normally eight mice at each dose.

Phase IV tests (Table VII) involved the same procedures described previously for phase II, except the test drug was administered to mice orally. Phase V entailed the administration of 85 mg/kg of Metrazol, 2.70 mg/kg of bicuculline, 3.15 mg/kg of picrotoxin, or 1.20 mg/kg of strychnine as a 0.5% solution subcutaneously in the posterior midline of mice. This amount of each convulsant was expected to produce seizures in 97% of the mice tested. The animal was isolated and observed for 30 min in the Metrazol, bicuculline, and strychnine tests and for 45 min in the case of the picrotoxin test. Protection in each of these tests was defined as the failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5-s duration) in the Metrazol test, complete absence of a seizure in the bicuculline and picrotoxin tests, and abolition of the hind-leg tonic extensor component of the seizure in the strychnine test. Eight mice were normally treated at each dose for both phase IV and V testing. The results obtained in this test are listed in Table VIII.

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Supplementary Material Available: Detailed experimental procedures and physical data for all new compounds (12 pages). Ordering information is given on any current masthead page.

Hydroxamic Acid Inhibitors of 5-Lipoxygenase

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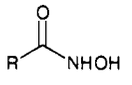
The hydroxamic acid functionality can be incorporated in a variety of simple molecules to produce potent inhibitors of 5-lipoxygenase. As an example of this, the structure–activity relationships in a series of ω-phenylalkyl and ω-naphthylalkyl hydroxamic acids are presented. Among the features described are the influence of hydrophobicity, aryl substitution, and modifications of the hydroxamate group on enzyme inhibitory potency. To assist in the selection of more potent hydroxamic acid inhibitors, a simple hypothesis about the nature of enzyme–inhibitor binding was devised. In this hypothesis, the structures of compounds were matched to a proposed geometry of arachidonic acid when bound to the enzyme. Compounds that match best without extending into disfavored regions were predicted to be the best inhibitors. Three series of hydroxamates selected according to this approach are described. Within these series are some of the most potent inhibitors of 5-lipoxygenase reported to date.

The enzyme 5-lipoxygenase catalyzes the first step of a biochemical pathway in which arachidonic acid is converted into the leukotrienes. Numerous biochemical effects have been associated with the leukotrienes and they have been implicated as important mediators in a variety of disease states including asthma, arthritis, psoriasis, and allergy.[1] As the first dedicated enzyme in the biosynthetic cascade leading to these important mediators, 5-lipoxygenase clearly represents an exciting target for therapeutic intervention.[2]

Hydroxamic acids are well-known to form strong complexes with a variety of transition metals. This property has been exploited in the use of hydroxamates as inhibitors of several metalloenzymes.[3] Since it is generally believed that 5-lipoxygenase contains a catalytically important iron atom,[4] this enzyme is a logical candidate for inhibition by hydroxamic acid containing molecules. In fact, Corey and co-workers[5] have reported that the hydroxamic acid of arachidonic acid is an inhibitor of 5-lipoxygenase. Kerdesky et al.[6] showed that when a hydroxamic acid group is positioned at C_5 of arachidonic acid, an inhibitor with a 10-fold enhancement in potency is obtained. This demonstrated that not only the presence but also the position of the hydroxamic acid moiety is important for 5-lipoxygenase inhibition. Corey et al.[5] has also shown that the full eicosenoid chain is not required for inhibition. A hydroxamate bearing the first 10 carbons of the arachidonate chain and capped by a phenyl ring inhibits 5-lipoxygenase.

Table I. ω-Arylalkyl Hydroxamic Acids: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R	IC_{50},[a] μM
1	C_6H_5	110 (94–120)
2	$C_6H_5CH_2$	300 (250–400)
3	$C_6H_5CH_2CH_2$	87 (81–93)
4	$C_6H_5CH_2CH_2CH_2$	27 (25–29)
5	2-naphthyl	14 (12–16)
6	(2-naphthyl)CH_2	19 (16–21)
7	(2-naphthyl)CH_2CH_2	9.7 (8.5–11)
8	1-naphthyl	43 (37–53)
9	(1-naphthyl)CH_2	27 (26–29)
10	*trans*-$C_6H_5CH{=}CH$-	12 (8–14)
11	*cis*-$C_6H_5CH{=}CH$-	31 (27–36)
12	$C_6H_5C{\equiv}C$-	46 (42–49)

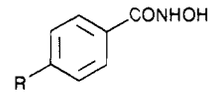
[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

While the compounds reported by Corey and Kerdesky are extremely potent inhibitors, they are unlikely to be of therapeutic value. As analogues of arachidonic acid they are prone to chemical oxidation and would almost certainly be degraded rapidly in vivo. It was our objective to identify simple, stable molecules containing the hydroxamic acid functionality that maintained the potent inhibition demonstrated by the arachidonic acid analogues referred to above. We report simple hydroxamates that not only inhibit 5-lipoxygenase, but surpass the potency of many of the commonly cited reference inhibitors of this enzyme.

(1) For a review of the biochemistry and pharmacology of the leukotrienes, see: Sirois, P. *Adv. Lipid Res.* **1985**, *21*, 79.
(2) Leukotriene biosynthesis inhibitors have been reviewed by Cashman: Cashman, J. R. *Pharm. Res.* **1985**, 253.
(3) Kiehl, H. *The Chemistry and Biology of Hydroxamic Acids*; Karger: Basel, 1982.
(4) Gibian, M. J.; Galaway, R. A. *Bio-Org. Chem.* **1977**, *1*, 117. Pistorius, E. K.; Axelrod, B. *J. Biol. Chem.* **1974**, *249*, 3183.
(5) Corey, E. J.; Cashman, J. R.; Kantner, S. R.; Wright, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 1503.
(6) Kerdesky, F. A. J.; Holms, J. H.; Schmidt, S. P.; Dyer, R. D.; Carter, G. W. *Tetrahedron Lett.* **1985**, 2143.

0022-2623/87/1830-0574$01.50/0

Table II. Substituent Effects on Benzohydroxamic Acid: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R	σ	π	IC_{50}, μM calcd[a]	IC_{50}, μM obsd[b]
13	NO_2	0.78	−0.28	40	23 (21–26)
14	CN	0.66	−0.57	62	61 (51–73)
15	CF_3	0.54	0.88	14	27 (24–29)
16	Br	0.23	0.86	19	14 (12–16)
17	I	0.18	1.12	15	15 (14–16)
1	H	0.00	0.00	63	110 (94–120)
18	C_6H_5	−0.01	1.96	6.9	4.1 (3.7–4.7)
19	CH_3	−0.17	0.56	40	65 (57–74)
20	OH	−0.37	−0.67	190	190 (160–230)
21	NH_2	−0.66	−1.23	490	≥400

[a] Log $(1/IC_{50}) = 0.43\ (\pm 0.16)\ \sigma + 0.49\ (\pm 0.07)\pi - 1.80\ (\pm 0.07)$ ($n = 10$, $s = 0.49$, $r = 0.95$). [b] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

Results and Discussion

Exploratory Studies. In order to establish the inhibitory utility of simple hydroxamates several ω-phenylalkyl and ω-naphthylalkyl hydroxamic acids were selected. The 5-lipoxygenase inhibitory potencies of these compounds were evaluated with the 20000*g* supernatant from RBL-1 cells according to the method of Dyer et al.[7] Results from this series are reported in Table I. All of these hydroxamic acids show some 5-lipoxygenase inhibition, and the potency is significantly affected by the nature of the groups attached to the hydroxamate. The introduction of methylene units between the hydroxamate moiety and a phenyl ring improves potency. Since methylene units add lipophilicity, this enhancement may be related to the lipophilic nature of lipoxygenase substrates. An exception to this trend occurs with the introduction of one methylene unit. Compound **2** is less active than either **1** (no methylene units) or **3** (two methylene units).

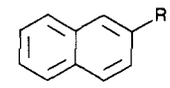
Compounds containing naphthyl groups are generally more potent than analogous phenyl hydroxamates. This observation is also consistent with a preference of the enzyme for more lipophilic compounds. Naphthalenes substituted at the 2-position are more active than those at the 1-position (**5** vs. **8**).

Unsaturated groups connecting the hydroxamate with the aryl ring also modify activity. The unsaturated hydroxamates **10–12** are more potent than their saturated analogue **3**. The trans configuration **10** is somewhat more potent than the cis isomer **11**.

Substituents on the aryl rings also affect the 5-lipoxygenase inhibitory potency of hydroxamic acids. In a series of para-substituted benzohydroxamic acids, IC_{50} values spanned 2 orders of magnitude (Table II) from about 400 μM with *p*-amino, **21**, to 4.1 μM with *p*-phenyl, **18**. In general, compounds with electron-withdrawing or hydrophobic groups are more potent inhibitors. A quantitative structure–activity analysis of these data shows a significant correlation of the logarithm of the 5-lipoxygenase IC_{50} with the Hammett electronic constant, σ, and the hydrophobicity constant, π, for each substituent.

The hydroxamic acid functionality is clearly the dominant feature responsible for the 5-lipoxygenase inhibitory activities of these molecules. Few modifications of the hydroxamate group yield a compound that maintains the potency observed in the parent. Table III summarizes the inhibitory potencies of several analogues of naphthalenecarbohydroxamic acid (**5**). The corresponding carboxylic acid **22**, amide **23**, hydrazide **24**, and keto alcohol **25** have essentially no inhibitory activity against 5-lipoxygenase. Methyl substitution on nitrogen (**26**) improves potency 10-fold. However, if the methyl group is on oxygen (**27**), the compound is inactive. A moderate degree of activity is associated with the hydroximic ester **28** and the hydroxy sulfonamides **29** and **30**.

Table III. Analogues of Naphthalenecarbohydroxamic Acid: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R	IC_{50},[a] μM
5	CONHOH	14 (12–16)
22	COOH	0% at 300 μM
23	$CONH_2$	9% at 300 μM
24	$CONHNH_2$	6% at 300 μM
25	$COCH_2OH$	15% at 100 μM
26	$CON(CH_3)OH$	1.3 (1.1–1.5)
27	$CONHOCH_3$	0% at 30 μM
28	$C(=NOH)OCH_3$	74% at 300 μM
29	SO_2NHOH	51% at 100 μM
30	$SO_2N(CH_3)OH$	6% at 30 μM

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

Hypothesis about Inhibitor Binding. The exploratory studies summarized above demonstrate that simple hydroxamic acids are inhibitors of 5-lipoxygenase. To assist in the efficient selection of yet more potent inhibitors, a simple predictive hypothesis about the nature of inhibitor–enzyme interactions was developed. Within this hypothesis a few theoretical arguments about the enzyme mechanism were used to postulate a partial geometry of arachidonic acid when bound to the enzyme. Compounds then were aligned with this geometry and those that fit best were predicted to be the best inhibitors. The predictive hypothesis makes use of a simplistic graphical comparison of bound substrate with inhibitors. As such it was neither intended to be a sophisticated representation of the active site nor to predict inhibitory potencies quantitatively or without exception. Nonetheless it has been a valuable tool in the selection of new hydroxamic acids of high potency. The main elements of this hypothesis are described below.

In a recent review article, Cashman[2] has summarized the key structural features of the 5-lipoxygenase active sites as a non-heme ferric iron, a hydrophobic domain, and a carboxylic acid binding site. Beyond this there is little

(7) Dyer, R. D.; Bornemeier, D. A.; Haviv, F.; Carter, G. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 904.

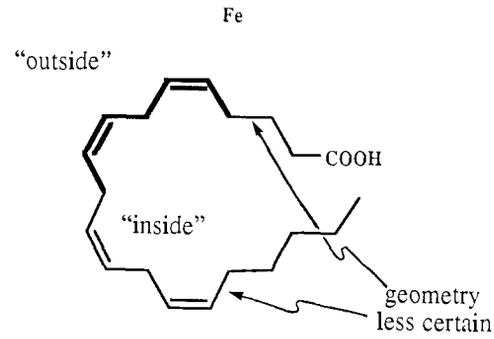
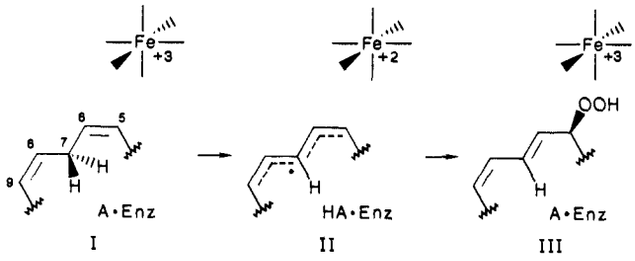


Figure 1. Graphical representation of the arachidonate conformation when bound to the active site of 5-lipoxygenase.

Scheme I



known about the enzyme. In particular, there is no experimental information available on the conformation of arachidonic acid when bound to 5-lipoxygenase. However, a partial hypothetical conformation can be proposed based on knowledge of the lipoxygenase reaction. The conversion of arachidonic acid, I, to 5-HPETE, III, involves the abstraction of the *pro-S* hydrogen atom from C_7 to produce a radical, II[8] (Scheme I). This radical can be stabilized by delocalization through the neighboring double bonds, extending the radical character from C_5 to C_9. Such stabilization requires that these double bonds be held in the same plane. In the binding hypothesis it was assumed that 5-lipoxygenase constrains the C_4–C_{10} atoms of arachidonic acid in a plane and thereby mediates a reduction in the activation energy for formation of the delocalized radical.

In the course of the lipoxygenase reaction a *cis,cis*-1,4-diene is converted into a *trans,cis*-1,3-diene. It was proposed, therefore, that the substrate be bound by the enzyme in a "W" conformation at C_5–C_9. This promotes the proper geometry for double bond migration to the product without a major conformational change within the active site.

Since 5-lipoxygenase is believed to contain a catalytically important iron, such an atom was postulated to be in the vicinity of C_5 where oxidation occurs. This is consistent with the observation of Kerdesky et al.,[6] which showed that a more potent inhibitor is obtained when a hydroxamate is appended to C_5 of arachidonate rather than to C_1.

Each of the elements described above can be combined into a simple graphical representation of features within the 5-lipoxygenase active site shown in Figure 1. No attempt was made to define the bound conformation of the C_1–C_3 or C_{11}–C_{20} portions of arachidonic acid. For the purposes of illustration, the remaining double bonds were also place in a planar "W" conformation.

Within the context of the hypothesis, inhibitors were aligned with the postulated bound arachidonic acid geometry such that the hydroxamate functionality of each compound was fixed in a position near the iron moiety.

(8) Corey, E. J.; Cashman, J. R.; Eckrich, T. M.; Corey, D. R. *J. Am. Chem. Soc.* **1985**, *107*, 713.

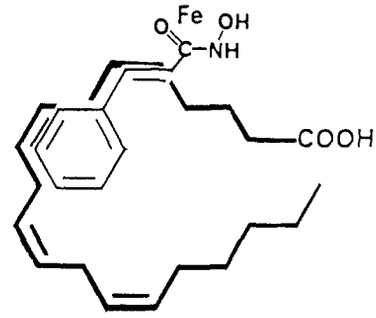


Figure 2. Alignment of **10** with hypothetical arachidonic acid conformation.

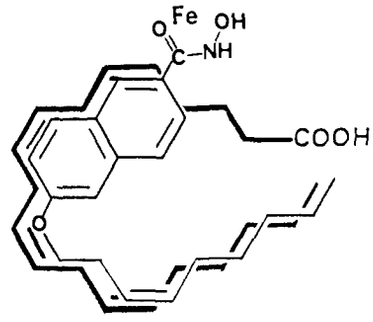


Figure 3. Alignment of **37** with arachidonic acid backbone according to the binding hypothesis.

The remainder of the inhibitors structure was matched as best as possible with the hypothetical enzyme-bound conformation of arachidonate. In this arrangement compounds that matched most precisely were predicted to be the best inhibitors. Specifically, compounds that matched aromatic systems or double bonds to the positions of double bonds in the substrate were expected to be more potent than analogous compounds that aligned saturated chains in double bond areas. Compounds that matched with a large portion of the substrate were expected to be more effective than those that did not.

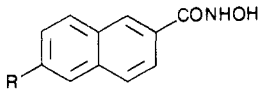
Figure 2 illustrates how **10** aligns with the hypothetical arachidonic acid conformation in the manner described above. When the other compounds listed in Table I were aligned in this way, the potencies observed were qualitatively consistent with those predicted. For example, the double bond of **10** aligns with the 5,6-double bond of arachidonic acid, while with **3** a saturated chain occupies this region. Consistent with the predictions of the hypothesis, **10** is nearly 8-fold more potent than **3**.

As compounds were aligned in the manner described above it became evident that compounds that must extend into regions "outside" the area defined by the substrate (Figure 1) were generally weaker inhibitors than those that can be placed onto the arachidonate backbone entirely on the "inside". For example, **10** can be aligned entirely on the "inside", but **11** must extend to the "outside". Hydroxamate **10** is more potent than **11**. Often it was possible to place these weaker compounds somewhere on the "inside", but not in a manner that allowed for the favorable alignment of the inhibitor's structure with the arachidonate chain.

The binding hypothesis described above has proven very useful in the identification of many very potent hydroxamic acid inhibitors. The following sections illustrate how three series of hydroxamates were selected through the application of this proposal.

(a) 6-Alkoxy-2-naphthalenecarbohydroxamic Acids. With the rules proposed as part of the binding hypothesis, the naphthyl rings of **5** might align with the C_4–C_{10} por-

Table IV. 6-Alkoxy-2-naphthalenecarbohydroxamic Acids: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R	IC_{50},[a] μM
5	H	14 (12–16)
31	OCH_3	8.2 (6.8–9.6)
32	$O(CH_2)_3CH_3$	2.0 (1.7–2.3)
33	$O(CH_2)_6CH_3$	6.5 (5.7–7.4)
34	$O(CH_2)_7CH_3$	7.1 (5.6–9.9)
35	$O(CH_2)_8CH_3$	33% at 10 μM
36	$O(CH_2)_{11}CH_3$	11% at 10 μM
37	$OCH_2CH_2CH{=}CH(CH_2)_4CH_3$ (cis)	2.9 (2.6–3.2)

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

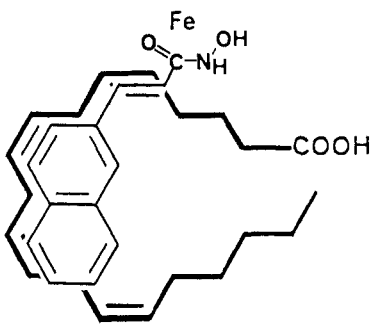


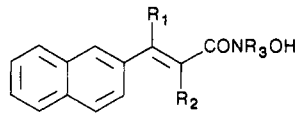
Figure 4. Alignment of **38** with arachidonic acid backbone according to the binding hypothesis.

tions of arachidonate. To mimic the C_{11}–C_{20} end of the substrate, compounds were selected with substitution from the 6-position of the naphthyl ring (Figure 3). To simplify the synthetic task of preparing this series, an ether linkage was used to attach an alkyl chain to the ring. Table IV presents the inhibitory potencies of ethers of varying chain lengths at the 6-position. Inhibitory potency improves as the chain is lengthened, peaking with approximately a four-carbon unit **32** and then decreasing again as the chain becomes longer. An alkyl chain that resembles the ω-end of arachidonate should also include unsaturation to mimic the C_{14}–C_{15} double bond. Such a double bond is included in **37** and an improvement in activity results (compared to **35**).

(b) 3-(2-Naphthyl)propenehydroxamic acids were selected on the basis of the binding hypothesis to mimic the C_5–C_{13} region of the bound substrate (Figure 4). The addition of the double bond spacer between the naphthyl and the hydroxamate improves potency (Table V). These compounds can be viewed as aligning with three double bonds of arachidonate and are approximately 10 times more potent than those aligning with two (e.g., **5**, IC_{50} = 14 μM) and 100 times more potent than those aligning with only one (e.g., **1**, IC_{50} = 110 μM). The double bond spacer leads to more potent inhibition than the saturated analogue (compare **38** with **7**). Alkyl substitution on nitrogen, e.g., **39**, improves activity about ninefold. This is reasonably independent of the nature of the alkyl chain (compare **39**, **40**, **41**, and **42**). Methyl substitution from the 3-position of the double bond (**44**) has little influence on potency. However, some activity is lost with 2-methyl or 2-phenyl substitution (**43**, **45**).

(c) Biaryl Hydroxamic Acids. The binding hypothesis includes no predictions about the conformation of the C_{11}–C_{20} region of the bound substrate. However it seemed reasonable that the C_5–C_7 and the C_{10}–C_{13} regions might be probed by a biaryl hydroxamate (Figure 5 shows a

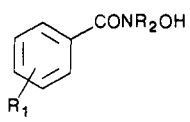
Table V. Naphthylpropenehydroxamic Acids: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities

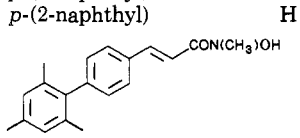


no.	R_1	R_2	R_3	IC_{50},[a] μM
38	H	H	H	0.95 (0.77–1.1)
39	H	H	CH_3	0.10 (0.091–0.11)
40	H	H	$CH(CH_3)_2$	0.082 (0.040–0.153)
41	H	H	c-C_6H_{11}	0.10 (0.085–0.12)
42	H	H	C_6H_5	0.052 (0.040–0.069)
43	H	CH_3	CH_3	0.47 (0.43–0.52)
44	CH_3	H	CH_3	0.12 (0.10–0.14)
45	H	C_6H_5	CH_3	0.50 (0.44–0.59)
7	CH_2CH_2		H	9.7 (8.5–11.0)

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

Table VI. Biaryl Hydroxamic Acids: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R_1	R_2	IC_{50},[a] μM
18	*p*-phenyl	H	4.1 (3.7–4.7)
46	*m*-phenyl	H	6.0 (5.2–6.3)
47	*p*-(2,4,6-trimethylphenyl)	H	0.29 (0.27–0.30)
48	*p*-(2,4,6-trimethylphenyl)	CH_3	0.064 (0.054–0.074)
49	*p*-(1-naphthyl)	H	0.18 (0.16–0.19)
50	*p*-(2-naphthyl)	H	0.33 (0.31–0.35)
51			0.022 (0.018–0.026)

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

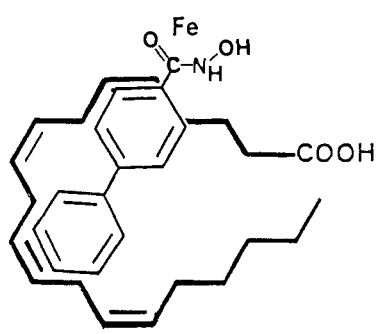


Figure 5. Alignment of **18** with arachidonic acid backbone according to the binding hypothesis.

possible alignment). To evaluate this possibility several biaryl hydroxamates were prepared and their inhibitory potencies are listed in Table VI. The *p*- and *m*-biphenyl hydroxamates **18** and **46** possess similar inhibitory activity. A greater than 10-fold improvement is achieved by replacing the *p*-phenyl ring with a *p*-(2,4,6-trimethylphenyl) (mesityl) (**47**). This modification holds the two aryl rings rigidly perpendicular. An improvement in activity is also achieved if the *p*-phenyl ring is replaced with a *p*-1-naphthyl (**49**) or 2-naphthyl (**50**).

The biaryl hydroxamate **51** is a remarkably potent 5-lipoxygenase inhibitor. It incorporates methyl substitution on nitrogen, a double bond spacer between the aryl system and the hydroxamate, and the mesityl group. It has an IC_{50} of 22 nM (0.022 μM). It is not clear what if any

Table VII. Comparison of the in Vitro 5-Lipoxygenase Inhibitory Potency of Two Simple Hydroxamic Acids with Some Common Reference Inhibitors

inhibitor	IC_{50},[a] μM
51	0.022 (0.018–0.026)
quercetin	0.3 (0.27–0.31)
nordihydroguaiaretic (NDGA)	0.4 (0.39–0.43)
26	1.3 (1.1–1.5)
BW-755C	1.3 (1.2–1.5)
phenidone	2.1 (2.0–2.2)
arachidonohydroxamic acid	2.2 (2.0–2.4)
15-HETE	7.3 (6.8–7.8)
Rev-5901	>45[b]
5,6-DHA	55[c] (46–64)

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses. [b] Insoluble above 45 μM. [c] The inhibition of 5,6-DHA is time dependent; therefore the IC^{50} varies with the conditions of the assay.

implications the activities of these biaryl hydroxamates have with respect to the nature of the C_{11}–C_{20} region of the bound substrate.

Activity in Other Enzyme Systems. In general, hydroxamic acids show very similar in vitro potency against 5-lipoxygenase in intact rat polymorphonuclear leukocytes as compared to the enzyme in broken cell preparations. For example, **18** has an IC_{50} of 3.3 μM against the whole cell and 4.0 μM against the isolated enzyme. These hydroxamates show some specificity with respect to related enzymes. While **18** is approximately equally potent against 5- and platelet 12-lipoxygenase (86% inhibition at 10 μM), it is less potent against soybean 15-lipoxygenase (30% at 100 μM) and sheep seminal vesicle cyclooxygenase (50% at 100 μM).

Activity Relative to Other Common Lipoxygenase Inhibitors. The compounds described in this paper rank among the most potent 5-lipoxygenase inhibitors ever reported. Table VII shows a comparison of the inhibitory potencies of two of the more potent hydroxamates with some common reference inhibitors measured under the same conditions. Simple hydroxamate **26** is more potent than Revlon-5901,[9] the endogenous regulator 15-hydroxyeicosatetraenoic acid (15-HETE),[10] and the reported suicide inhibitor 5,6-DHA.[11] It is equipotent with BW-755C,[12] phenidone,[13] and also the hydroxamic acid of arachidonic acid. Hydroxamate **51** is nearly 100 times more potent than any of these. It is even 10 times more potent than the antioxidants nordihydroguaiaretic acid[14] and quercetin.[15]

Summary

Simple molecules containing a hydroxamic acid functionality have been demonstrated to be potent inhibitors of 5-lipoxygenase. Groups attached to the hydroxamate moiety have a great influence on the magnitude of this potency, but only a few modifications to the hydroxamate itself maintain inhibition.

A predictive binding hypothesis has been presented that involves a crude graphical representation of features within the active site. This hypothesis has been a useful tool for the identification of potent inhibitors. In fact this approach has resulted in the selection of some of the most potent 5-lipoxygenase inhibitors reported to date.

Experimental Section

Determination of 5-Lipoxygenase Inhibitory Potencies. Assays to determine 5-lipoxygenase activity were performed in 200-μL incubations containing the 20000*g* supernatant from 1.5 million homogenized RBL-1 cells and various concentrations of the test compound. Reactions were initiated by addition of radiolabeled arachidonic acid and terminated by acidification and ether extraction. Reaction products were separated from nonconverted substrate by thin-layer chromatography and measured by liquid scintillation spectroscopy. All incubations are performed in triplicate. Inhibition of 5-lipoxygenase activity was calculated as the ratio of the amounts of product formed in the presence and absence of inhibitor. IC_{50} values and 95% confidence limits were computed from linear regression analysis of percentage inhibition vs. log concentration plots.[16]

Inhibitory potencies against related enzymes were determined in assays similar to that described above, but substituting 12-lipoxygenase obtained from human platelets,[17] 15-lipoxygenase from soybean (Sigma), or cyclooxygenase from sheep seminal vesicles in place of the RBL-1 preparation.

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 the 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.

The synthesis of hydroxamic acid **5** is given as an example. Other hydroxamates were prepared according to this procedure from the corresponding carboxylic acid. The synthesis of noncommercially available carboxylic acids are also described. Physical data for compounds not explicitly described in this section are included in Table VIII.

2-Naphthalenecarbohydroxamic Acid (5). 2-Naphthoic acid (2.5 g, 14.5 mmol) and DMF (1.06 g, 14.5 mmol) were dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. Oxalyl chloride (4.4 g, 32.6 mmol) was added slowly. Vigorous gas evolution was noted. After being stirred for 40 min, this solution was added to a solution of hydroxylamine hydrochloride (3.75 g, 58 mmol) and triethylamine (8.8 g, 87 mmol) in THF (50 mL)/H_2O (10 mL). After being stirred an additional 30 min, the mixture was poured into 2 N HCl and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The residue was recrystallized from aqueous ethanol: yield 2.2 g (82%); mp 169–170

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Table VIII. Summary of Physical Data for Compounds Not Included in the Experimental Section

no.	mp, °C	formula	analysis[a]
2	122–123[b]	$C_8H_9NO_2$	C, H, N
3	70–71[c]	$C_9H_{11}NO_2$	C, H, N
4	77.5–79[d]	$C_{10}H_{13}NO_2$	C, H, N
6	182–184	$C_{12}H_{11}NO_2$	C, H, N
8	191–192 dec[e]	$C_{11}H_9NO_2$	C, H, N
9	179–180[f]	$C_{12}H_{11}NO_2$	C, H, N
10	108–109[g]	$C_9H_9NO_2$	C, H, N
11	87–89	$C_9H_9NO_2$	C, H, N
12	114–115	$C_9H_7NO_2$	C, H, N
13	174–175[h]	$C_7H_6N_2O_4$	C, H, N
14	174–175[i]	$C_8H_6N_2O_2$	C, H, N
15	165–166	$C_8H_6F_3NO_2$	C, H, N, F
16	185[j]	$C_7H_6BrNO_2$	C, H, N, Br
17	200 dec[k]	$C_7H_6INO_2$	C, H, N, I[l]
18	182–183	$C_{13}H_{11}NO_2$	C, H, N
19	147–148[m]	$C_8H_9NO_2$	C, H, N
20	178 dec[n]	$C_7H_7NO_3$	C, H, N
21	190 dec[o]	$C_7H_8N_2O_2$	C, H, N
23	193[p]	$C_{11}H_9NO$	C, H, N
24	146–147[q]	$C_{11}H_{10}N_2O$	C, H, N
26	113–114	$C_{12}H_{11}NO_2$	C, H, N
27	127–128	$C_{12}H_{11}NO_2$	C, H, N
28	oil	$C_{12}H_{11}NO_2$	C, H, N
31	190 dec	$C_{12}H_{11}NO_3$	C, H, N
32	184–185	$C_{15}H_{17}NO_3$	C, H, N
33	169–170	$C_{18}H_{23}NO_3$	C, H, N
34	170–171	$C_{19}H_{25}NO_3$	C, H, N
36	171–172	$C_{23}H_{33}NO_3$	C, H, N
37	166–167	$C_{20}H_{25}NO_3$	C, H, N
39	138–140	$C_{14}H_{13}NO_2$	C, H, N
40	184–185	$C_{16}H_{17}NO_2$	C, H, N
41	187–188	$C_{19}H_{21}NO_2$	C, H, N
42	175–177	$C_{19}H_{15}NO_2$	C, H, N
43	140–141	$C_{15}H_{15}NO_2$	C, H, N
44	129–131	$C_{15}H_{15}NO_2$	C, H, N
46	171–173	$C_{13}H_{11}NO_2$	C, H, N
48	150–151	$C_{17}H_{19}NO_2$	C, H, N
49	169–170	$C_{17}H_{13}NO_2$	C, H, N
50	190 dec	$C_{17}H_{13}NO_2$	C, H, N

[a] Elemental analyses were within ±0.4% of the theoretical value unless otherwise indicated. [b] Lit.[18] 121 °C. [c] Lit.[18] 78 °C. [d] Lit.[19] 85 °C. [e] Lit.[20] 186–187 °C dec. [f] Lit.[21] 164–165 °C. [g] Lit.[18] 111 °C. [h] Lit.[22] 176–177 °C. [i] Lit.[23] 169 °C. [j] Lit.[22] 189–190 °C. [k] Lit.[24] 195–196 °C. [l] C: calcd, 31.96; found, 31.22. I: calcd, 48.25; found, 47.09. [m] Lit.[25] 148 °C. [n] Lit.[26] 179 °C. [o] Lit.[26] 185 °C. [p] Lit.[27] 192 °C. [q] Lit.[28] 146.5 °C.

°C (lit.[20] mp 168 °C); 1H NMR (Me_2SO-d_6) δ 7.2–8.3 (m, 7 H), 9.04 (br s, 1 H), 11.3 (s, 1 H); MS, *m/e* 187, 155, 127. Anal. ($C_{11}H_9NO_2$) C, H, N.

6-(1-Nonyloxy)-2-naphthalenecarbohydroxamic acid (35) was prepared from the corresponding carboxylic acid as described above for **5**. The carboxylic acid was prepared as follows: Potassium *tert*-butoxide (1.67 g, 14.8 mmol) was added to a solution of 6-bromo-2-naphthol (3.0 g, 13.5 mmol) in Me_2SO (30 mL). After the mixture was stirred for 10 min, 1-bromononane (3.63 g, 17.5 mmol) was added to the solution. The reaction mixture was stirred for an additional 15 min and then was poured into 2 N HCl solution (75 mL). Ether (75 mL) was added and the organic phase was washed with 2 N NaOH and saturated NaCl, dried over $MgSO_4$, and evaporated. The solid residue was chromatographed on 100 g of silica gel, eluting with 3% ether in hexanes. Yield 3.1 g (66%) of 2-bromo-6-(1-nonyloxy)naphthalene; MS, *m/e* 350, 348, 224, 222.

The bromo ether prepared as above (1.2 g, 2.4 mmol) in THF (40 mL) was cooled to −78 °C and *tert*-butyllithium (4.4 mL, 1.6 M) was added. After being stirred for 60 min at −78 °C, the mixture was transferred via cannula to a 250-mL Erlenmeyer flask containing dry ice (ca. 20 g) covered with anhydrous ether. After the transfer was complete and the mixture had been allowed to warm to room temperature, the solution was poured into 2 N HCl (75 mL). The organic phase was dried over $MgSO_4$ and evaporated. The residue was recrystallized from aqueous ethanol, yielding 1.0 g (94%) of 6-(1-nonyloxy)-2-naphthoic acid: 1H NMR (Me_2SO-d_6) δ 0.87 (t, 3 H), 1.23–1.25 (m, 12 H), 1.78 (m, 2 H), 4.10 (t, 2 H), 7.20–8.51 (m, 6 H), 9.05 (br s, 1 H), 12.9 (br s, 1 H); MS, *m/e* 314, 270, 188. Anal. ($C_{20}H_{27}NO_3$) C, H, N.

Hydroxamates 31–34, 36, and 37 were prepared as described for **35**.

3-(2-Naphthyl)propenehydroxamic Acid (38). The corresponding carboxylic acid was prepared as follows: Malonic acid (7.5 g, 72.1 mmol) and 2-naphthaldehyde (5.0 g, 32.0 mmol) were dissolved in pyridine, and the mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was poured into 2 N HCl (200 mL). The product precipitated immediately. It was collected by filtration and recrystallized from aqueous ethanol to give colorless needles (5.2 g, 82%): mp 207–208 °C; 1H NMR (Me_2SO-d_6) δ 6.68 (d, 1 H), 7.5–8.2 (m, 8 H), 12.5 (br s, 1 H); MS, *m/e* 198, 181, 153.

This acid was converted to **38** by using the method described for **5**: mp 145–146 °C; 1H NMR (Me_2SO-d_6) δ 6.6 (d, 1 H), 7.3–8.0 (m, 8 H), 9.4 (br s, 1 H), 10.5 (br s, 1 H); MS, *m/e* 213, 196. Anal. ($C_{13}H_{11}NO_2$) C, H, N.

Hydroxamates 39–43 were prepared in a manner similar to **38** with the appropriately substituted hydroxylamine or malonic acid.

3-(2-Naphthyl)propanehydroxamic Acid (7). The corresponding acid was prepared by catalytic hydrogenation of 3-(2-naphthyl)propenoic acid (prepared as for **38**) over 5% palladium on carbon: MS, *m/e* 200, 155.

This acid was converted to **7** by using the standard method: mp 158–159 °C; 1H NMR (Me_2SO-d_6) δ 2.35 (t, 2 H), 2.91 (t, 2 H), 7.4–7.9 (m, 7 H), 8.7 (s, 1 H), 10.4 (s, 1 H); MS, *m/e* 215. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

3,*N*-Dimethyl-3-(2-naphthyl)propenehydroxamic Acid (44). The corresponding carboxylic acid was prepared as previously described.[29]

***N*-Methyl-3-(2-naphthyl)-2-phenylpropenehydroxamic Acid (45).** A mixture of 2-naphthaldehyde (5 g, 32 mmol), phenylacetic acid (4.4 g, 32 mmol), triethylamine (5 mL), and acetic anhydride (10 mL) was refluxed for 5 h. The solution was poured into 2 N HCl (150 mL) and a precipitate formed. It was collected by filtration and recrystallized from 50% aqueous ethanol to yield 7.8 g (89%) of 3-(2-naphthyl)-2-phenylpropenoic acid: MS, *m/e* 274.

This material was converted to the hydroxamic acid as described above for **5**, with *N*-methylhydroxylamine hydrochloride: mp 156–158 °C; 1H NMR (Me_2SO-d_6) δ 3.33 (s, 3 H), 7.04 (s, 1 H), 7.13–7.84 (m, 12 H), 8.97 (br s, 1 H); MS, *m/e* 303, 257, 229. Anal. ($C_{20}H_{17}NO_2$) C, H, N.

4-(2,4,6-Trimethylphenyl)benzenecarbohydroxamic acid (47) was prepared from the corresponding carboxylic acid as described above for **5**. This acid was prepared as follows: *tert*-Butyllithium (14.3 mL, 1.6 M) was added at −78 °C to a THF (15 mL) solution of 2-bromomesitylene (2.3 g, 11.5 mmol). After 15 min this solution was transferred via cannula to a suspension of zinc chloride (1.6 g, 11.5 mmol) in THF (10 mL). The resulting mixture was stirred for 1 h at room temperature. In a separate flask, diisobutylammonium hydride (1.5 mmol) was added to a solution of palladium bis(triphenylphosphine) dichloride (535 mg, 0.76 mmol) in THF (25 mL). To this was added a THF (20 mL) solution of methyl 4-iodobenzoate (2.0 g, 7.6 mmol). The zinc reagent prepared above was added via cannula to the palladium mixture. After being stirred for 1 h, the reaction was poured into 2 N HCl and the product extracted into ether. The organic phase was evaporated in vacuo and the residue flash chromatographed on 120 g of silica gel, eluting with 2% ether in hexanes. Methyl 4-(2,4,6-trimethylphenyl)benzoate was obtained (1.5 g, 52%): 1H NMR δ 1.9 (s, 6 H), 2.3 (s, 3 H), 3.8 (s, 2 H), 7.1 (d, 2 H), 7.9 (d, 2 H).

This ester was hydrolyzed with LiOH (1.3 g) in 50% 2-propanol/water (20 mL). The resulting acid was converted to **47** as described for **5**: mp 129–131 °C; 1H NMR (Me_2SO-d_6) δ 1.92 (s, 6 H), 2.27 (s, 3 H), 6.95 (s, 2 H), 7.2 (d, 2 H), 7.83 (d, 2 H), 9.1 (br s, 1 H), 11.27 (br s, 1 H); MS, *m/e* 255, 223, 180. Anal. ($C_{16}H_{17}NO_2$) C, H, N.

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Hydroxamates 48–50 were prepared as described for **47**.

***N*-Methyl-2-[4-(2,4,6-trimethylphenyl)phenyl]propenehydroxamic Acid (51).** The corresponding carboxylic acid was prepared from methyl 4-(2,4,6-trimethylphenyl)benzoate (prepared as described for **47**). The ester (3.0 g, 11.8 mmol) was dissolved in CH_2Cl_2 and cooled to −78 °C. Diisobutylaluminum hydride (27.1 mmol) was added and the reaction mixture stirred for 40 min. After warming to room temperature, the mixture was poured into 2 N HCl and the organic phase was dried with $MgSO_4$ and evaporated. 4-(2,4,6-Trimethylphenyl)benzyl alcohol was obtained in 88% yield (2.4 g) and was carried on without purification.

A mixture of the alcohol (2.0 g, 8.8 mmol) prepared above and pyridinium chlorochromate (3.36 g, 15.6 mmol) in CH_2Cl_2 (100 mL) was stirred for 2 h at room temperature. Ether was added and the mixture was filtered through a pad of silica gel to remove chromium salts. After removal of the solvent in vacuo, 1.57 g (79%) of nearly pure 4-(2,4,6-trimethylphenyl)benzaldehyde was obtained.

The benzaldehyde prepared above was converted to 2-[4-(2,4,6-trimethylphenyl)phenyl]propenoic acid in a manner similar to that described for **38** and then to hydroxamate **51** by using the standard procedure: mp 170–171 °C; ^1H NMR (Me_2SO-d_6) δ 1.92 (s, 6 H), 2.27 (s, 3 H), 3.23 (s, 3 H), 6.95 (s, 2 H), 7.18 (d, 2 H), 7.27 (d, 1 H), 7.56 (d, 1 H), 7.77 (d, 2 H), 10.12 (br s, 1 H); MS, *m/e* 295, 245. Anal. ($C_{19}H_{21}NO_2$) C, H, N.

2-(2-Naphthyl)-2-oxoethanol (25). 2-Naphthoyl chloride (2.9 g, 15.2 mmol) in ether (50 mL) was added to a solution of triethylamine (1.69 g, 16.7 mmol) and diazomethane (75 mL, ~0.5 M) in ether (100 mL) at 0 °C. After 1 h the triethylamine hydrochloride was filtered away and the solvent removed in vacuo. The resulting diazo ketone was dissolved in THF (50 mL), and a few drops of $HClO_4$ were added. Fifteen minutes later the solvent was evaporated and the residue chromatographed on 100 g of SiO_2, eluting with 25% ether in hexanes: MS, *m/e* 186, 155, 127. Anal. ($C_{12}H_{10}O_2$) C, H.

***N*-Hydroxy-2-naphthalenesulfonamide (29).** 2-Naphthalenesulfonyl chloride (2.0 g, 8.82 mmol) in CH_2Cl_2 (25 mL) was added to a solution of hydroxylamine hydrochloride (2.45 g, 35.3 mmol) and triethylamine (5.4 g, 53 mmol) in THF (30 mL)/H_2O (10 mL). After being stirred for 30 min, the mixture was poured into 2 N HCl and the organic layer was dried over $MgSO_4$ and evaporated. The residue was flash chromatographed on 80 g of SiO_2, eluting with 50% ether in hexanes. The yield was 1.51 g (77%). **29**: mp 158–160 °C; ^1H NMR (Me_2SO-d_6) δ 7.65–8.2 (m, 6 H), 8.5 (s, 1 H), 9.66 (dd, 2 H); MS, *m/e* 223, 191. Anal. ($C_{11}H_9NO_3S$) C, H, N; S: calcd, 13.63; found, 12.91.

***N*-Methyl-*N*-hydroxy-2-naphthalenesulfonamide (30)** was prepared in the same manner as for **29** with *N*-methylhydroxylamine: mp 134–135 °C. Anal. ($C_{12}H_{11}NO_3S$) C, H, N, S.

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Notes

Synthesis of D-Oxa Tricyclic Partial Ergolines as Dopamine Agonists

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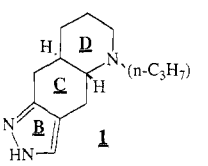
A series of hetero fused hexahydro-1,4-benzoxazines has been synthesized and evaluated for dopamine agonist activity. This class of compounds is another example in which an oxygen substitution in the D ring of a partial ergoline or ergoline retains dopaminergic properties. Compound **10**, *trans*-(±)-4,4a,5,6,8a,9-hexahydro-5-propyl-2*H*,7*H*-pyrazolo[4,3-*g*][1,4]benzoxazine, is a D-ring analogue of *trans*-(±)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2*H*-pyrazolo[3,4-*g*]quinoline (**1**, LY141865) and also a des-A-ring analogue of 9-oxaergoline (**2**). Compounds **10**, 2-aminohexahydrothiazolo[1,4]benzoxazine **11**, and 2-aminohexahydropyrimido[1,4]benzoxazine **12** possess dopaminergic activity in prolactin inhibition and 6-hydroxydopamine lesioned rat turning assays.

Ergoline derivatives such as bromocryptine, lergotrile, and pergolide possess dopaminergic activity.[1,2] These dopamine agonists have therapeutic utility in the treatment of Parkinson's disease,[3] inhibition of postpartum lactation,[1] and galactorrhea–amenorrhea syndrome.[1]

The BCD partial ergoline structure **1** (LY141865)[4] demonstrated that the aromatic A ring of the ergoline molecule is not necessary to retain dopaminergic properties. Furthermore, recent reports by Jones and co-workers[5,6] described new classes of dopamine agonists that are



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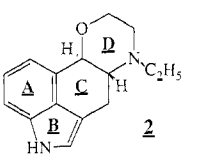

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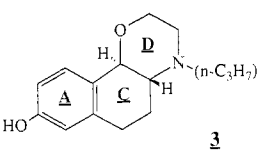

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