Synthesis, Chemical, and Biological Properties of Vinylogous Hydroxamic Acids: Dual Inhibitors of 5-Lipoxygenase and IL-1 Biosynthesis

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Vinylogous hydroxamic acids (3-(*N*-hydroxy-*N*-alkylamino)-2-propen-1-ones, VHA) were prepared as antiinflammatory agents. The synthesis, chemical properties, and in vitro biological activities of these relatively unexplored compounds are described. The VHAs were prepared by condensation of the appropriate N-substituted hydroxylamine with any of the three reagents: a 1,3-dicarbonyl compound (method A); a vinylogous amide (method B); or an alkynone (method C). The VHAs exist as one or more tautomers in solution with the relative proportions of each being dependent upon the structure of the VHA, solvent, and pH. VHAs undergo some of the typical reactions of hydroxamic acids as well as those of vinylogous amides. VHAs are active as inhibitors of 5-lipoxygenase and of IL-1 biosynthesis in vitro, which do not inhibit other enzymes of the arachidonic acid cascade. They have been shown by ESR studies to bring about inhibition of soybean type 1 15-lipoxygenase by reduction of the active site iron.

Introduction

Hydroxamic acids (1) have received considerable attention recently as enzyme inhibitors, particularly of the enzyme 5-lipoxygenase, which is thought to play a key role in mediating certain inflammatory diseases.[1a-f] While hydroxamic acids are well known and characterized compounds, the corresponding vinylogs of hydroxamic acids, 3-(*N*-hydroxy-*N*-alkylamino)-2-propen-1-ones (VHA, 2), represent an almost entirely unknown class of compounds.[2] By analogy to other vinylogous systems, VHAs would be expected to possess some properties similar to those of hydroxamic acids. For example, VHAs may form a bidentate chelate with transition metal ions, such as iron(III). VHAs should likewise share the acidic and reducing properties of hydroxamic acids.[3] The olefin in the VHAs allows additional substituents to be incorporated into the molecule. The greater diversity of structures than is possible with simple hydroxamic acids allows for additional interesting chemical and/or biological properties. The synthesis, chemical properties, and preliminary in vitro characterization of these compounds is reported herein.

Synthesis

We envisioned the synthesis of VHAs to be feasible via five possible routes: (a) condensation of an *N*-substituted hydroxylamine (R_4NHOH) with a 1,3-dicarbonyl compound (3); (b) addition–elimination of an *N*-substituted hydroxylamine with an 2-propen-1-one bearing a suitable β-leaving group (4) such as chloro, methanesulfonate, alkoxy, or dialkylamino; (c) the addition of an *N*-substituted hydroxylamine to a 2-propyn-1-one (5); (d) the oxidation of a *N*-hydroxyl-3-(alkylamino)propanone (6); or (e) the oxidation of a 3-(alkylamino)-2-propen-1-one (7). The *N*-alkyl-substituted hydroxylamines used in this work were prepared by the reduction of the corresponding oximes[4a,b] 1, or nitro compounds,[4c] or by the alkylation of a suitably protected hydroxylamine.[4d] *N*-Aryl-substituted hydroxylamines were prepared by the reduction of the corresponding nitro compounds.[4e] The utility of each of these routes was studied; our findings are summarized below.

A simple and inexpensive entry into the VHA series from appropriately substituted 1,3-dicarbonyl compounds and hydroxylamines was found to be convenient and generally furnished the desired VHA in good yield (method

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(2) (a) These compounds are the subject of a recent U.S. patent 5,110,831. (b) A series of cyclic vinylogous *N*-hydroxy-*N*-methylureas have recently been described as inhibitors of 5-lipoxygenase: Flynn, G. A.; Beight, D. W. Novel Cyclic N-Hydroxy-N-methylureas Useful as 5-Lipoxygenase Inhibitors. *Eur. Pat. Appl.* 90113677, filed July 17, 1990.

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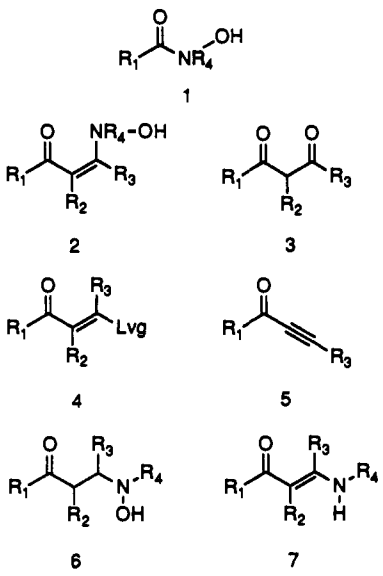
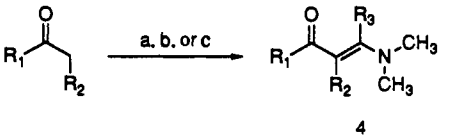


A).[5] The reaction proceeded rapidly at 25 °C and was frequently complete within minutes. This reaction proceeded best in protic solvents, particularly methanol, from which the analytically pure VHAs usually crystallized directly from the reaction mixture.[6] The required β-keto aldehyde and β-diketones (3) were readily prepared by acylation of the appropriate ketones ($R_1COCH_2R_2$) using standard methods.[7]

The successful synthesis of VHAs from 2-propen-1-ones with various β-leaving groups (4) and hydroxylamines, by an addition–elimination reaction, was dependent upon the nature of the leaving group (Lvg). For example, the amine exchange reaction of vinylogous amides (3-(*N*,*N*-dimethylamino)-2-propen-1-ones 4, Lvg = $N(CH_3)_2$) and hydroxylamines provided a convenient route to VHA (method B).[8] The reaction of 4 with hydroxylamines to give 2 occurred rapidly in the presence of 1 equiv of acid, usually *p*-toluenesulfonic or hydrochloric acid, in protic solvents. Methanol was again the solvent of choice for this reaction, as the VHAs generally precipitated directly from the reaction mixture in pure form. The reaction of 4 with hydroxylamines would not form 2 in the absence of acid. In fact the reaction of 2 with excess dimethylamine was found to furnish 4. The vinylogous dimethylamides were prepared in high yields from the corresponding ketones ($R_1COCH_2R_2$) by any one of three methods (Scheme I). The use of dimethylamide dimethyl acetals, such as DMF dimethyl acetal, was found to be the most convenient method for the preparation of 4 (Lvg = $N(CH_3)_2$).

Scheme I [a]



[a] (a) $R_3C(OCH_3)_2N(CH_3)_2$, reflux; (b) Gold's reagent, CH_3OH, $NaOCH_3$, reflux (R_3 = H); (c) (i) $NaOCH_3$, $R_3CO_2C_2H_5$, THF; (ii) $(CH_3)_2NH$ HCl, CH_3OH.

The reaction of 3-methoxy-2-propen-1-ones[9] (4, Lvg = OCH_3) with hydroxylamines in methanol to give 2 was likewise successful. In this case, as with method A, no acid was necessary for the reaction to occur. However, the reactions of 3-chloro-2-propen-1-ones[10] (4, Lvg = Cl), 3-(*p*-tolylsulfonoxy)-2-propen-1-ones[11] (4, Lvg = *p*-TsO), or 3-(methylsulfonoxy)-2-propen-1-ones (4, Lvg = MsO) with hydroxylamines in a variety of solvents resulted in the formation of complex mixtures from which only low yields of the desired VHAs could be isolated. Attempts to improve the outcome of these reactions by the addition of an auxiliary base, such as triethylamine or pyridine, to scavenge the HCl, *p*-TsOH, or MsOH liberated during the reaction were likewise unsuccessful. Examination of the product mixture from these reactions indicated that products resulting from nucleophilic attack of the hydroxylamine N or O atom, or both, upon 4 (Lvg = Cl, *p*-TsO, MsO) were present. This loss of selectivity is likely due to the higher susceptibility of these 3-substituted 2-propen-1-ones to addition–elimination reactions.

The addition of hydroxylamines to suitable acetylenic precursors 5 was successful (method C).[12] This route provided certain VHAs that would have otherwise been difficult to obtain by methods A and B, (e.g. 2, $R_1 = OCH_3$). The addition of the hydroxylamine to the alkyne could be controlled to give a good yield of 2 by slow addition of the alkyne to the hydroxylamine with cooling. Dichloromethane was the solvent of choice, and the use of protic solvents gave lower yields. In general, however, acetylenic starting materials 5 were not used because of the ready availability of more convenient precursors such as 3 and 4.

Attempts to synthesize VHAs by the oxidation of 3-(*N*-hydroxy-*N*-alkylamino)-2-propan-1-ones 6 were unsuccessful. While similar transformations have been reported to occur, product 2 could not be detected upon treatment of 6 with a variety of oxidants, including *m*-CPBA in CH_2-

(5) The preparations of three VHAs by this method have been reported: Woodward, R. B.; Woodman, D. J.; Kobayashi, Y. The Reaction of 3-Unsubstituted N-Arylisoxazolium Salts with Carboxylic Acid Anions. *J. Org. Chem.* **1967**, *32*, 388–391. (b) De Sarlo, F.; Renzi, G. Reaction of Benzoyl(phenyl)acetaldehyde with N-Phenylhydroxylamine: a Re-examination. *J. Chem. Soc., Perkin Trans. I* **1978**, 1113–1116.

(6) The reaction of 1,3-dicarbonyl compounds and hydroxylamines to give 2 also occurred in other non-protic solvents such as $CHCl_3$, CH_2Cl_2, and THF; however, the VHAs generally did not precipitate from these mixtures. Isolation in these cases required extraction and chromatography.

(7) House, H. O. *Modern Synthetic Reactions, 2nd ed.*; Benjamin: Menlo Park, CA, 1972.

(8) The preparation of one VHA by this method has been reported: Lin, Y.; Lang, S. A. Jr. Novel Two Step Synthesis of Pyrazoles and Isoxazoles from Aryl Methyl Ketones. *J. Heterocycl. Chem.* **1977**, *14*, 345–347.

(9) (a) Hallberg, A.; Andersson, C.-M. Palladium Catalyzed Aroylation of Alkyl Vinyl Ethers. An Entry to Monoprotected 1-Aryl-1,3-dicarbonyl Equivalents. *J. Org. Chem.* **1988**, *53*, 4257–4263. (b) Hudlicky, T.; Olivo, H. F.; Natchus, M. G. Synthesis of β-Methoxy Enones via a New Two-Carbon Extension of Carboxylic Acids. *J. Org. Chem.* **1990**, *55*, 4767–4770.

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(11) Jalander, L.; Mattinen, J.; Oksanen, L.; Rosling, A. Synthesis of Enol Tosylates of β-Keto Esters. *Synth. Commun.* **1990**, *20*, 881–886.

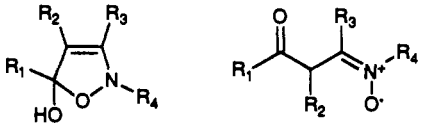
(12) The synthesis of three VHAs by this method have been reported: Padwa, A.; Wong, G. S. K. 1,3-Dipolar Cycloadditions of Nitrones Derived from the Reaction of Acetylenes with Hydroxylamines. *J. Org. Chem.* **1986**, *51*, 3125–3133.

Cl_2,[13a] H_2O_2 and Na_2WO_4 in CH_3CN,[13a,b] $K_3Fe(CN)_6$,[13b] or PbO_2 in $CHCl_3$.[13c] In each case TLC analysis indicated that a complex mixture of products was formed. Attempts to prepare VHAs by the oxidation of the readily accessible vinylogous amides **7**, using conditions reported to give *N,N*-dialkylhydroxylamines from secondary amines, were likewise unsuccessful, including the use of H_2O_2 and Na_2WO_4 in EtOH,[14a] dimethyldioxirane,[14b] and *N*-benzoylperoxycarbamic acid.[14c] In these instances, no reaction could be detected by TLC and the starting materials **7** were recovered in high yield.

Physical Properties

The VHAs were obtained as crystalline solids which in most cases were stable indefinitely at room temperature in the solid state. In those VHAs wherein R_1 was an aromatic residue, the VHA were bright yellow or orange, while those VHAs in which R_1 was an alkyl or alkoxy group were colorless. Aromatic (R_1 = aryl) VHAs exhibited a characteristic band in their UV spectrum (EtOH) at ca. 360 nm ($\epsilon \approx 19\,000$; R_4 = alkyl) or 385 nm ($\epsilon \approx 26\,000$; R_4 = aryl). Aliphatic (R_1 = cyclopropyl) VHAs absorbed at ca. 315 nm ($\epsilon \approx 15\,000$). The infrared spectra (KBr pellet) were distinguished by the presence of a relatively weak OH band (3500 cm^{-1}) and strong C=O and C=C absorbances (1620, 1590 cm^{-1}; R_1 = aryl or 1695, 1685 cm^{-1}; R_1 = alkyl). The mass spectra (NH_3Cl) of the VHAs were notable for the presence of both the expected $M + H^+$ ion and a strong fragment ion at $M + H^+ - 16$, corresponding to loss of oxygen from the parent ion.

Several possible tautomeric forms for the VHA may be considered. These include the (*Z*)-VHA **2** or 5-hydroxyisoxazolidine **8**, the nitrone **9**, the oxaziridine **10**, and the (*E*)-VHA **11**. Examination of the ^1H and ^{13}C NMR spectra



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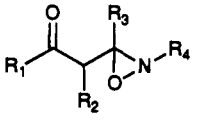

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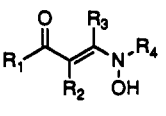

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of the VHAs revealed that each of the tautomeric possibilities were present in solution in varying proportions. The relative proportion of each tautomer was found to be dependent upon the solvent in which the spectrum was recorded as well as the structure of the particular VHA. For example, a typical aromatic VHA (**2f**, R_1 = 4-($C_6H_4CH_2O$)C_6H_4, $R_2 = R_3$ = H, $R_4 = CH_3$) existed as a 55:35:10 mixture of **9f–2f–11f** in $CDCl_3$ at 25 °C.[15] The R_2 proton was exchangeable with D_2O, suggesting that equilibration between these tautomers is rapid. Addition of 10% CD_3OD to the $CDCl_3$ solution caused the proportion of nitrone present to increase at the expense of **2f** and **11f** to give a 70:25:5 mixture of **9f–2f–11f**. Further addition of CD_3OD to the solution did not cause a significant change in the relative proportions of the tautomers present. In the presence of a trace of base (Et_3N in $CDCl_3$ or Na_2CO_3 in CD_3OD), the spectrum was considerably simplified and only the (*E*)-VHA **11f** was detected. In the presence of a trace of acid (CF_3CO_2D in $CDCl_3$), only the nitrone **9f** was noted. The proportion of **2f** (or **8f**) was increased in C_6D_6; in this solvent a 85:15 mixture of **2f–9f** was present and **11f** could not be detected. In both acetone-d_6 and DMSO-d_6 only **2f** was present. In no case could the oxaziridine **10f** be detected. Other aromatic VHAs behaved similarly, with the proportion of **2** to **9** varying from 3:1 to 1:2 in $CDCl_3$. By contrast, an aliphatic VHA (**2uu**, R_1 = $(CH_3)_3C$, $R_2 = R_3$ = H, R_4 = $CH_2C_6H_5$) was found to exist exclusively as a mixture of diastereomeric oxaziridines **10uu** in acetone-d_6, while in $CDCl_3$ a mixture of **2uu** and only one oxaziridine diastereomer was noted. Not unexpectedly, those VHAs which lacked a substituent on nitrogen (R_4 = H) existed exclusively as a mixture of the *syn*- and *anti*-oxime tautomers in all solvents examined. VHAs with methyl or acetyl groups on the hydroxylamine oxygen atom, lacking the NOH moiety, existed exclusively in the *E*-VHA **11** tautomer.

Chemical Properties

Like hydroxamic acids, VHAs are weak acids that do not react with mild bases, such as $NaHCO_3$, but can be dissolved with strong bases (NaOH or $NaOCH_3$), with which they form salts. Like the corresponding vinylogous amides **7**, the VHAs are not basic and are not extracted by aqueous acids.[16] Also similarly to **7**, the VHAs are not stable upon prolonged exposure to either strong acids or strong bases, in which they undergo hydrolysis to the hydroxylamine and corresponding 1,3-dicarbonyl compound. Unlike **7**, which react with electrophiles at the α-carbon atom (similar to enamines),[17] VHAs react with acylating agents primarily on oxygen. The oximes (R_4 = H) are alkylated primarily at the α-carbon and are acylated on oxygen. The VHAs were found to be mild reducing agents similar to hydroxamic acids, reducing such reagents as $K_2Cr_2O_7$, $KMnO_4$, Fehling's solution, and Tollen's reagent. The potential ability of VHAs to act as oxidizing agents was also examined. No oxidation of either dibenzyl sulfide or triphenylphosphine could be detected even upon

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(15) Characteristic ^1H NMR resonances for the (*E*)-VHA **11f**: δ 6.98 (d, 1 H, J = 14 Hz, R_3 = H), 5.05 (d, 1 H, J = 14 Hz, R_2 = H), 5.15 (s, 2 H, $PhCH_2O$), 2.78 (d, 3 H, NCH_3); for the (*Z*)-VHA (or 5-hydroxyisoxazolidine) **2f** (**8f**): δ 6.87 (d, 1 H, J = 6.6 Hz, R_3 = H), 5.31 (d, 1 H, J = 6.6 Hz, R_2 = H), 5.10 (s, 2 H, $PhCH_2O$), 3.62 (s, 3 H, NCH_3); for the nitrone **9f**: δ 7.43 (m, 1 H, MeN(O)=C*H*CH_2), 5.14 (s, 2 H, $PhCH_2O$), 4.17 (d, 2 H, $COCH_2$), 3.78 (s, 3 H, NCH_3); for the *cis*-oxaziridine **10uu**: 4.93 (d, J = 13.2 Hz, 1 H, $PhCH_2$), 4.87 (d, J = 13.2 Hz, 1 H, $PhCH_2$), 4.00 (d of d, J = 5.5, 9.8 Hz, 1 H, oxaziridine CH), 2.84 (m, 2 H, $COCH_2$); for the *trans*-oxaziridine **10uu**: 4.20 (d, J = 13.9 Hz, 1 H, $PhCH_2$), 3.87 (d of d, J = 7.7, 9.9 Hz, oxaziridine CH), 2.84 (m, 2 H, $COCH_2$). Characteristic ^{13}C NMR resonances for the (*Z*)-VHA (or 5-hydroxyisoxazoline) **2f** (**8f**): δ 135 (CR_1(OH)), 114 (CR_3), 88 (CR_2), 69 ($PhCH_2O$), 45 (NCH_3); for the nitrone **9f**: δ 192 (C=O), 127 (CR_3), 114 (CR_2), 35 (NCH_3).

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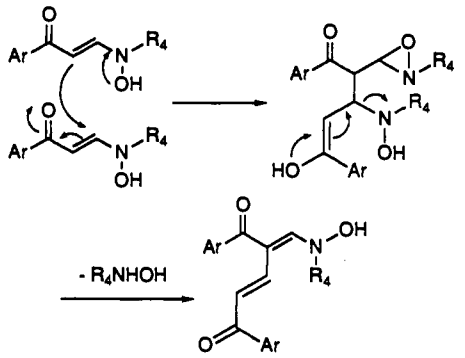
Scheme II



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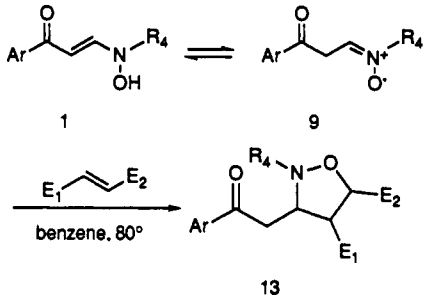
heating in toluene at reflux. In contrast to hydroxamic acids, the VHAs generally did not give a color change upon treatment with aqueous solutions of transition metals, such as Cu(II) and Fe(III), suggesting that they do not chelate the metal ion.[18]

In general, aliphatic VHAs (**2**, R_1 = alkyl) are considerably more reactive and prone to self-condensation reactions than the corresponding aromatic VHAs (**2**, R_1 = aryl). The aliphatic VHAs are stable enough to permit isolation and characterization only when the aliphatic residue R_1 was cyclopropyl or *tert*-butyl. Self-condensation of aromatic VHAs was enhanced if the conjugation of the carbonyl group to the R_1 aromatic ring was disrupted by ortho substituents on the aromatic ring.[19] In these cases, both the bis-condensation product **12** and a small amount of the expected **2** could be isolated upon reaction of either **3** or **4** with *N*-benzylhydroxylamine (Table II). This occurred only when at least one ortho substituent (Cl, F, NO_2, OCH_3, CH_3) was present in R_1. Formation of the diene **12** can be rationalized by an intermolecular Michael addition of two equiv of **2** (possibly via the tautomeric nitrone **10**) followed by the elimination of 1 equivalent of *N*-benzylhydroxylamine (Scheme II). Attempts to change the outcome of these reactions by the use of *O*-trimethylsilyl-*N*-benzylhydroxylamine or *O*-(*tert*-butyldimethylsilyl)-*N*-benzylhydroxylamine were unsuccessful, with the diene **12** again being isolated as the major product. However, the use of *O*-methyl-*N*-benzylhydroxylamine under the same conditions did afford the expected *O*-methoxy VHAs (R_4 = $CH_2C_6H_5$) as the major product (Table I: **2bbb**, **2ccc**).

Aromatic VHAs (**2**, R_1 = aryl; R_2 = R_3 = H) were found to undergo [3 + 2] cycloaddition reactions with electron deficient olefins such as diethyl fumarate, *N*-phenylmaleimide, and dimethyl acetylenedicarboxylate to afford the expected cycloadducts in good yield (Scheme III). These reactions likely occur via the nitrone tautomer **9**.[20] They occurred slowly at 25 °C but were completed within 1 h in benzene at 80 °C. Chromatography of the reaction mixture afforded the cycloadducts **13**, which in most cases

Scheme III



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could be crystallized from an appropriate solvent to give analytically pure samples. The vinylogous amides **4** (Lvg = $N(CH_3)_2$) and **7** failed to react under the same conditions, with the starting materials being recovered in high yield.

Biological Evaluation

The VHAs were examined for their ability to inhibit various enzymes in the arachidonic acid cascade thought to play a role in inflammatory diseases. They were found as a class to be inactive as inhibitors of cyclooxygenase[21] (bovine seminal vesicles, IC_{50} generally >750 μM) and PLA_2[22] (*Croatalus adamanteus*, IC_{50} generally >1 mM). As expected, they were active as inhibitors of 5-lipoxygenase (5-LO)[23] (rat basophilic leukemia cell line) (Table I).

Two of the VHAs (**2a**, 5-LO K_i = 1.8 μM; **2f**, 5-LO K_i = 1.1 μM) were selected for mechanistic studies with soybean type 1 lipoxygenase (SBLO).[24] These VHAs were found to inhibit SBLO (**2a**, K_i = 20 μM; **2f**, K_i = 40 μM) in 0.1 M pH 9.0 borate buffer. Furthermore, these two compounds were shown by ESR experiments to bring about SBLO inhibition by reduction of the catalytically critical active site iron atom of SBLO from the active Fe(III) state to the inactive Fe(II) state.[25,26] The corresponding vinylogous *N*-methylamides (**7a** and **7f**) did not inhibit either SBLO ($K_i \gg 100$ μM in each case) or 5-LO ($K_i > 25$ μM in each case). The vinylogous amides **7a** and **7f** also failed to reduce the active site iron atom of SBLO in ESR experiments. The reduction of the active site iron by the VHAs is similar to the previously reported reduction of the active site iron by other lipoxygenase inhibitors,

(18) We have so far been unable to fully characterize the products formed upon the reaction of **2** with $FeCl_3$. The formation of 1,3-dicarbonyl chelate complexes from vinylogous amides **7** and transition metal salts by in situ hydrolysis of the vinylogous amide has been reported: Gash, V. W. Convenient Synthesis of Metal Chelates. *Can. J. Chem.* **1967**, *45*, 2109–2112.

(19) Unlike other aromatic VHAs (**2**, R_1 = aryl), aromatic VHAs in which R_1 bore one or more ortho substituents were colorless.

(20) Some [3 + 2] cycloadditions of this type have been reported: Padwa, A.; Wong, G. S. K. 1,3-Dipolar Cycloadditions of Nitrones Derived from the Reaction of Acetylenes with Hydroxylamines. *J. Org. Chem.* **1986**, *51*, 3125–3133.

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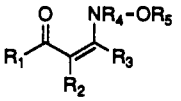
(26) No attempt was made to isolate and characterize the oxidized VHA product(s) resulting from these incubations.

Table I. Physical and in Vitro Data for VHAs (2)



entry	R_1	R_2	R_3	R_4	R_5	5-LO, IC_{50}[a]	% yield[b]	method	mp, °C
2a	2-naphthyl	H	H	CH_3	H	1.8	58	A	100–102
2b	2-naphthyl	H	H	c-C_6H_{11}	H	2.6	74	B	168–170
2c	2-naphthyl	H	H	C_6H_5	H	0.81	49	A	168–170
2d	2-naphthyl	H	H	1-(2-naphthyl)ethyl	H	0.75	61	B	127–129
2e	2-naphthyl	H	H	$CH_2C_6H_5$	H	1.6	80	A	125–127
2f	4-(BzO)C_6H_4[c]	H	H	CH_3	H	1.1	76	B	125–127
2g	c-C_3H_5	H	H	4-(BzO)$C_6H_4CH_2$[d]	H	2.6	64	B	107–109
2h	4-$BzOC_6H_4$	H	H	CH_3	$COCH_3$	>25	31	D	61–63
2i	CH_3O	H	H	4-(BzO)$C_6H_4CH_2$	H	4.8	63	C	121–123
2j	4-(BzO)C_6H_4	CH_3	H	CH_3	H	8.9	36	B	104–106
2k	4-FC_6H_4	H	H	n-$C_{10}H_{21}$	H	13	18	B	52–54
2l	4-(BzO)C_6H_4	H	H	CH_3	Na	1.9	90	E	230 dec
2m	4-$PicOC_6H_4$[e]	H	H	CH_3	H	10	82	B	131–133
2n	2-thianaphthyl	H	H	CH_3	H	0.24	87	B	139–141
2o	c-C_3H_5	H	H	CH_2(2-naphthyl)	H	1.5	80	B	133–135
2p	c-C_3H_5	H	H	4-$C_6H_4C_6H_4CH_2$	H	1.2	72	B	129–131
2q	c-C_3H_5	H	H	4-(C_6H_5O)$C_6H_4CH_2$	H	1.4	81	B	98–101
2r	4-(CH_3O)C_6H_4	4-(CH_3O)C_6H_4	H	CH_3	H	>25	10	B	182–184
2s	4-(CH_3S)C_6H_4	4-FC_6H_4	H	CH_3	H	>25	11	B	219–221
2t	C_6H_5	C_6H_5	H	CH_3	H	>25	9	B	162–164
2u	4-FC_6H_4	H	H	CH_3	H	5.0	20	B	81–82
2v	4-FC_6H_4	H	H	$CH_2C_6H_5$	H	0.15	34	B	98–100
2w	4-FC_6H_4	H	H	c-C_6H_{11}	H	0.27	78	A	117–119
2x	–$C_6H_4CH_2CH_2$–[f]		H	$CH_2C_6H_5$	H	1.3	52	A	102–104
2y	4-ClC_6H_4	H	H	$CH_2C_6H_5$	H	1.5	74	A	120–122
2z	4-$C_6H_5C_6H_4$	H	H	$CH_2C_6H_5$	H	0.25	79	A	170–172
2aa	4-(NO_2)C_6H_4	H	H	CH_3	H	1.5	61	B	146–148
2bb	4-$C_6H_5C_6H_4$	H	H	CH_3	H	0.06	65	B	143–145
2cc	4-(CH_3O)C_6H_4	H	H	CH_3	H	6.0	44	B	112–114
2dd	4-ClC_6H_4	H	H	CH_3	H	0.55	35	B	112–114
2ee	4-(NO_2)C_6H_4	H	H	$CH_2C_6H_5$	H	2.5	48	B	151–153
2ff	$(CH_3)_3C$	H	H	$COCH_3$	$CH_2C_6H_5$	>25	30	D	52–53
2gg	4-pyridyl	H	H	$CH_2C_6H_5$	H	2.0	73	B	140–142
2hh	4-FC_6H_4	H	H	4-$C_6H_5C_6H_4CH_2$	H	3.0	72	A	159–160
2ii	4-FC_6H_4	H	H	CH_2(2-naphthyl)	H	0.40	61	A	128–130
2jj	2,6-$Cl_2C_6H_3$	H	H	$CH_2C_6H_5$	H	1.7	56	A	106–107
2kk	3,4-$Cl_2C_6H_3$	H	H	$CH_2C_6H_5$	H	0.61	15	A	112–113
2ll	4-FC_6H_4	H	H	$CH_2C_6H_5$	$COCH_3$	>25	44	D	115–117
2mm	4-(NO_2)C_6H_4	H	H	$CH_2C_6H_5$	$COCH_3$	>25	84	D	122–123
2nn	4-$C_6H_5C_6H_4$	H	H	$CH_2C_6H_5$	$COCH_3$	>25	42	D	121–122
2oo	3-thienyl	H	H	4-$C_6H_5C_6H_4CH_2$	H	3.0	70	B	152–154
2pp	2-furyl	H	H	4-$C_6H_5C_6H_4CH_2$	H	3.1	54	B	117–119
2qq	3,5-$(CF_3)_2C_6H_3$	H	H	CH_3	H	0.91	54	B	119–121
2rr	2-thienyl	H	H	4-$C_6H_5C_6H_4CH_2$	H	>25	62	B	139–141
2ss	4-FC_6H_4	H	H	C_6H_5	H	0.20	50	A	157–158
2tt	4-FC_6H_4	H	H	$CH_2CH_2C_6H_5$	H	0.12	77	A	122–123
2uu	$(CH_3)_3C$	H	H	$CH_2C_6H_5$	H	5.0	47	A	100–101
2vv	$(CH_3)_3C$	H	H	$CH_2C_6H_5$	$COCH_3$	>25	60	D	126–127
2ww	4-ClC_6H_4	H	H	$CH_2C_6H_5$	$COCH_3$	>25	15	D	89–90
2xx	4-ClC_6H_4	H	H	CH_3	$COCH_3$	>25	27	D	79–80
2yy	4-(NO_2)C_6H_4	H	H	CH_3	$COCH_3$	>25	31	D	155–156
2zz	4-$C_6H_5C_6H_4$	H	H	CH_3	$COCH_3$	>25	21	D	67–68
2aaa	4-FC_6H_4	H	CH_3	$CH_2C_6H_5$	H	0.25	14	A	95–96
2bbb	2-(CH_3O)C_6H_4	H	H	$CH_2C_6H_5$	CH_3	>25	84	A	oil
2ccc	4-FC_6H_4	H	H	$CH_2C_6H_5$	CH_3	>25	45	A	76–77
2ddd	$(CH_3)_3C$	H	H	H	$CH_2C_6H_5$	>25	14	A	oil
2eee	2-(CH_3O)C_6H_4	H	H	H	$CH_2C_6H_5$	>25	41	B	oil
2fff	4-$C_6H_5C_6H_4$	H	H	H	$CH_2C_6H_5$	>25	23	B	88–89
2ggg	4-FC_6H_4	H	H	H	$CH_2C_6H_5$	>25	90	A	oil

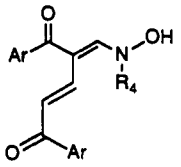
[a] Values listed in μM concentration; averages of two or more determinations. [b] All compounds gave satisfactory ^{1}HNMR, CIMS, and elemental analyses. [c] 4-(Benzyloxy)phenyl. [d] 4-(Benzyloxy)benzyl. [e] 4-(4-Picolyloxy)phenyl. [f] 1-tetralonyl.

such as *N*-alkylhydroxylamines,[27a] phenidone,[27b] and guiacol.[27b] This is also consistent with the observation that NR_4O(alkyl) and NR_4O(acyl) VHAs, such as **2h**, **2ff**, **2ll–nn**, **2vv–zz**, **2bbb**, and **2ccc**, which lack the NR_4OH moiety, are inactive as inhibitors of 5-LO (Table I).

Some preliminary in vitro 5-lipoxygenase inhibition structure–activity relationships (SAR) are evident from the data in Table I. In particular, the NR_4OH moiety is critical for enzyme inhibition to occur. Compounds lacking the hydroxylamino functionality are uniformly inactive.

(27) (a) Clapp, C. H.; Banerjee, A.; Rotenberg, S. A. Inhibition of Soybean Lipoxygenase 1 by N-Alkylhydroxylamines. *Biochemistry* **1985**, *24*, 1826–1830. (b) Mansuy, D.; Cucurou, C.; Biatry, B.; Battioni, J. P. Soybean Lipoxygenase-catalyzed Oxidations by Linoleic Acid Hydroperoxide: Different Reducing Substrates and Dehydrogenation of Phenidone and BW 755C. *Biochem. Biophys. Res. Commun.* **1988**, *151*, 339–346.

Table II. Physical Data for Dienes 12



entry	Ar	R_4	% yield[a]	mp, °C
12a	2-$(CH_3O)C_6H_4$	$CH_2C_6H_5$	25	179–181
12b	2,4,6-$(CH_3O)_3C_6H_2$	$CH_2C_6H_5$	43	214–216
12c	2,6-$F_2C_6H_3$	$CH_2C_6H_5$	30	173–175
12d	2-$CH_3C_6H_4$	$CH_2C_6H_5$	56	174–175
12e	2-ClC_6H_4	$CH_2C_6H_5$	39	164–166
12f	2,6-$(CH_3O)_2C_6H_3$	$CH_2C_6H_5$	67	218–220

[a] All compounds gave satisfactory ^1H NMR, CIMS, and elemental analyses.

Sterically bulky (e.g., aryl) substituents on the olefin at R_2 have a very negative effect upon the enzyme inhibitory activity (**2r–t**). This is possibly due to excessive steric hindrance of the hydroxylamine moiety by these substituents at R_2 and consequent failure of the compound to be able to approach and reduce the lipoxygenase active site iron. Smaller (CH_3) substituents at R_2 and R_3 result in but a slight loss of potency (**2j**, **2aaa**). The introduction of a heterocyclic residue in R_1 (**2n**, **2gg**, **2oo**, **2pp**) generally yields activity similar to that observed for similar alicyclic compounds. Electron donating groups on R_1 appear to be somewhat detrimental to activity (**2cc**); however, this can be compensated for to some extent by making the VHA more lipophilic (**2f**).[28] Substitution of a variety of groups at R_4 (Me, Ph, Bz) appears to be generally well tolerated.

The VHAs were also examined for their ability to inhibit the release of IL-1β by human monocytes in vitro.[29] Unlike simple hydroxamic acids (**1**), which showed no inhibitory effects upon IL-1β release, the VHAs were found to inhibit IL-1β biosynthesis (Table III). Some preliminary structure–activity relationships pertaining to IL-1β biosynthesis inhibition are apparent from the data in Table III. In particular, it may be noted that IL-1β inhibition is optimal when R_1 is an aromatic residue (e.g., **2v**, **2y**, **2z**). VHAs in which R_1 is alkyl (**2o–q**, **2uu**) are ineffective. The aromatic residue R_1 must remain in conjugation to the carbonyl group for activity. This is shown by **2jj**, which has two chloro substituents next to the carbonyl and is inactive, while the corresponding 3,4-dichloro analog **2kk** is active. The nature of the substituent R_4 on nitrogen is also critical to IL-1β inhibition. It may be seen that optimal activity is found when R_4 is benzylic (**2e**, **2v**, **2y**, **2z**, **2ee**). More lipophilic benzyl-type residues are less active (**2hh**, **2ii**). Substitution of methyl (**2a**, **2u**, **2aa**, **2dd**) at R_4 leads to a significant decrease in activity, while substitution of a saturated alicyclic residue (**2b**, **2w**) results in a complete loss of activity. Even substitution of phenyl (**2ss**) or phenethyl (**2tt**) at R_4 results in the loss of activity. Similarly to the 5-lipoxygenase SAR, the NR_4OH moiety is critical for IL-1β inhibition. VHAs with acyl (**2ll**, **2mm**, **2nn**, **2ww**) or alkyl (**1cc**) R_5 residues on oxygen were inactive.

(28) Log p calculations on these compounds suggest that the optimum log p for 5-lipoxygenase inhibition by these compounds lies between 2 and 3. Log p values were calculated using the MEDCHEM Software CLOGP 3 program, release 3.54 (1989) from Daylight Chemical Information Systems, Inc. (18500 Von Karman, Ste. no. 450, Irvine, CA 92715).

(29) Newton, R. C. Human Monocyte Production of Interleukin-1: Parameters of the Induction of Interleukin-1 Secretion by Lipopolysaccharides. *J. Leukocyte Biol.* **1986**, *39*, 299–311.

Table III. Inhibition of IL-1β Biosynthesis by Selected VHAs

entry	R_1	R_2	R_3	R_4	R_5	IL-1β, IC_{50}[a]
2a	2-naphthyl	H	H	CH_3	H	>10
2b	2-naphthyl	H	H	c-C_6H_{11}	H	>10
2e	2-naphthyl	H	H	$CH_2C_6H_5$	H	5.4
2f	4-(BzO)C_6H_4[b]	H	H	CH_3	H	>10
2n	2-thianaphthyl	H	H	CH_3	H	>10
2o	c-C_3H_5	H	H	CH_2(2-naphthyl)	H	9.9
2p	c-C_3H_5	H	H	4-$C_6H_4C_6H_4CH_2$	H	>10
2q	c-C_3H_5	H	H	4-$(C_6H_5O)C_6H_4CH_2$	H	>10
2u	4-FC_6H_4	H	H	CH_3	H	5.5
2v	4-FC_6H_4	H	H	$CH_2C_6H_5$	H	2.8
2w	4-FC_6H_4	H	H	c-C_6H_{11}	H	>10
2y	4-ClC_6H_4	H	H	$CH_2C_6H_5$	H	2.1
2z	4-$C_6H_5C_6H_4$	H	H	$CH_2C_6H_5$	H	3.6
2aa	4-$(NO_2)C_6H_4$	H	H	CH_3	H	>10
2bb	4-$C_6H_5C_6H_4$	H	H	CH_3	H	>10
2dd	4-ClC_6H_4	H	H	CH_3	H	>10
2ee	4-$(NO_2)C_6H_4$	H	H	$CH_2C_6H_5$	H	1.7
2gg	4-pyridyl	H	H	$CH_2C_6H_5$	H	8.9
2hh	4-FC_6H_4	H	H	4-$C_6H_5C_6H_4CH_2$	H	5.2
2ii	4-FC_6H_4	H	H	CH_2(2-naphthyl)	H	7.7
2jj	2,6-$Cl_2C_6H_3$	H	H	$CH_2C_6H_5$	H	>10
2kk	3,4-$Cl_2C_6H_3$	H	H	$CH_2C_6H_5$	H	3.6
2ll	4-FC_6H_4	H	H	$CH_2C_6H_5$	$COCH_3$	>10
2mm	4-$(NO_2)C_6H_4$	H	H	$CH_2C_6H_5$	$COCH_3$	>10
2nn	4-$C_6H_5C_6H_4$	H	H	$CH_2C_6H_5$	$COCH_3$	>10
2ss	4-FC_6H_4	H	H	C_6H_5	H	>10
2tt	4-FC_6H_4	H	H	$CH_2CH_2C_6H_5$	H	>10
2uu	$(CH_3)_3C$	H	H	$CH_2C_6H_5$	H	>10
2ww	4-ClC_6H_4	H	H	$CH_2C_6H_5$	$COCH_3$	>10
2ccc	4-FC_6H_4	H	H	$CH_2C_6H_5$	CH_3	>10

[a] Values listed in μM concentration; averages of two or more determinations. [b] 4-(Benzyloxy)phenyl.

Table IV. Data for Standard Drugs and Selected Vinylogous Hydroxamic Acids

	ID_{50}[e]			
compound	PLA_1[a]	CO[b]	5-LO[c]	IBI[d]
A64077	>1000	>750	0.14	>30
indomethacin	>1000	0.43	>25	>30
phenidone	>1000	6.9	0.48	>30
2v	>1000	>750	0.15	2.8
2y	>1000	>750	1.50	2.1

[a] PLA_2 inhibition. [b] Cyclooxygenase inhibition. [c] 5-Lipoxygenase inhibition. [d] IL-1 biosynthesis inhibition. [e] Values listed in μM concentration; averages of two or more determinations.

The optimum substitution pattern for dual 5-LO/IL-1β inhibition is found in those compounds in which R_1 is phenyl-substituted with halogen, nitro, or phenyl; R_2 and R_3 are H, and R_4 is benzyl. These are exemplified by **2v**, **2y**, **2z**, **2ee**, and **2kk**. A comparison of the in vitro activities of selected VHAs and several standard drugs is given in Table IV. The hydroxamic acid A64077 (Zileuton) was chosen as a selective 5-lipoxygenase inhibitor, while indomethacin was chosen as a selective cyclooxygenase inhibitor and phenidone as a dual 5-LO/CO inhibitor. Studies are currently in progress to examine the in vivo biological properties of these compounds, particularly their effects upon various models of inflammatory diseases.

Experimental Section

^1H NMR spectra were recorded on Varian Gemini 200 (200 MHz) or IBM 200 SY (200 MHz) spectrometers using tetramethylsilane as an internal standard. Infrared spectra were recorded as neat films or KBr pellets as noted on a Perkin-Elmer 1710 FT spectrometer. Mass spectral data was recorded on Finnigan-MAT 8230 or Du Pont DP-1 instruments, using the indicated ionization techniques. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. A Packard Prias liquid scintillation counter was used to measure radioactivity of labeled enzyme reaction products and

was programmed to convert cpm to dpm to account for variable quenching by solvents. Microanalyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ and were within 0.4% of the calculated values. Thin-layer chromatography was carried out with E. Merck 15327 silica gel plates.

All reactions were carried out with continuous magnetic stirring under an atmosphere of dry nitrogen. All solutions were dried over anhydrous magnesium sulfate unless otherwise noted; all evaporations were carried out on a rotary evaporator at ca. 30 Torr. Commercial reagents were used as received without additional purification. Ether and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl. Unlabeled arachidonic acid was obtained from NuChekPrep. Linoleic acid was obtained from Sigma. [^{14}C]Arachidonic acid was obtained from DuPont/NEN Products.

Method A: 1-(4-Chlorophenyl)-3-oxopropan-1-one (3y, R_1 = 4-ClC_6H_4; $R_2 = R_3 = H$). Sodium methoxide (7.56 g, 0.14 mol) was suspended in 140 mL of THF and treated at 25 °C with ethyl formate (11.3 mL, 0.14 mol), followed by dropwise addition of 4-chloroacetophenone (15.5 mL, 0.12 mol). The reaction mixture was stirred for 2.5 h at 25 °C, during which time the reaction mixture became more viscous. The mixture was dissolved in water (500 mL) and was extracted with Et_2O (2 × 100 mL). These extracts were discarded, and the aqueous phase was acidified with 25 mL of 6 M H_2SO_4. The mixture was then extracted with Et_2O (120 mL), and the extract was washed with water and brine, dried, and concentrated to give a yellow oil, which was crystallized from 1:1 1-chlorobutane–hexane to provide 16.9 g (77%) of yellow crystals; mp 46–48 °C; ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1 H), 7.84 (d, 2 H), 7.44 (d, 2 H), 6.19 (d, 1 H); CIMS (CH_4) m/z = 183, 185 (M + H$^+$). Anal. ($C_9H_7ClO_2$) C, H, N.

1-(4-Chlorophenyl)-3-(*N*-hydroxy-*N*-benzylamino)-2-propen-1-one (2y). A solution of **3y** (1.82 g, 10 mmol) in 3 mL of methanol was treated at 25 °C with a solution of 1.23 g (10 mmol) of *N*-benzylhydroxylamine in 4 mL of methanol. The mixture was warmed to 40 °C for 5 min and then cooled to 25 °C. The mixture turned bright red, and after 1 min a precipitate began to form. The mixture was filtered after 1 h, and the precipitate was washed with methanol and dried to give 2.14 g (74%) of yellow crystals: mp 120–122 °C; ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 2 H), 7.45–7.38 (m, 5 H), 7.33 (d, 2 H), 6.96 (d, J = 6.6 Hz, 1 H), 5.36 (d, J = 6.6 Hz, 1 H), 4.87 (s, 2 H); CIMS (NH_3) m/z = 288, 290 (M + H$^+$), 272, 274 (M + H$^+$ − O). Anal. ($C_{16}H_{14}ClNO_2$) C, H, N.

Method B: (*E*)-1-(2-Furyl)-3-(dimethylamino)-2-propen-1-one (4n, R_1 = 2-Furyl; $R_2 = R_3 = H$). 2-Acetylfuran (12.0 g, 0.11 mol) was dissolved in 50 mL of *N*,*N*-dimethylformamide dimethylacetal, and the mixture was heated under reflux for 14 h. The mixture was then cooled and concentrated, and the crystalline residue was recrystallized from 50 mL of 1:1 1-chlorobutane–hexane to supply the product as orange crystals (15.06 g, 84%): mp 85–87 °C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 12.5 Hz, 1 H), 7.49 (br s, 1 H), 7.06 (m, 1 H), 6.48 (m, 1 H), 5.67 (d, J = 12.5 Hz, 1 H), 3.12–2.91 (br d, 6 H); CIMS (NH_3) m/z = 166 (M + H$^+$). Anal. ($C_9H_{11}NO_2$) C, H, N.

1-(2-Furyl)-3-[*N*-hydroxy-*N*-(4-phenylbenzyl)amino]-2-propen-1-one (2pp). A solution of *N*-(4-phenylbenzyl)hydroxylamine (0.298 g, 1.5 mmol) and *p*-TsOH (0.285 g, 1.5 mmol) in 5 mL of methanol was treated at 20 °C with a solution of **4n** (0.248 g, 1.5 mmol) in 2 mL of methanol. The mixture was allowed to stand at 20 °C for 30 min and then was cooled to −15 °C for 30 min. Water (2 mL) was added dropwise to the mixture with continued cooling to precipitate the product, which was filtered and washed with cold 2:1 methanol–water to give 0.259 g (54%) of yellow crystals: mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 7.65–7.28 (m, 11 H), 6.95 (d, J = 6.7 Hz, 1 H), 6.45 (m, 1 H), 5.43 (d, J = 6.7 Hz, 1 H), 5.01 (s, 2 H); CIMS (NH_3) m/z = 320 (M + H$^+$); 304 (M + H$^+$ − O). Anal. ($C_{20}H_{17}NO_3$) C, H, N.

Method C: Methyl 3-[*N*-Hydroxy-*N*-[4-(benzyloxy)benzyl]amino]-2-propenoate (2i). A solution of *N*-[4-(benzyloxy)-benzyl]hydroxylamine (5.00 g, 20 mmol) in 60 mL of dichloromethane was treated at 0 °C with a solution of methyl propiolate (2.13 mL, 24 mmol) in 10 mL of dichloromethane. The reaction mixture was kept 3 h at 0 °C and then concentrated. The resulting foam was triturated with 35 mL of Et_2O and the flask stoppered and allowed to stand at 0 °C for 30 min. The white precipitate which formed was filtered and washed with Et_2O and 1-chlorobutane and dried to give the product as a white powder (4.31 g, 63%): mp 121–123 °C; ^{1}H NMR ($CDCl_3$) δ 7.43–7.13 (m, 7 H), 6.95 (d, 2 H), 6.85 (d, J = 6.4 Hz, 1 H), 5.00 (s, 2 H), 4.80 (d, J = 6.4 Hz, 1 H), 3.67 (s, 3 H), 3.42 (s, 2 H); CIMS (CH_4) 314 (M + H$^+$) 298 (M + H$^+$ − O). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

Method D: 1-[4-(Benzyloxy)phenyl]-3-(*N*-acetoxy-*N*-methylamino)-2-propen-1-one (2h). Four grams (14.1 mmol) of **2f** was suspended in 60 mL of dichloromethane at 20 °C. Acetic anhydride (1.46 mL, 15.5 mmol) was added, and the mixture became homogeneous, after which triethylamine (2.55 mL, 18.3 mmol) was added. A transient orange color faded quickly, and the mixture became yellow. The mixture was stirred at 20 °C for 30 min and then was concentrated, and the residue was taken up in ether. The ethereal solution was washed with water, 1 M hydrochloric acid, water, 1 M sodium bicarbonate, and brine, dried, and evaporated. The remaining yellow oil was chromatographed on silica gel, eluting with 1:1 hexane–ethyl acetate to give pure product that was crystallized from 1-chlorobutane–hexane to give the product as a yellow powder (1.40 g, 31%): mp 61–63 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 2 H), 7.65 (d, J = 12.5 Hz, 1 H), 7.48–7.32 (m, 5 H), 7.01 (d, 2 H), 6.03 (d, J = 12.5 Hz, 1 H), 5.15 (s, 2 H), 3.30 (s, 3 H), 2.23 (s, 3 H); CIMS (CH_4) m/z = 326 (M + H$^+$), 268 (M + H$^+$ − CH_2=C=O). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

Method E: Sodium 1-[4-(Benzyloxy)phenyl]-3-(*N*-hydroxy-*N*-methylamino)-2-propen-1-one Sodium Salt (2l). A solution of 1.50 g (5.3 mmol) of **2f** in 100 mL of methanol and 20 mL of dioxane was treated with sodium methoxide (0.286 g, 5.3 mmol). The base dissolved at once to give a deep red solution. The mixture was concentrated to dryness, and the residue was triturated with benzene and filtered to give 1.45 g (90%) of yellow crystals: mp 230 °C dec; ^{1}H NMR (CD_3OD) δ 7.77 (d, 2 H), 7.72 (s, 1 H), 7.46–7.27 (m, 6 H), 6.95 (d, 2 H), 5.09 (s, 2 H), 3.47 (s, 3 H). Anal. ($C_{17}H_{16}NNaO_3$) C, H, N.

Reaction of 2f with *N*-Phenylmaleimide (13a; E_1, E_2 = CON(C_6H_5)CO). A mixture of **2f** (0.283 g, 1 mmol) and *N*-phenylmaleimide (0.173 g, 1 mmol) in 10 mL of benzene was heated under reflux for 1 h, at which point consumption of the imide was complete by TLC analysis. The reaction mixture was cooled and chromatographed on silica eluting with 3:1 benzene–EtOAc to give 0.402 g (88%) of pale yellow crystals. Recrystallization from 1-chlorobutane gave white crystals: mp 186–188 °C; ^{1}H NMR ($CDCl_3$) 7.96 (d, 2 H), 7.50–7.26 (m, 10 H), 7.03 (d, 2 H), 5.14 (s, 2 H), 4.89 (d, 1 H), 4.35 (m, 1 H), 3.66 (m, 1 H), 3.54 (m, 1 H), 3.03 (m, 1 H), 2.72 (s, 3 H); CIMS (NH_3) m/z = 457 (M + H$^+$). Anal. ($C_{27}H_{24}N_2O_5$) C, H, N.

Reaction of 2f with Diethyl Fumarate (13b; $E_1 = E_2$ = $CO_2C_2H_5$). A mixture of **2f** (0.283 g, 1 mmol) and diethyl fumarate (0.164 mL, 1 mmol) in 10 mL of benzene was heated under reflux for 1 h, at which point consumption of the ester was complete by TLC analysis. The reaction mixture was cooled and chromatographed on silica eluting with 3:1 benzene–EtOAc to give 0.250 g (55%) of an oil that crystallized upon standing. This was recrystallized from 1:1 1-chlorobutane–hexane to give white crystals: mp 122–124 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 2 H), 7.44–7.32 (m, 5 H), 7.01 (d, 2 H), 5.14 (s, 2 H), 4.88 (d, 1 H), 4.27 (q, 2 H), 4.22 (q, 2 H), 4.03 (m, 1 H), 3.51 (m, 1 H), 3.32 (m, 1 H), 3.24 (m, 1 H), 2.76 (s, 3 H), 1.31 (t, 3 H), 1.26 (t, 3 H); CIMS (NH_3) 456 (M + H$^+$). Anal. ($C_{25}H_{29}NO_7$) C, H, N.

Synthesis of Dienes 12: 2,4-Bis(2-methoxybenzoyl)-1-(*N*-hydroxy-*N*-benzylamino)-1,3-butadiene (12a, Ar = 2-CH_3O-C_6H_4; R_4 = $CH_2C_6H_5$). A solution of 1-(2-methoxyphenyl)-3-oxopropan-1-one (1.16 g, 6.51 mmol) in 50 mL of methanol was treated with 0.80 g (6.51 mmol) of *N*-benzylhydroxylamine. The reaction mixture was stirred at 25 °C for 24 h, and the crystalline precipitate was filtered and washed with cold methanol to give 0.73 g (25%) of yellow crystals: mp 179–181 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (s, 1 H), 7.43 (s, 4 H), 7.38–7.20 (m, 5 H), 7.13 (d, 1 H), 6.85 (q, 2 H), 6.78 (d of d, 2 H), 6.18 (d, 1 H), 6.17 (d, 1 H), 3.70 (s, 6 H); CIMS (NH_3) m/z = 444 (M + H$^+$). Anal. ($C_{27}H_{25}NO_5$) C, H, N.

RBL-1 5-Lipoxygenase Assay. The enzyme was prepared as a 10000*g* supernatant from homogenized RBL-1 cells. Because of variability in enzyme content from culture to culture, an amount of supernatant was chosen to give a net production of 3300–3800

dpm of 5-HETE under the assay conditions (total cell protein 9–20 μg/assay). All reactions were run in duplicate. In a total volume of 100 μL, the appropriate amount of enzyme was incubated with test compound (prepared in 5% DMSO, 95% 0.2 M Tris, pH 8.5) in a phosphate buffer (45 mM sodium phosphate, 0.83 mM EDTA, 0.083% gelatin, 0.1 mM glutathione, 0.83 mM calcium chloride, 0.012 mM indomethacin) at pH 7.0 and 37 °C for 5 min. The reaction was initiated by the addition of 20 μL of a solution of arachidonic acid in phosphate buffer. The final concentration of substrate in the assay solution was 0.042 mM, including 0.167 μCi of [^{14}C]arachidonic acid (specific activity 50 mCi/mmol). The reaction was terminated after 2 min by freezing in CO_2–ethanol. 5-LO products were separated from unreacted arichidonic acid on silica gel columns with hexane–ethyl acetate–acetic acid (82:17:1). 5-HETE was eluted with hexane–THF–ethyl acetate–acetic acid (65:30:10:1). Remaining products were eluted with methanol–water–acetic acid (70:30:1). Activity was measured as the total reactivity in the 5-LO products, and inhibition was calculated as $(1 - D/C) \times 100\%$, where D is the activity in the presence of the test compound and C is the control activity. IC_{50} values were calculated by linear regression analysis using three concentrations of drug, spanning the 50% inhibition point.

Soybean Lipoxygenase Assay. The enzyme was assayed at 25 °C with linoleic acid in 0.05 M borate buffer at pH 9.0. The production of hydroperoxide was followed by monitoring the change in absorbance at 234 nm (ϵ = 23600 M^{-1} cm^{-1}). Values for K_i were obtained by analysis of double reciprocal plots (1/velocity vs 1/[substrate]) at several concentrations of inhibitor. The inhibitors were prepared as concentrated solutions in ethanol; the final concentration of ethanol in all assays was 0.1 M, below the level at which it has an effect on the assay.

Preparation of EPR Samples and Spectroscopy. Samples of ferrous lipoxygenase were oxidized to the ferric state by addition of linoleic acid in the presence of oxygen and dialyzed twice against 1000 volumes of 0.05 M pH 9.0 borate buffer. Aliquots of the enzyme (300 μL, approximately 0.1 mM) were placed in serum-stoppered EPR tubes that had been evacuated and flushed with argon. Two equivalents of the inhibitor were added, and the tube was gently agitated to mix the solution. The sample was frozen in liquid nitrogen after approximately 30 s. EPR spectra were obtained at X-band at 5 K as previously described.[30]

IL-1 Biosynthesis Inhibition in Vitro Assay. Normal human blood is layered over ficoll-hypaque and centrifuged to isolate the white blood cells.[31] The white blood cells are further separated by elutriation, and the monocyte population is used.[32] The monocytes in RPMI 1640 + 5% FBS are plated at 2×10^6 cells mL^{-1} in a 12-well tissue culture plate. The drugs (diluted in DMSO) are added to the monocytes at concentrations of 10, 3, 1, 0.3, and 0.1 μM. Incubation of compounds is continued for 1 h before LPS (*Salmonella typhemurium*) is added to the cells for stimulation of IL-1 production.[29] LPS incubation is continued for approximately 20 h, after which the supernatants are collected and tested or presence of IL-1 by ELISA. The cells are tested for viability by MTT assay.[33] A compound is considered active if it inhibits IL-1 production by >50% and leaves the cells >90% viable.

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Supplementary Material Available: Methods, yields, melting points, spectral data, and analytical data for **2a–2ggg** and yields and melting points for vinylogous dimethylamides (**4**, Lvg = $N(CH_3)_2$) (10 pages). Ordering information is given on any current masthead page.

Registry No. 2a, 143620-89-7; **2aa**, 143621-13-0; **2aaa**, 143620-71-7; **2b**, 143620-90-0; **2bb**, 143621-14-1; **2bbb**, 143620-72-8; **2c**, 143620-91-1; **2cc**, 143621-15-2; **2ccc**, 143620-73-9; **2d**, 143620-92-2; **2dd**, 143621-16-3; **2ddd**, 143620-74-0; **2e**, 143631-85-0; **2ee**, 143621-17-4; **2eee**, 143631-83-8; **2f**, 143620-93-3; **2ff**, 143621-18-5; **2fff**, 143620-75-1; **2g**, 143620-94-4; **2gg**, 143621-19-6; **2ggg**, 143620-76-2; **2h**, 143620-95-5; **2hh**, 143621-20-9; **2i**, 143620-96-6; **2ii**, 143621-21-0; **2j**, 143620-97-7; **2jj**, 143621-22-1; **2k**, 143620-98-8; **2kk**, 143621-23-2; **2l**, 143620-99-9; **2ll**, 143621-24-3; **2m**, 143621-00-5; **2mm**, 143621-25-4; **2n**, 143621-01-6; **2nn**, 143621-26-5; **2o**, 143621-02-7; **2oo**, 143621-27-6; **2p**, 143621-03-8; **2pp**, 143631-87-2; **2q**, 143621-04-9; **2qq**, 143621-28-7; **2r**, 143621-05-0; **2rr**, 143621-29-8; **2s**, 143621-06-1; **2ss**, 143621-30-1; **2t**, 143621-07-2; **2tt**, 143620-64-8; **2u**, 143621-08-3; **2uu**, 143620-65-9; **2v**, 143621-09-4; **2vv**, 143620-66-0; **2w**, 143621-10-7; **2ww**, 143620-67-1; **2x**, 143621-11-8; **2xx**, 143620-68-2; **2y**, 143631-86-1; **2yy**, 143620-69-3; **2z**, 143621-12-9; **2zz**, 143620-70-6; **3y**, 56856-73-6; **4n**, 109482-86-2; **9f**, 143620-87-5; **10uu**, 143620-88-6; **11f**, 142556-94-3; **12a**, 143620-77-3; **12b**, 143620-78-4; **12c**, 143620-79-5; **12d**, 143631-84-9; **12e**, 143620-80-8; **12f**, 143620-81-9; **13a**, 143620-82-0; **13b**, 143620-83-1; 5-LO, 9029-60-1; MeNHOH, 593-77-1; c-C_6H_{11}NHOH, 2211-64-5; C_6H_5NHOH, 100-65-2; $C_6H_5CH_2$NHOH, 622-30-0; 4-(BzO)$C_6H_4CH_2$NHOH, 106328-99-8; n-$C_{10}H_{21}$NHOH, 26228-72-8; 4-$C_6H_5C_6H_4CH_2$NHOH, 143620-84-2; 4(C_6H_5O)$C_6H_4CH_2$NHOH, 143620-86-4; $C_6H_5(CH_2)_2$NHOH, 3217-93-4; H_2NOH, 7803-49-8; 4-(BzO)$C_6H_4COCH_3$, 54696-05-8; c-C_3H_5-$COCH_3$, 765-43-5; 4-(BzO)$C_6H_4COCH_3$, 4495-66-3; 4-FC_6H_4-$COCH_3$, 403-42-9; 4-Pic$OC_6H_4COCH_3$, 143620-85-3; 4-(CH_2O)$C_6H_4COCH_2C_6H_4$-p-OCH_3, 120-44-5; 4-(CH_3S)C_6H_4-$COCH_2C_6H_4$-p-F, 87483-29-2; $C_6H_5COCH_2C_6H_5$, 451-40-1; 4-(NO_2)$C_6H_4COCH_3$, 100-19-6; 4-(CH_3O)$C_6H_4COCH_3$, 100-06-1; 4-$ClC_6H_4COCH_3$, 99-91-2; 3,5-$(CF_3)_2C_6H_3COCH_3$, 30071-93-3; $C_6H_5(CH_2)_2COCH_3$, 529-34-0; 4-$ClC_6H_4COCH_3$, 99-91-2; 4-$C_6H_5C_6H_4COCH_3$, 92-91-1; 2,6-$Cl_2C_6H_3COCH_3$, 2040-05-3; 3,4-$Cl_2C_6H_3COCH_3$, 2642-63-9; $(CH_3)_3CCOCH_3$, 75-97-8; 2-(CH_3O)$C_6H_4COCH_3$, 579-74-8; 2-acetylfuran, 1192-62-7; *N*,*N*-dimethylformamide dimethylacetal, 4637-24-5; methyl propiolate, 922-67-8; *N*-phenylmaleimide, 941-69-5; diethyl fumarate, 623-91-6; 1-(2-methoxyphenyl)-3-oxopropan-1-one, 67860-32-6; 2-acetylnaphthalene, 93-08-3; 2-acetylthianaphthalene, 22720-75-8; 4-acetylpyridine, 1122-54-9; 3-acetylthiophene, 1468-83-3; *N*-hydroxy-1-(2-naphthyl)ethylamine, 111525-02-1; *N*-hydroxy-(2-naphthyl)methylamine, 134796-86-4; 2-acetylthiophene, 88-15-3.

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