.1 g (39%) of pure product as a white solid: mp 144 °C; ^1H NMR ($CDCl_3$/Me_2SO-d_6, 1:1) δ 3.25 (s, 3 H), 3.75 (s, 2 H), 5.33 (s, 2 H), 6.87–8.3 (m, 10 H).

Compounds **5–7**, **19**, **21**, **26**, and **28** were prepared according to the methodology described above.

***N*-Hydroxy-*N*-(2-phenylethyl)-4-(benzyloxy)phenylacetamide (11).** **(a) *N*-(Benzyloxy)-*N*-(1-phenylethyl)-4-(benzyloxy)phenylacetamide (11a).** A mixture of 2.42 g (0.01 mol) of 4-(benzyloxy)phenylacetic acid and 1.62 g (0.01 mol) of carbonyldiimidazole was heated at reflux for 1 h. Then 2.27 g (0.01 mol) of *O*-benzyl-*N*-(2-phenylethyl)hydroxylamine was added, and the reaction mixture was refluxed for 4 days. After evaporation of solvent, the crude product was purified by column chromatography to give 3.4 g (75%) of oily product: NMR ($CDCl_3$) δ 1.6 (d, 3 H), 3.7 (d, 2 H), 4.4 (q, 2 H), 5.05 (s, 4 H), 5.75 (q, 1 H).

Compounds **34–36** were prepared according to the methodology described above from appropriate starting materials.

(b) Hydroxamate 11. To a solution of 3.3 g (7.32 mmol) of the compound obtained above in 20 mL of CH_2Cl_2 at −40 °C was added dropwise a solution of BCl_3 (7.3 mL, 1 M) in 15 min. The reaction mixture was allowed to warm to room temperature slowly and then stirred for an additional 3 h. Excess reagent was decomposed by CH_3OH (3 mL). After evaporation of solvent, the crude product was purified by column chromatography to give 1.5 g (57%) of solid: mp 140–141 °C; ^1H NMR ($CDCl_3$) δ 1.6 (d, 3 H), 3.7 (s, 2 H), 4.9 (s, 2 H), 5.5 (q, 1 H), 7.0 (q, 4 H), 7.3 (m, 10 H).

Compounds **8–16**, **18**, **22**, **23**, **27**, and **29–33** were prepared according to the methodology described above with the appropriate starting materials.

Typical Procedure for the Preparation of *O*-Benzyl-*N*-substituted-hydroxylamines 2. Most other hydroxylamines were prepared according to this method.

***O*-Benzyl-*N*-(1-phenylethyl)hydroxylamine.** A mixture of 3.6 g (0.03 mol) of acetophenone, *O*-benzylhydroxylamine hydrochloride (4.8 g, 0.03 mol), and K_2CO_3 (4.2 g, 0.031 mol) in 50 mL of 95% ethanol was refluxed for 5 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic solution was washed with water, dried, and evaporated to give 4.4 g (65%) of oily product.

To a stirred solution of the crude product obtained above and 6.5 mL (0.21 mol) of borane/pyridine in 25 mL of ethanol cooled below 5 °C was added dropwise 65 mL of 10% HCl during 15 min, and the mixture was then stirred for an additional 10 min at room temperature. The reaction mixture was made alkaline with sodium carbonate solution and then extracted with CH_2Cl_2. The combined extracts were dried, evaporated, and purified by column chromatography to give 2.7 g (61%) of oily product: ^1H NMR ($CDCl_3$) δ 1.25 (d, 3 H), 4.2 (q, 1 H), 4.6 (s, 2 H), 7.2 (m, 10 H).

Biological Assays. Rat Polymorphonuclear Leukocyte (PMN) 5-Lipoxygenase. This assay, in which intact, glycogen-elicited PMNs, obtained from four or five Sprague-Dawley rats (250 g), were challenged with exogenous [^{14}C]arachidonic acid (4 μM) and calcium ionophore A23187 (0.8 μM), in a total volume of 250 μL, was performed as described.[16] Cells were preincubated

for 5 min at 30 °C with test compounds and 1 μM indomethacin, which were dissolved in dimethyl sulfoxide (final assay concentration ≤ 0.1%). Three minutes after challenge, the reaction was terminated by the addition of 10 μL of 1 M citric acid/5 mM NDGA, and tritiated (10 000 dpm) 5-HETE and unlabeled (35 μg) 5-HETE were added prior to extraction with 500 μL of chloroform/methanol (5:1). Radioactivity in the organic layer was chromatographed on flexible sheets of silica gel developed with the upper phase of ethyl acetate/isooctane/water/acetic acid (11:5:10:1). Spots of 5-HETE were located by iodine staining and were cut out and quantitated by two-channel scintillation spectroscopy. All assays were performed in triplicate, and the average amount of 5-HETE produced per million cells was 79 ± 2 pmol (mean ± SE; N = 162). For all enzyme assays, compounds were tested in duplicate at three or more concentrations and IC_{50} values graphically determined if significant inhibition was obtained. Values of IC_{50} differing by a factor of 2 or more were significantly different. The standard inhibitors RG 5901 and NDGA had IC_{50} values of 0.12 ± 0.04 μM (N = 6) and 0.61 ± 0.15 μM (N = 3), respectively.

Human PMN 5-Lipoxygenase. For each experiment, 50 mL of blood was taken by venipuncture from a healthy volunteer. Clotting was prevented by 1 mL of 0.1 M EDTA. PMN were purified by a two-step sedimentation procedure employing dextran/saline to separate white cells from red cells followed by a discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient, formed from layers having densities of 1.100, 1.082, and 1.072. Leukocytes from the first step were suspended in 2 mL of saved dextran/plasma and were layered over Percoll and centrifuged at 400g for 20 min at 4 °C. Cells at the interface of the 1.082 and 1.100 density layers were collected and washed in buffer [25 mM Hepes, 120 mM NaCl, 5 mM KCl, and 0.03% human serum albumin (Sigma), pH 7.6]. Contaminating red blood cells were lysed by 1-min exposure to 0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 M EDTA, pH 7.0. Purified PMNs (>90%) were washed and resuspended in PBS, supplemented with calcium and magnesium (Grand Island Biological Co., Grand Island, NY), as well as 1 mg/mL glucose and 1 μM indomethacin. The assay measured the production of 5-HETE after challenge with calcium ionophore and [^{14}C]arachidonic acid as with rat PMNs above with a 3-min incubation.

In 94 experiments, 92 ± 3 (mean ± SE) pmol of 5-HETE per million PMNs was produced by control cells; standard inhibitors RG 5901 (10 μM) and NDGA (1 μM) gave 82 ± 2% (N = 58) and 56 ± 2% inhibition (N = 39) (mean ± SE), respectively.

Cell-Free Guinea Pig PMN 5-Lipoxygenase. Guinea pig peritoneal PMNs were elicited by ip injection of 0.1% casein in Penn-Dutch guinea pigs followed 18 h later by peritoneal lavage with calcium- and magnesium-free PBS supplemented with 1.0% sodium citrate. Cells were washed three times in 50 μM phosphate buffer (pH 7.4) containing 0.5 mM EDTA by sequentially pelleting at 250g for 5 min and resuspension in buffer. Cells were broken by sonication (Branson microprobe for 50 s at setting 4 with 50% duty cycle) at 0 °C, and the cytosol was collected after centrifugation at 43000g for 15 min. The cytosol (about 0.1 mg of protein in 250 μL of 50 mM potassium phosphate buffer, pH 7.4) was preincubated at 0 °C for 5 min with test compound dissolved in DMSO (final concentration = 0.1%). The reaction was initiated by the addition of 6 μM [^{14}C]arachidonic acid supplemented with 1.5 mM $CaCl_2$, 1 mM glutathione, 2 mM ATP, and 0.02 mg/mL BSA. The reaction was quenched after 2 min by the addition of 5 μL of 2 M citric acid (final pH brought to 3) and 10 μM NDGA. The extraction, isolation, and quantitation of [^{14}C]-5-HETE was as described for the rat PMN 5-lipoxygenase assay. The enzyme preparation had a K_m of 24 μM and a V_{max} of 8.5 nmol/(min·mg of protein). Activities of compounds were determined as described above. The standards RG 5901 and NDGA had IC_{50} values of 3.9 ± 1.6 μM (N = 4) and 0.16 ± 0.04 μM (N = 3), respectively.

Cell-Free Rat Platelet 12-Lipoxygenase. Platelets were obtained from Sprague-Dawley rats, and cell-free 12-lipoxygenase was prepared according to Chang et al.[17] In the presence of 100 μM glutathione, the soluble enzyme converted arachidonic acid to 12-HETE as the only detectable product. Tris buffer (25 mM, pH 7.4, supplemented with 1 μM indomethacin), test compound (dissolved in DMSO; final DMSO concentration ≤ 0.1%), glutathione (100 μM), and cytosolic enzyme (0.4 mg/mL) were preincubated in a total volume of 250 μL for 5 min at 30 °C. [^{14}C]Arachidonic acid (6 μM in IC_{50} determinations, and 0.5–8 μM in kinetic determinations) was added, and the reaction was quenched with 10 μL of 1 M citric acid/5 mM NDGA after 3 min. Test compounds were dissolved in DMSO (final concentration ≤ 0.1%). Samples were extracted with chloroform/methanol (5:1; 500 μL) containing 10 000 dpm of [^{3}H]-12-HETE and 30 μg of 15-HETE (which cochromatographs with 12-HETE in the TLC system). The organic layer was evaporated to dryness, and the residue was chromatographed on flexible silica gel sheets as with the 5-lipoxygenase assay above. Spots of 12-HETE were located by iodine staining, and the amount of ^{14}C-labeled product was quantitated by two-channel scintillation spectroscopy. The enzyme preparation had a K_m of 16 μM and a V_{max} of 0.8 pmol/(min·mg of protein). At 1 μM, NDGA caused 51 ± 2% inhibition (N = 20) of 12-lipoxygenase run under the screening conditions to compare activities of compounds.

Rat PMN Cyclooxygenase. Glycogen-elicited peritoneal PMNs from three Sprague-Dawley rats were homogenized with a Polytron in pH 8.2, 0.1 M Tris·HCl buffer containing 10 μM NDGA (to inhibit 5-lipoxygenase) at 0 °C. Test compounds (dissolved in DMSO; final DMSO concentration ≤ 0.1%) and homogenate were preincubated for 5 min at 37 °C, followed by the addition of [^{14}C]arachidonic acid (8 μM) and glutathione and epinephrine (both 4 mM) in a total volume of 250 μL. The reaction was quenched after 10 min by the addition of 10 μL of 1 M citric acid, as well as 10 000 dpm of [^{3}H]TxB_2 and 8 μg each of unlabeled TxB_2 and $PGF_{2\alpha}$. Extraction of the assay mixture with chloroform/methanol and chromatography of the residue from the organic phase were as with the rat PMN 5-lipoxygenase assay, except the flexible thin-layer sheets were developed with the upper phase of ethyl acetate/isooctane/water/acetic acid (11:5:10:2). The amount of TxB_2 and $PGF_{2\alpha}$ synthesized was determined by two-channel scintillation spectroscopy. Such preparations were run in triplicate and produced, on the average, 150 ± 49 pmol of TxB_2 (mean ± SE), and the ratio of $PGF_{2\alpha}$ to TxB_2 was ca. 0.6. Smaller amounts of PGD_2 and PGE_2 were also detected. Indomethacin had an IC_{50} of 0.1 μM.

Leukotriene D_4 Binding Assay. The procedure of Mong et al.[18] with modifications[14] was employed in measuring the effect of test compounds on the binding interaction of LTD_4 to receptors in homogenates of guinea pig lungs. Incubations were performed at 25 °C in 10 mM PIPES, 5 mM Ca and $MgCl_2$, and 1 mM glycine and cysteine. Specific binding was defined as that inhibited by a 1000-fold excess of unlabeled LTD_4. LTD_4 had a K_d of 0.2 nM in this assay, and the standard leukotriene antagonist FPL 55712 gave a K_i of 0.94 ± 0.02 μM (mean ± SD) in three experiments.[14]

Leukotriene-Induced Contraction of Guinea Pig Lung Strips. Parenchymal strips were cut from guinea pig lungs and suspended in tissue bath as described previously.[14] The myotropic response to 1 μM histamine was used to normalize the responses to other spasmogens and to verify tissue responsiveness throughout the experiment. Tissues were challenged with 0.2 nM LTC_4 or 0.4 nM LTD_4. Under these conditions conversion to LTD_4 and LTE_4 was possible. Most likely the responses of LTC_4 and LTD_4 were not due to conversion to LTE_4 as the first two are more potent than LTE_4. Whether the guinea pig lung contains distinct LTC_4 and LTD_4 receptors is controversial. The use of serine borate to prevent conversion from LTC_4 to LTD_4 makes the data difficult to interpret since tissues characterized prior to the ad-

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dition of serine borate respond appreciably differently to all spasmogens after addition of serine borate. The tissues were preincubated with test compounds for 5 min before challenge with leukotriene. Values of IC_{50} were reproducible in this assay within an error of less than 30%. FPL 55712, a standard antagonist of LTC_4-induced contractions, had an IC_{50} of 0.51 ± 0.13 μM (mean ± SD; N = 9) against 0.2 nM LTC_4 in this assay.

Leukotriene-Mediated Anaphylaxis in Guinea Pigs. Male Hartley guinea pigs were immunized by ip injections of ovalbumin and *Salmonella typhosa* lipopolysaccharide adjuvant (2.7 mg/kg and 20 μg, ip, respectively). Two weeks later, six guinea pigs were placed into separate compartments of an aerosol chamber and were challenged for 30 s with ovalbumin solution (1% w/v) aerosolized by a DeVilbiss nebulizer. Ten minutes after the start of the challenge, the animals were removed from the aerosol chamber and the incidence of deaths at 30 min postchallenge was recorded. Test compounds were administered orally as solutions in PEG 400 1 h before antigen challenge. Twenty minutes before challenge, the animals were pretreated with pyrilamine (2 mg/kg, ip), indomethacin (10 mg/kg, ip), and propranolol (1 mg/kg, ip). Activity is expressed as the percent inhibition of mortality compared to vehicle-treated animals. Significance was determined by a χ^2 test. The standard inhibitor phenidone (50 mg/kg, ip) inhibited mortality 83% ($p < 0.05$) with this protocol.

Arachidonic Acid Induced Murine Ear Inflammation. This assay was carried out according to Young et al.[12] Topically applied arachidonic acid produces an inflammatory reaction characterized by increased vascular permeability and infiltration of PMNs. One ear of male DBA/2J mice was treated topically with test compound or ethanol vehicle 60 min before the application of arachidonic acid (2 mg/ear). Increases in ear weight due to arachidonic acid treatment were determined 1 h later. Ear swelling, taken as a measure of inflammation, was compared in drug- and vehicle-treated groups. Statistical differences were determined by using Student's t test. AA-861, a standard inhibitor of 5-lipoxygenase, gave an ED_{50} of 0.15 mg/ear by this protocol.

Acknowledgment. We thank the following individuals for their excellent technical skills: J. Auerbach, M. Clearfield, V. Dally-Meade, D. Donigi-Gale, S. Hyman, R. Ingram, N. Jariwala, Y. Karode, R. Liu, G. Schuessler, D. Sweeney, and J. Travis. The members of the Analytical Department at Rorer Central Research are gratefully acknowledged for their high-quality and timely analyses of the synthesized compounds. The excellent advice and comments of I. Weinryb and A. Khandwala are also gratefully acknowledged.

3,4-Dihydro-2*H*-1-benzopyran-2-carboxylic Acids and Related Compounds as Leukotriene Antagonists

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Evaluation of a series of 3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acids linked to the 2-hydroxyacetophenone pharmacophore present in the standard peptidoleukotriene antagonist FPL 55712 (1) has led to the discovery of Ro 23-3544 (7), an antagonist possessing greater potency and duration of action vs LTD_4 than the standard (aerosol route of administration, guinea pig bronchoconstriction model). Interestingly, this compound also potently inhibited bronchoconstriction induced by LTB_4 whereas 1 did not. Attempts to establish structure–activity relationships in this series involved modifications in the 2-hydroxyacetophenone moiety, the linking chain, and the chroman system. All variations produced analogues which were either inactive or possessed reduced potency relative to acid 7. Optical resolution of 7 was achieved by two methods. Absolute configurations of the enantiomers were determined via X-ray crystallographic analyses of an intermediate as well as a salt of the *S* enantiomer. Although the enantiomers exhibited similar potencies in in vitro assays and in vivo when administered intravenously, significant differences were observed in the guinea pig bronchoconstriction model vs LTC_4 and LTD_4 when administered by the aerosol route (*S* antipode 15-fold more potent). The properties of 7 have been compared with several recently reported leukotriene antagonists.

Nearly a decade has passed since Samuelsson's elucidation of the peptidoleukotriene (LT) structures and the confirmation that these novel lipid derivatives comprise the slow-reacting substance of anaphylaxis (SRS, SRS-A).[1] During this period, pure LTC_4, LTD_4, LTE_4, and radiolabeled versions thereof have become readily available through total synthesis[2] thus facilitating the establishment of pharmacological assays to detect novel LT antagonists and biosynthesis inhibitors. Not surprisingly, a major worldwide effort to discover such compounds has ensued.[3] The motivation for this explosion in synthetic, medicinal, and biochemical research has been provided by the steadily accumulating evidence that the LTs are intimately involved in the mediation of many serious allergic and inflammatory disorders.[4] On the other hand, since mediators such as histamine, platelet activating factor, thromboxane, and chemotactic peptides may also be involved, the relative importance of the LTs in these disease states will only be defined upon clinical evaluation of selective LT antagonists and biosynthesis inhibitors. It appears that we are finally approaching the time when such information will be available as clinical results involving the first

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