Structure-Activity Analysis of a Class of Orally Active Hydroxamic Acid Inhibitors of Leukotriene Biosynthesis

James B. Summers,* Bruce P. Gunn, Jonathan G. Martin, Michael B. Martin, Hormoz Mazdiyasni, Andrew O. Stewart, Patrick R. Young, Jennifer B. Bouska, Andrew M. Goetze, Richard D. Dyer, Dee W. Brooks, and George W. Carter

Immunosciences Research Area, Department 47K, Abbott Laboratories, Abbott Park, Illinois 60064. *Received March 30, 1988*

The nature of the carbonyl and nitrogen substituents of hydroxamic acids has a major influence on the biological profile of these compounds. Hydroxamates with small groups such as methyl appended to the carbonyl and relatively large nitrogen substituents generally have longer duration in vivo, produce greater plasma concentrations, and often are more potent inhibitors of in vivo leukotriene biosynthesis than hydroxamic acids with the opposite arrangement. The structure-activity relationships that describe in vitro 5-lipoxygenase inhibitory activity and in vivo leukotriene biosynthesis inhibitory potency for a group of these hydroxamic acids were investigated. While most of the compounds examined were potent in vitro inhibitors of 5-lipoxygenase, their in vivo potencies varied widely. This discrepancy was usually attributable to differences in bioavailability. Substitution patterns are described that produce potent, orally active inhibitors of leukotriene biosynthesis.

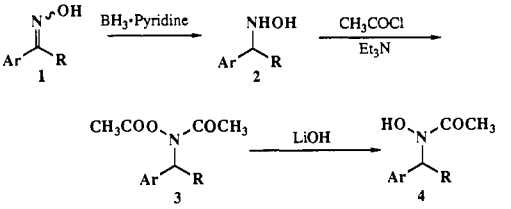
The control of leukotriene biosynthesis through the inhibition of the enzyme 5-lipoxygenase represents a potential new method of treating diseases such as asthma, rheumatoid arthritis, and psoriasis. The hydroxamic acid functionality can be incorporated into a wide variety of molecules to produce potent inhibitors of 5-lipoxygenase.[1-4] We have shown that although many hydroxamic acids are potent inhibitors of the enzyme in vitro, they fail to produce significant inhibition in vivo because they are rapidly metabolized to the corresponding carboxylic acid.[2] Recently we reported that hydroxamic acids having a small group attached to the carbonyl of the hydroxamate functionality and a relatively large substituent appended to the nitrogen display superior pharmacokinetic characteristics compared to compounds with the opposite substitution pattern.[3] These so called "type B hydroxamic acids"[5] are much less prone to rapid metabolism to the corresponding carboxylic acid and are often potent orally active inhibitors of leukotriene biosynthesis.

We now wish to report structure-activity relationships that describe the in vitro 5-lipoxygenase inhibitory activity and in vivo leukotriene biosynthesis inhibitory potency of some type B hydroxamic acids.

Chemistry

The compounds reported here were generally prepared as illustrated in Scheme I. Oxime 1 is reduced with borane-pyridine complex in the presence of 6 N HCl.[6] The resulting hydroxylamine **2** was converted to **3** by treatment with 2.2 equiv of the appropriate acid chloride. The use of this *N,O*-diacyl intermediate **3** avoided the mixtures of *N*- and *O*-monoacyl compounds that often resulted when only 1 equiv of acid chloride was used. The *O*-acetate was then selectively removed with lithium hydroxide in 2:1 2-propanol-water to produce the desired hydroxamic acid **4**.

Scheme I



Biological Results and Discussion

Type A versus Type B Hydroxamic Acids. Two of the more potent compounds described in our previous report[3] were **5** (Abbott-63162) and **6** (Abbott-62800). When administered orally to rats, these type B hydroxamic acids produced higher plasma concentrations and longer duration than their type A analogues in which the nitrogen and carbonyl substituents are transposed, **7** and **8**. For example, **5** produced peak plasma concentrations in the rat of 140 μM at 3 h after a 100 mg/kg oral dose, while its congener **7** reached a peak level of only 15 μM 30 min after dosing.[3] When administered intravenously (iv), **5** exhibited a plasma half-life 2.5 times that of **7** (1.1 and 0.4 h, respectively, following a 20 mg/kg dose, iv). Although **7** is extensively metabolized to the corresponding carboxylic acid, **5** primarily undergoes other routes of metabolism (vide infra).

As a result of their superior pharmacokinetic behavior, type B hydroxamic acids **5** and **6** were significantly more potent than **7** and **8** in inhibiting in vivo leukotriene biosynthesis in the rat peritoneal anaphylaxis model.[7-9] Following oral administration, **5** was 5-fold more potent than **7** [ED_{50} values of 28 μmol/kg (8 mg/kg) and 141 μmol/kg (40 mg/kg), respectively]. Hydroxamate **6** had an ED_{50} of 68 μmol/kg (17 mg/kg) in this model, while **8**

(1) Summers, J. B.; Mazdiyasni, H.; Holms, J. H.; Ratajczyk, J. D.; Dyer, R. D.; Carter, G. W. *J. Med. Chem.* **1987**, *30*, 574.
(2) Summers, J. B.; Gunn, B. P.; Mazdiyasni, H.; Goetze, A. M.; Young, P. R.; Bouska, J. B.; Dyer, R. D.; Brooks, D. W.; Carter, G. W. *J. Med. Chem.* **1987**, *30*, 2121.
(3) Summers, J. B.; Gunn, B. P.; Martin, J. G.; Mazdiyasni, H.; Stewart, A. O.; Young, P. R.; Goetze, A. M.; Bouska, J. B.; Dyer, R. D.; Brooks, D. W.; Carter, G. W. *J. Med. Chem.* **1988**, *31*, 3.
(4) Other examples of hydroxamic acid containing inhibitors of 5-lipoxygenase include: (a) Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Wright, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 1503. (b) Kerdesky, F. A. J.; Schmidt, S. P.; Holms, J. H.; Dyer, R. D.; Carter, G. W.; Brooks, D. W. *J. Med. Chem.* **1987**, *30*, 1177. (c) Musser, J. H.; Kubrak, D. M.; Chang, J.; Lewis, A. J. *J. Med. Chem.* **1986**, *29*, 1429. (d) Sweeney, D.; Travis, J.; Gordon, R.; Coutts, S.; Jariwala, N.; Haung, F.; Carnathan, G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 540. (e) Jackson, W. P.; Islip, P. J.; Kneen, G.; Pugh, A.; Wates, P. J. *J. Med. Chem.* **1988**, *31*, 499.
(5) The terms type A and type B hydroxamic acids are used as defined previously.[3] Type A hydroxamates have small substituents on the hydroxamic acid nitrogen and large groups appended to the carbonyl. Type B hydroxamates have the reverse substitution pattern.
(6) Kawase, M.; Kikugawa, Y. *J. Chem. Soc., Perkin Trans. 1* **1979**, 643.
(7) Young, P. Rr.; Dyer, R. D.; Carter, G. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 1185.
(8) Rapp, H. J. *J. Physiol. (London)* **1961**, *158*, 35.
(9) Orange, R. P.; Valentine, M. D.; Austen, K. F. *Science (Washington, D.C.)* **1967**, *157*, 318.

0022-2623/88/1831-1960$01.50/0

Table I. Inhibitory Activities of Hydroxamic Acids of Structure I

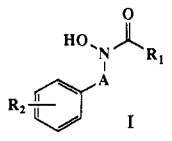
no.	R_1	A	R_2	mp, C	method[a]	formula[b]	in vitro 5-LO inhibn: IC_{50},[c] μM	in vivo LT inhibn: ED_{50},[d] μmol/kg
5	CH_3	$CHCH_3$	4-$C_6H_5CH_2O$	115–117	5	$C_{17}H_{19}NO_3$	0.37 (0.24–0.51)	28 (17–45) (8 mg/kg)
6	CH_3	$CHCH_3$	4-C_4H_9O	68–70	5	$C_{14}H_{21}NO_3$	0.79 (0.55–1.1)	68 (44–124) (17 mg/kg)
7[e]	CH_3	$CHCH_3$	4-$C_6H_5CH_2O$	121–125	7	$C_{17}H_{19}NO_3$	0.28 (0.27–0.28)	141 (63–1100) (40 mg/kg)
8[e]	CH_3	$CHCH_3$	4-C_4H_9O	oil	8	$C_{14}H_{21}NO_3$	0.78 (0.70–0.88)	62 ± 5% at 400 μmol/kg
9	H	$CHCH_3$	4-$C_6H_5CH_2O$	107-109	5	$C_{16}H_{17}NO_3$	0.94 (0.86–1.1)	NS at 200 μmol/kg[f]
10	H	$CHCH_3$	4-C_4H_9O	75–77	5	$C_{13}H_{19}NO_3$	1.9 (1.7–2.2)	NS at 200 μmol/kg[f]
11	$CH(CH_3)_2$	$CHCH_3$	4-$C_6H_5CH_2O$	141–143	5	$C_{19}H_{23}NO_3$	0.47 (0.41–0.57)	NS at 200 μmol/kg[f]
12	C_6H_5	$CHCH_3$	4-$C_6H_5CH_2O$	134–135	5	$C_{22}H_{21}NO_3$	0.69 (0.64–0.74)	NS at 200 μmol/kg[f]
13	C_6H_5	$CHCH_3$	4-C_4H_9O	69–71	5	$C_{19}H_{23}NO_3$	1.3 (1.1–1.4)	NS at 200 μmol/kg[f]
14	CH_3	CH_2	4-$C_6H_5CH_2O$	112–114	5	$C_{16}H_{17}NO_3$	0.46 (0.31–0.61)	48 ± 8% at 200 μmol/kg
15	CH_3	CH_2	4-C_4H_9O	70–72	5	$C_{13}H_{19}NO_3$	1.1 (0.9–1.3)	71 ± 3% at 200 μmol/kg[g]
16	CH_3	$CH(CH_2CH_3)$	4-C_4H_9O	77–79	5	$C_{15}H_{23}NO_3$	0.74 (0.60–1.0)	188 (142–241) (50 mg/kg)
17	CH_3	$CHCH(CH_3)_2$	4-C_4H_9O	oil	5	$C_{16}H_{25}NO_3$	0.88 (0.82–0.95)	40 ± 8% at 200 μmol/kg
18	CH_3	$CH(C_4H_9)$	4-C_4H_9O	oil	5	$C_{17}H_{27}NO_3$	0.57 (0.47–0.70)	NS at 200 μmol/kg[f]
19	CH_3	$CHCH(CH_3)_2$	4-$C_6H_5CH_2O$	136–139	5	$C_{19}H_{23}NO_3$	0.78 (0.75–0.81)	NS at 200 μmol/kg[f]
20	CH_3	$C(CH_3)_2$	4-C_4H_9O	87–88	20	$C_{15}H_{23}NO_3$	0.65 (0.47–0.82)	NS at 200 μmol/kg[f]
21	CH_3	CH_2CH_2	4-C_4H_9O	68–69	21	$C_{14}H_{21}NO_3$	0.38 (0.31–0.45)	NS at 200 μmol/kg[f]
22	CH_3	$CH_2CH_2CH_2$	4-C_4H_9O	77–79	22	$C_{15}H_{23}NO_3$	0.32 (0.28–0.36)	56 ± 5% at 200 μmol/kg
23	CH_3	CH_2CHCH_3	4-C_4H_9O	58–60	21	$C_{15}H_{23}NO_3$	0.54 (0.45–0.60)	74 ± 2% at 200 μmol/kg
24	CH_3	$CH_2CH_2CHCH_3$	4-C_4H_9O	58–59	24	$C_{16}H_{25}NO_3$	0.36 (0.33–0.40)	120 (80–280) (35 mg/kg)
25	CH_3	OCH_2CH_2	4-C_4H_9O	72–74	25	$C_{14}H_{21}NO_4$	0.75 (0.62–0.88)	84 (50–180) (22 mg/kg)
26	CH_3	$CHCH_3$	2-$C_6H_5CH_2O$	87–89	5	$C_{17}H_{19}NO_3$	0.76 (0.66–0.88)	NS at 200 μmol/kg[f]
27	CH_3	$CHCH_3$	3-$C_6H_5CH_2O$	77–78	5	$C_{17}H_{19}NO_3$	0.33 (0.28–0.37)	68 (45–124) (19 mg/kg)
28	CH_3	$CHCH_3$	3-C_4H_9O	68–71	5	$C_{14}H_{21}NO_3$	0.39 (0.32–0.46)	57 ± 16% at 200 μmol/kg
29	CH_3	$CHCH_3$	4-(4-pyridyl)CH_2O	152–153	5	$C_{16}H_{18}N_2O_3$	2.6 (2.2–3.0)	32 (25–42) (9 mg/kg)
30	CH_3	$CHCH_3$	4-(2-quinolyl)CH_2O	144–145	5	$C_{20}H_{20}N_2O_3$	0.40 (0.35–0.45)	51 (36–71) (17 mg/kg)
31	CH_3	$CHCH_3$	4-$C_6H_5CH_2S$	119–121	31	$C_{17}H_{19}NO_2S$	0.19 (0.15–0.23)	NS at 200 μmol/kg[f]
32	CH_3	$CHCH_3$	4-$C_6H_5CH{=}CH$	176–178	32	$C_{18}H_{19}NO_2$	0.29 (0.25–0.32)	NS at 200 μmol/kg[f]
33	CH_3	$CHCH_3$	4-C_6H_5O	97–98.5	5	$C_{16}H_{17}NO_3$	0.37 (0.32–0.43)	47 (27–77) (12 mg/kg)
34	CH_3	$CHCH_3$	4-$C_6H_5CH_2CH_2O$	90–92	5	$C_{13}H_{21}NO_3$	0.41 (0.36–0.48)	49 (27–91) (15 mg/kg)
35	CH_3	$CHCH_3$	4-$C_6H_5CH(CH_3)O$	oil	5	$C_{18}H_{21}NO_3$	0.50 (0.50–0.64)	92 (67–136) (28 mg/kg)
36	CH_3	$CHCH_3$	4-$C_6H_5CH(CH(CH_3)_2)O$	oil	5	$C_{20}H_{25}NO_3$	0.61 (0.51–0.73)	NS at 200 μmol/kg[f]
37	CH_3	$CHCH_3$	4-(2,4,6-$CH_3)_3C_6H_2CH_2O$	132–138	5	$C_{20}H_{25}NO_3$	0.62 (0.54–0.71)	NS at 200 μmol/kg[f]
38	CH_3	$CHCH_3$	4-(9-fluorenyl)O	oil	5	$C_{23}H_{21}NO_3$	4.9 (4.2–6.2)	NS at 200 μmol/kg[f]
39	CH_3	$CHCH_3$	4-$C_6H_5OCH(CH_3)$	oil	39	$C_{18}H_{21}NO_3$	0.46 (0.43–0.50)	47 ± 8% at 200 μmol/kg

[a] See compound number in the Experimental Section for analogous preparation. [b] Elemental analysis (C, H, N) within ±0.4 of the theoretical value. [c] IC_{50} with 95% confidence limits in parentheses for the in vitro inhibition of 5-lipoxygenase from the 20000*g* supernatant of RBL-1 cells. [d] ED_{50} with 95% confidence limits in parentheses or mean percent inhibition values ± SEM for inhibition in the rat peritoneal anaphlaxis model. [e] Type A hydroxamic acid. [f] Nonsignificant ($p > 0.05$) inhibition (typically <40%). [g] NS at 60 μmol/kg.

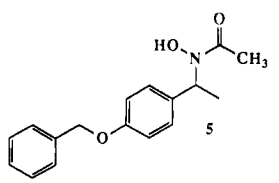
exhibited less than 40% inhibition at 120 μmol/kg.

Hydroxamates **5** and **6** were relatively specific in vitro inhibitors of 5-lipoxygenase. Both compounds displayed less than 50% inhibition at 100 μM against sheep seminal vesical cyclooxygenase.

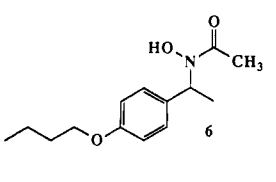
In view of the favorable oral activity and bioavailability of type B hydroxamic acids **5** and **6**, an investigation of their structure–activity relationships was conducted. Three sites on these molecules were systematically modified in order to define the optimum substitution pattern. These sites (R_1, A, and R_2) are shown in generic structure I. Data for the compounds in this study are summarized in Tables I and II.



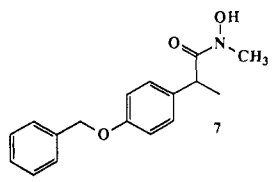
I



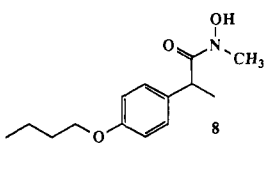
5



6



7



8

Table II. Rat Plasma Concentration of Selected Compounds 1 h following an Oral Dose of 200 μmol/kg

no.	plasma concn,[a] μM	no.	plasma concn,[a] μM
5	70.0 ± 2.6	17	<2.0[b]
6	65.7 ± 2.0	**29**	152 ± 1
9	2.3 ± 2.2	**33**	52 ± 7
12	<2.0[b]	**35**	106 ± 20
14	2.1 ± 0.6	**36**	10.7 ± 1.7

[a] Average concentrations ± the range of values from two determinations. [b] Plasma concentration for all determinations were less than the indicated level of detection.

Acyl Substituent R_1. When R_1 is hydrogen, as in formyl hydroxamates **9** and **10**, a dramatic loss of in vivo potency relative to acetyl hydroxamic acids **5** and **6** was observed. Both **9** and **10** failed to produce significant inhibition of leukotriene biosynthesis in the rat peritoneal anaphylaxis model when administered orally at 200 μmol/kg. Although **9** and **10** are about 2–3-fold less potent than **5** and **6** as in vitro inhibitors of RBL-1 5-lipoxygenase, this alone did not appear to account for the marked loss of in vivo activity. Therefore the bioavailability of **9** in rat was examined. One hour following an oral dose of 200 μmol/kg (the same time and dose at which in vivo activity was measured), plasma concentrations of **9** were only 2.3 μM. This is 30 times less than that observed for **5** and is consistent with the lack of significant in vivo inhibitory activity.

When R_1 is a group larger than methyl, compounds exhibiting properties inferior to **5** and **6** also resulted. Isobutyrate **11** and benzoates **12** and **13** produced no significant in vivo inhibition in the rat peritoneal anaphylaxis model following an oral dose of 200 μmol/kg. This oc-

curred even though the in vitro inhibitory activity of these compounds was similar to that of **5** and **6**. Bioavailability again appeared to account for the different in vivo potencies. When **12** was administered orally at 200 μmol/kg to rats, no compound could be detected in the plasma 1 h after dosing. Although we cannot rule out rapid metabolism, elimination, or tissue distribution, the fact that we are unable to detect any metabolites in the plasma suggests that **12** was poorly absorbed.

Among the variations evaluated at R_1, the acetyl hydroxamic acids provided the best activity.

Linker Group, A. A single-carbon linker with a methyl side chain ($CHCH_3$) connects the aryl ring to the hydroxamic acid group in **5** and **6**. In compounds such as **14** and **15**, where a methylene unit was used as the linker, lower in vivo activities were observed. Hydroxamate **14** produced only 48% inhibition in the rat peritoneal anaphylaxis model at 200 μmol/kg, po, while **15** produced 71% at this dose and no significant inhibition at 60 μmol/kg, po. Both compounds were potent inhibitors of 5-lipoxygenase and exhibited similar activities to those of **5** and **6**, respectively. The discrepancy between in vitro and in vivo activity again appeared to be attributable to differences in bioavailability. The plasma concentrations of **14** in the rat were only 2.1 μM 1 h after 200 μmol/kg, po.

The in vivo potencies of **16–19** appeared to be inversely related to the size of the side chain attached to the one-carbon linker. As the side chain was increased from methyl (**6**) to ethyl (**16**) to isopropyl (**17**) to butyl (**18**), the potency in the peritoneal anaphylaxis model decreased steadily (ED_{50} = 68 μmol/kg, 188 μmol/kg, 40% inhibition at 200 μmol/kg, and no significant inhibition at 200 μmol/kg, respectively). As with previous examples, decreased bioavailability appears to be consistent with this observation. Hydroxamate **19**, having an isobutyl side chain, exhibited no significant inhibitory activity in vivo and could not be detected in the plasma 1 h after an oral dose of 200 μmol/kg.

In **20** the hydroxamic acid and aryl ring are connected by a single-carbon linker bearing two methyl side chains. The addition of the second methyl branch reduced in vivo potency compared with **6**. No significant inhibition of leukotriene biosynthesis was achieved following a 200 μmol/kg oral dose in the rat.

Linker groups of more than one carbon atom were also evaluated. In hydroxamate **21** and **22** the hydroxamic acid nitrogen is connected to the aryl ring by two- and three-carbon linkers, respectively. Although these compounds were potent in vitro inhibitors of 5-lipoxygenase, they were relatively weak in vivo. Hydroxamic acids **23** and **24** also incorporate two- and three-carbon linkers, but they have a methyl side chain branching from the carbon adjacent to the hydroxamic acid nitrogen. This side chain improved in vivo potency; **24** exhibited an ED_{50} of 120 μmol/kg (35 mg/kg) in the rat peritoneal anaphylaxis model. The OCH_2CH_2 group links the hydroxamate and aryl portions of **25**. This compound displayed good in vitro potency (IC_{50} = 0.75) and also inhibited leukotriene biosynthesis in the rat (ED_{50} = 84 μmol/kg, 22 mg/kg).

The greatest in vivo leukotriene inhibitory potency was achieved by the $CHCH_3$ spacer found in **5** and **6**.

Phenyl Ring Substituent, R_2. Analogues of **5** and **6** were prepared in which the R_2 substituents were moved to the ortho and meta positions. All of the isomers were potent in vitro inhibitors of 5-lipoxygenase. However, the ortho analogue of **5** (**26**) exhibited no significant inhibition of leukotriene biosynthesis following oral dosing at 200 μmol/kg. The meta compounds, **27** and **28**, exhibited in vivo activity, albeit less than their para analogues.

R_2 substituents related to the benzyloxy group found in **5** were also studied. In hydroxamate **29**, the benzyloxy group was replaced with a 4-pyridylmethoxy group. Although **29** was 7-fold less potent than **5** as an inhibitor of 5-lipoxygenase in vitro (2.6 and 0.37 μM, respectively), it exhibited nearly identical activity in vivo (ED_{50} = 32 and 28 μmol/kg, respectively). This discrepancy can be at least partially explained by differences in bioavailability. The lower in vitro activity of **29** was compensated by plasma concentrations more than twice those of **5** 1 h after a 200 μmol/kg oral dose (152 versus 70 μM). Replacement of the benzyloxy group of **5** with a 2-quinoylmethoxy, **30**, also produced a compound with oral activity in the rat peritoneal anaphylaxis model.

Modifications to the CH_2O portion of the benzyloxy R_2 substituent of **5** were investigated. Replacement of the CH_2O with CH_2S (**31**) or with CH=CH (**32**) produced compounds that were slightly more potent than **5** in vitro but which produced no significant inhibition in vivo at 200 μmol/kg, po. However, when the benzyloxy group of **5** was shortened to a phenoxy (**33**) or lengthened to a 2-phenylethoxy (**34**), compounds with both in vitro and in vivo potencies similar to **5** were obtained. Consistent with their similar potencies in the rat peritoneal anaphylaxis model, **33** and **5** exhibited similar plasma concentrations following a 200 μmol/kg dose (52 and 70 μM, respectively).

One of the most interesting modifications at R_2 was the 1-phenylethoxy group employed in **35**. The addition of a methyl side chain at the benzyloxy methylene resulted in a compound with profoundly improved pharmacokinetic properties compared to those of **5**. Hydroxamate **35** exhibited 1.5 times the plasma levels of **5** in the rat 1 h after oral administration of 200 μmol/kg. These levels remained relatively constant for 8 h after dosing and the compound could still be detected 15 h after oral administration. In fact the iv plasma half-life of **35** was nearly 5 times that of **5** (5.3 and 1.1 h, respectively). This difference in duration can be attributed to a difference in the metabolism of the compounds. In the rat, ^{14}C-labeled **5** was observed to undergo oxidation at the benzylic position of R_2 and subsequently hydrolyzed to benzoic acid which in turn was excreted as hippuric acid.[10] The methyl side chain attached to the benzylic position of **35** apparently limits this metabolism and resulted in a longer lived compound. Despite this improved duration, **35** is less potent in vivo than **5**. For reasons that are not readily apparent, the ED_{50} of **35** against in vivo leukotriene biosynthesis was only 92 μmol/kg (28 mg/kg), po.

Other modifications to R_2 that might limit the oxidative metabolism of **5** were explored, but **35** appears to incorporate the optimum substitution. An isopropyl side chain was introduced at the benzylic position, **36**, but this compound failed to produce significant inhibition of leukotriene biosynthesis in vivo after a 200 μmol/kg oral dose. This can be attributed to the low bioavailability of the compound. Attempts to block benzylic oxidation through the steric hindrance of neighboring ortho methyl substituents as in **37** or through the incorporation of a 9-fluorenyloxy substituent at R_2 as in **38** also resulted in compounds that were inactive in vivo. In the case of **38**, in vitro potency was also markedly reduced. Some in vivo inhibition of leukotriene biosynthesis was obtained with **39** in which the oxygen and $CHCH_3$ of **35** were reversed.

Conclusions

The in vitro 5-lipoxygenase inhibitory activities of the

(10) Machinist, J., unpublished results.

compounds reported here are similar. All of the compounds are reasonably potent and with the exception of **29** and **38** their IC_{50} values fall within 1 order of magnitude of each other. However, in vivo, these compounds displayed activities ranging from no significant inhibition at 200 μmol/kg, po to those with ED_{50} values well below 50 μmol/kg. The discrepancies between high in vitro potency and weak in vivo activity appeared to be attributable to differences in bioavailability. Hydroxamates with high plasma concentrations generally produced potent inhibition in vivo, while compounds that exhibited small or nondetectable levels were weak or inactive.

This analysis of the structure–activity relationships that describe the biological activities of compounds with generic structure I has revealed **5**, A-63162, as a relatively optimized, potent orally active inhibitor of leukotriene biosynthesis.

Experimental Section

Biological Assays. Detailed experimental procedures for the rat peritoneal anaphylaxis model, the method of determining plasma concentration, and the RBL-1 20000*g* supernatant 5-lipoxygenase inhibitory assay have been previously reported.[2]

Synthesis. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded at 300 MHz on a GE QE300 instrument with $(CH_3)_4Si$ as the internal standard and are reported in units of δ. Mass spectra were obtained on a Kratos MS-50 instrument with EI ion source (70 eV). Analytical data indicated by elemental symbols were within ±0.4% theoretical values unless noted.

The purity of compounds was checked by TLC analysis on silica gel F_{254} (Merck), and compounds were visualized with UV fluorescence inhibition or $Ce(SO_4)_3$ spray. Flash column chromatography was conducted on silica gel 60 (Merck, 40–60 μm) under 10–20 psi of pressure.

Table I summarizes the physical data and methods of preparation for compounds of structure I. Compounds **7** and **8** were prepared as previously described.[2]

***N*-Hydroxy-*N*-[1-[4-(phenylmethoxy)phenyl]ethyl]acetamide (5).** 4-Hydroxyacetophenone (5.0 g, 36.7 mmol) was dissolved in dimethyl sulfoxide (50 mL) and potassium *tert*-butoxide (4.73 g, 42.2 mmol) was added. Twenty minutes later benzyl bromide (7.85 g, 45.8 mmol) was added. After an additional hour, the reaction mixture was poured into water and extracted with ether. The ether layer was dried with magnesium sulfate and evaporated to give 4-(benzyloxy)acetophenone as an off white solid which was carried on without further purification.

The material prepared as described above (3.8 g, 17 mmol) and hydroxylamine hydrochloride (2.4 g, 35 mmol) were dissolved in a mixture of ethanol (25 mL) and pyridine (25 mL) and heated at 50 °C for 2 h. Most of the solvent was removed in vacuo and the residue dissolved in ether. After being washed with 2 N HCl (50 mL), the solution was dried over $MgSO_4$ and evaporated. The residue was recrystallized from ethanol to give 4-(benzyloxy)-acetophenone oxime as a white crystalline solid (3.7 g, 90%).

The oxime (3.7 g, 15.3 mmol) was dissolved in ethanol (30 mL) and cooled to 0 °C. Borane–pyridine complex (4.6 mL, 46 mmol) was added via syringe under nitrogen followed 10 min later by 6 N HCl (15 mL). Within 30 min the reaction was complete and was brought to pH 9 with the addition of solid sodium carbonate or 2 N NaOH. The mixture was extracted into ether and dried over $MgSO_4$. After evaporation, the residue was recrystallized from 95% aqueous ethanol to provide 1-[4-(benzyloxy)phenyl]-ethylhydroxylamine (2.3 g, 62%).

Acetyl chloride (2.11 g, 27.1 mmol) was added to a solution of triethylamine (3.7 g, 36.9 mmol) and 1-[4-(benzyloxy)phenyl]-ethylhydroxylamine (3.0 g, 12.3 mmol) in methylene chloride (50 mL) at 0 °C. After being allowed to stir for 30 min, the mixture was added to 2 N HCl (50 mL). The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo. The residue was chromatographed on 100 g of silica gel, eluting with 67% ether in hexanes to give *N*-acetoxy-*N*-[1-[4-(phenylmethoxy)phenyl]-ethyl]acetamide (3.6 g, 92%).

The *N*,*O*-diacetate prepared above was dissolved in 2-propanol (30 mL) and lithium hydroxide (3.1 g) in water (15 mL) was added. The mixture was allowed to stir for 30 min and then partitioned between 2 N HCl (60 mL) and ether (100 mL). The organic layer was dried and evaporated and the residue was recrystallized from 40% aqueous ethanol to yield **5** (2.5 g, 80%): mp 120–122 °C; ^1H NMR (DMSO-d_6) δ 1.41 (d, 3 H), 1.97 (s, 3 H), 5.08 (s, 2 H), 5.56 (br m, 1 H), 6.95 (d, 2 H), 7.23 (d, 2 H), 7.3–7.47 (m, 5 H), 9.47 (br s, 1 H); IR ($CDCl_3$) 3240, 3090, 3060, 3030, 2980, 1610, 1510, 1245, 1180 cm^{-1}; MS, *m/e* 285 (M^+), 268, 226, 211, 91. Anal. ($C_{17}H_{19}NO_3$) C, H, N.

***N*-Hydroxy-*N*-[1-methyl-1-(4-butoxyphenyl)ethyl]acetamide (20).** Methyllithium (1.2 M in hexanes, 62.5 mmol) was added to a solution of 4-butoxyacetophenone (8.0 g, 41.7 mmol) in ether (100 mL) at 0 °C. The mixture was allowed to stir for 30 min and 2 N HCl was added and the ether layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with 40% ether in hexanes to provide 1-methyl-1-(4-butoxyphenyl)ethanol (63%).

O-Benzylhydroxylamine hydrochloride (7.35 g, 46.1 mmol) was suspended in THF (200 mL). Triethylamine (6.5 mL, 46.1 mmol) was added and the mixture stirred for 1 h. The suspension was then filtered to remove triethylamine hydrochloride and the filtrate was concentrated in vacuo. The residue was redissolved in benzene (20 mL) and added to a stirred solution of 1-methyl-1-(4-butoxyphenyl)ethanol, prepared as described above (3.55 g, 17.1 mmol) in benzene (50 mL). Trifluoroacetic acid (1.3 mL, 16.9 mmol) was added neat and the mixture stirred for 48 h. At that time the mixture was concentrated to dryness and then redissolved in ether (150 mL). This solution was dried over $MgSO_4$ and evaporated to produce *N*-(benzyloxy)-1-methyl-1-(4-butoxyphenyl)ethylamine as an oil, which was carried on without purification. The *O*-benzylhydroxylamine was dissolved in THF (200 mL), and triethylamine (7.8 g, 77 mmol) and acetyl chloride (6.0 g, 77 mmol) were added. The mixture was allowed to stir for 2 h and was then filtered. The solvent was evaporated and the residue chromatographed on silica gel, eluting with 25% ether in hexanes to give *N*-(benzyloxy)-*N*-[1-methyl-1-(4-butoxyphenyl)ethyl]acetamide (3.75 g, 71% over two steps).

The *N*-benzyloxy group was removed by catalytic hydrogenation with 10% Pd/C (2.5 mol % catalyst) to give **20** as a white solid (67%): mp 87–88 °C, ^1H NMR (DMSO-d_6) δ 0.92 (t, 3 H), 1.42 (m, 2 H), 1.58 (s, 6 H), 1.68 (m, 2 H), 1.95 (s, 3 H), 3.92 (t, 2 H), 6.80 (d, 2 H), 7.18 (d, 2 H), 9.72 (s, 1 H); MS, *m/e* 265 (M^+), 249, 206, 191, 135, 119. Anal. ($C_{15}H_{23}NO_3$) C, H, N.

***N*-Hydroxy-*N*-[2-(4-butoxyphenyl)ethyl]acetamide (21).** Potassium hydroxide (13 g, 200 mmol) in ethanol (100 mL) was added to a solution of 4-butoxybenzaldehyde (18 g, 10 mmol) and nitromethane (6.0 g, 100 mmol) in ethanol (300 mL) and a white solid mass formed. The mixture was poured into 6 N HCl (500 mL) and the white mass turned to a yellow solid. This material was collected by filtration and dissolved in ether (300 mL). The solution was dried with $MgSO_4$ and evaporated. The residue was chromatographed on silica gel, eluting with 10% ether in hexanes to afford 1-nitro-2-(4-butoxyphenyl)ethene as a yellow solid (10.0 g, 45%).

Borane–THF (28.4 mmol) was added to a solution of the vinyl nitro compound prepared above (6.0 g, 27.1 mmol) in THF (80 mL) at 0 °C. Sodium borohydride (10 mg) was then added and the mixture allowed to stir for 8 h. Most of the yellow color of the starting material was gone. Water (75 mL) and 2 N HCl were added and the mixture was heated to 60 °C for 45 min. The reaction mixture was washed with ether and the neutralized with 2 N NaOH. The product was extracted into ether which was dried over $MgSO_4$ and evaporated. A white solid, *N*-hydroxy-2-(4-butoxyphenyl)ethylamine, (2.2 g, 39%) was obtained. This hydroxylamine was converted to **21** without further purification by using the procedure described above for the synthesis of **5** (53%): mp 68–69 °C; ^1H NMR (DMSO-d_6) δ 0.93 (t, 3 H), 1.44 (m, 2 H), 1.65 (m, 2 H), 1.9 (s, 3 H), 2.73 (t, 2 H), 3.63 (t, 2 H), 3.91 (t, 2 H), 6.82 (d, 2 H), 7.10 (d, 2 H), 9.78 (s, 1 H); IR ($CDCl_3$) 3250, 2980, 2930, 1610, 1510, 1245 cm^{-1}; MS, *m/e* 251 (M^+), 177, 176, 163, 120, 107. Anal. ($C_{14}H_{21}NO_3$) C, H, N.

***N*-Hydroxy-*N*-[3-(4-butoxyphenyl)propyl]acetamide (22).** Lithium aluminum hydride (1 M in THF, 42 mL) was added rapidly to a solution of methyl 3-(4-butoxyphenyl)propionate (10

g, 42 mmol) in THF (50 mL). After being allowed to be stirred for 15 min, 2 N HCl (100 mL) was added to quench the reaction. The organic layer was separated and dried with $MgSO_4$ and evaporated to give 3-(4-butoxyphenyl)-1-propanol as a colorless oil (8.5 g). This intermediate, without further purification was dissolved in methylene chloride (100 mL) and pyridinium chlorochromate (18 g, 84 mmol) was added. Three hours later ether was added and the mixture was filtered through silica gel. The filtrate was evaporated and the residue chromatographed on silica gel, eluting with 50% ether in hexanes. 3-(4-Butoxyphenyl)-propanal was obtained (7.5 g, 86%). This aldehyde was converted to **22** by using the procedure described for the preparation of **5** (77%): mp 77–79 °C, ^1H NMR (DMSO-d_6) δ 0.92 (t, 3 H), 1.4 (m, 2 H), 1.60–1.80 (m, 6 H), 1.92 (s, 3 H), 2.48 (t, 2 H), 3.90 (t, 2 H), 6.80 (d, 2 H), 7.10 (d, 2 H), 9.72 (br s, 1 H); MS, m/e 265 (M^+), 247, 190, 177, 134, 121, 107. Anal. ($C_{15}H_{23}NO_3$) C, H, N.

***N*-Hydroxy-*N*-[1-methyl-3-(4-butoxyphenyl)propyl]-acetamide (24).** Methyllithium in hexanes (27 mmol) was added to a solution of 3-(4-butoxyphenyl)propanal (5.0 g, 24 mmol), prepared as described for the preparation of **22**, at −78 °C. The reaction was allowed to warm to room temperature and then was quenched with 2 N HCl. The organic layer was separated, dried over $MgSO_4$, and evaporated to give a colorless oil, 4-(4-butoxyphenyl)-2-butanol (3.8 g, 71%). This material was homogeneous by TLC and therefore converted without purification to 4-(4-butoxyphenyl)-2-butanone by oxidation with pyridinium chlorochromate by using the procedure described above for the preparation of **22** (81%). This ketone was converted to **24** with the procedure used to prepare **5** (40%): mp 58–59 °C; ^1H NMR (DMSO-d_6) δ 0.92 (t, 3 H), 1.03 (d, 3 H), 1.42–1.85 (m, 6 H), 2.00 (s, 3 H), 2.40 (m, 2 H), 3.90 (t, 2 H), 4.40 (m, 1 H), 6.80 (d, 2 H), 7.08 (d, 2 H), 9.38 (s, 1 H); MS, m/e 279 (M^+), 261, 220, 204, 177, 163, 148. Anal. ($C_{16}H_{25}NO_3$) C, H, N.

***N*-Hydroxy-*N*-[2-(4-butoxyphenoxy)ethyl]acetamide (25).** 4-Butoxyphenol (10 g, 60 mmol) was dissolved in DMSO and potassium *tert*-butoxide (6.75 g, 60 mmol) was added followed by bromoacetaldehyde dimethyl acetal (60 mmol). After 30 min, the mixture was poured into water and the product extracted into ether. After drying with $MgSO_4$ the ether was evaporated in vacuo to give a solid (7.2 g). Without purification the acetal was removed by refluxing the solid with sulfuric acid (5 drops) in acetone (50 mL) and water (5 mL). The solvent was evaporated and the residue chromatographed on silica gel eluting with 40% ether in hexanes to give a crystalline solid, (4-butoxyphenoxy)acetaldehyde (5.0 g, 57%). This was converted to **25** by using the procedure described for the preparation of **5** (35%): mp 72–74 °C; ^1H NMR (DMSO-d_6) δ 0.92 (t, 3 H), 1.42 (m, 2 H), 1.65 (m, 2 H), 2.00 (s, 3 H), 3.82 (t, 2 H), 3.89 (t, 2 H), 4.05 (t, 2 H), 6.85 (s, 4 H), 9.90 (br s, 1 H); MS, m/e 267 (M^+), 251, 166, 151, 136, 110, 102. Anal. ($C_{14}H_{21}NO_4$) C, H, N.

***N*-Hydroxy-*N*-[1-[4-(benzylthio)phenyl]ethyl]acetamide (31).** Benzyl phenyl sulfide (10.0 g, 50 mmol) was added at 0 °C to a solution of acetyl chloride (4.3 g, 55 mmole) and aluminum chloride (28 g, 204 mmol) in nitroethane (75 mL). The mixture was allowed to stir for 3 h at 0 °C and then slowly poured over ice and 3 N HCl. The resulting mixture was extracted with ether, which was then dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with 10% ethyl acetate in hexanes to give 4-(benzylthio)acetophenone as a white solid (3.41 g, 22%). This ketone was converted to **29** by using the procedure described above for **5** (35%): mp 119–121 °C; ^1H NMR (DMSO-d_6) δ 1.41 (d, 3 H), 1.99 (s, 3 H), 4.23 (s, 2 H), 5.55 (m, 1 H), 7.20–7.40 (m, 9 H), 9.56 (br s, 1 H); MS, m/e 301 (M^+), 284, 227, 91. Anal. ($C_{17}H_{19}NO_2S$) C, H, N.

***N*-Hydroxy-*N*-[1-[4-(2-phenylethenyl)phenyl]ethyl]-acetamide (32).** 4-Stilbenecarboxaldehyde was converted to 4-(2-phenylethenyl)acetophenone in the same manner that 3-(4-butoxyphenyl)propanal was converted to 4-(4-butoxyphenyl)-2-butanone as described above in the preparation of **24** (77%). 4-(2-Phenylethenyl)acetophenone was converted to **32** by using the method described for the synthesis of **5** (11%): mp 176–178 °C; ^1H NMR (DMSO-d_6) δ 1.45 (d, 3 H), 2.03 (s, 3 H), 5.60 (m, 1 H), 7.20–7.41 (m, 7 H), 7.53–7.63 (m, 4 H), 9.54 (br s, 1 H); MS, m/e 282 $(M + H)^+$, 264, 207. Anal. ($C_{18}H_{19}NO_2$) C, H, N.

***N*-Hydroxy-*N*-[1-[4-(1-phenoxyethyl)phenyl]ethyl]acetamide (39).** 4-Ethylacetophenone (5.0 g, 33.7 mmol) was added to a solution of *N*-bromosuccinimide (5.7 g, 32.1 mmol) and benzoyl peroxide (0.08 g, 0.32 mmol) in CCl_4 (50 mL). The reaction mixture was allowed to stir for 15 h and then filtered and dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed on silica gel (500 g), eluting with 25% ether in hexanes. 4-(1-Bromoethyl)acetophenone was obtained as a yellow liquid (5.7 g, 78%).

Potassium *tert*-butoxide (2.5 g, 22 mmol) was added to a solution of phenol (2.1 g, 22 mmol) in DMSO (50 mL). This mixture was allowed to stir for 15 min and then 4-(1-bromoethyl)acetophenone (5.0 g, 22 mmol) prepared as described above was added dropwise. After being allowed to stir for an additional 2 h, the mixture was poured into water (200 mL). The aqueous layer was extracted with ethyl acetate (2 × 100 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to afford 4-(1-phenoxyethyl)acetophenone (4.8 g). This ketone was converted to **39** without purification by using the procedure described for the synthesis of **5** (26%): ^1H NMR (DMSO-d_6) δ 1.41 (d, 3 H), 1.52 (d, 3 H), 1.99 (s, 3 H), 5.48 (q, 1 H), 5.58 (m, 1 H), 6.87 (m, 3 H), 7.20 (m, 2 H), 7.32 (m, 4 H), 9.7 (s, 1 H); MS, m/e 300 $(M + 1)^+$, 225, 206, 189, 131. Anal. ($C_{18}H_{21}NO_3$) C, H, N.

Acknowledgment. We thank Jennifer Barlow, Dirk Bornemeier, Thomas Clark, Wendy Rach, Ellen Roberts, and Sharyl Culbertson for very capable technical assistance.

Registry No. **5**, 111525-11-2; **6**, 111525-08-7; **7**, 110319-82-9; **8**, 110319-79-4; **9**, 115513-76-3; **10**, 115513-77-4; **11**, 115513-78-5; **12**, 115513-79-6; **13**, 115513-80-9; **14**, 106328-28-3; **15**, 115513-81-0; **16**, 115513-82-1; **17**, 115513-83-2; **18**, 115513-84-3; **19**, 115513-85-4; **20**, 115513-86-5; **21**, 115513-87-6; **22**, 115513-88-7; **23**, 115513-89-8; **24**, 115513-90-1; **25**, 115513-91-2; **26**, 115513-92-3; **27**, 115513-93-4; **28**, 115513-94-5; **29**, 115513-95-6; **30**, 115513-96-7; **31**, 115513-97-8; **32**, 115513-98-9; **33**, 115513-99-0; **34**, 115514-00-6; **35**, 115514-01-7; **36**, 115514-02-8; **37**, 115514-03-9; **38**, 115514-04-0; **39**, 115514-05-1; 4-hydroxyacetophenone, 99-93-4; benzyl bromide, 100-39-0; 4-(benzyloxy)acetophenone, 54696-05-8; 4-(benzyloxy)acetophenone oxime, 75408-90-1; 1-[4-(benzyloxy)phenyl]ethylhydroxylamine, 111525-03-2; C_4H_9O-*p*-$C_6H_4OCH_2CH(OCH_3)_2$, 115514-14-2; 5-lipoxygenase, 80619-02-9; *N*-acetoxy-*N*-[1-[4-(phenylmethoxy)-phenyl]ethyl]acetamide, 111525-07-6; 4-butoxyacetophenone, 5736-89-0; 1-methyl-1-(4-butoxyphenyl)ethanol, 93308-49-7; *O*-benzylhydroxylamine hydrochloride, 2687-43-6; *N*-(benzyloxy)-1-methyl-1-(4-butoxyphenyl)ethylamine, 115514-06-2; *N*-(benzyloxy)-*N*-[1-methyl-1-(4-butoxyphenyl)ethyl]acetamide, 115514-07-3; 4-butoxybenzaldehyde, 5736-88-9; 1-nitro-2-(4-butoxyphenyl)ethene, 115514-08-4; nitromethane, 75-52-5; *N*-hydroxy-2-(4-butoxyphenyl)ethylamine, 115514-09-5; methyl 3-(4-butoxyphenyl)propionate, 115514-10-8; 3-(4-butoxyphenyl)-1-propanol, 92101-88-7; 3-(4-butoxyphenyl)propanal, 115514-11-9; 4-(4-butoxyphenyl)-2-butanol, 115514-12-0; 4-(4-butoxyphenyl)-2-butanone, 115514-13-1; 4-butoxyphenol, 122-94-1; (4-butoxyphenoxy)acetaldehyde, 101397-94-8; bromoacetaldehyde dimethyl acetal, 7252-83-7; benzyl phenyl sulfide, 831-91-4; (benzylthio)acetophenone, 57366-87-7; stilbenecarboxaldehyde, 32555-96-7; 4-(2-phenylethenyl)acetophenone, 3112-03-6; 4-ethylacetophenone, 937-30-4; 4-(1-bromoethyl)acetophenone, 115514-15-3; 4-(1-phenoxyethyl)acetophenone, 115514-16-4; nitroethane, 79-24-3.