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Novel Bishydroxamic Acids as 5-Lipoxygenase Inhibitors

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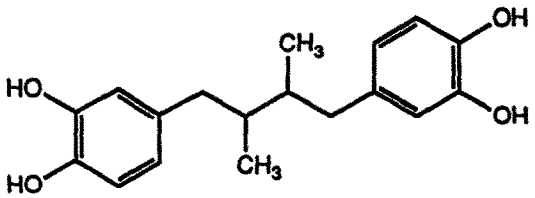
Abstract—Two series of novel bishydroxamic acids **2** and **3** (types A and B) were synthesized and tested for inhibition of 5-lipoxygenase from rat basophile leukemia (RBL) cells. Both series were potent inhibitors of the isolated enzyme but only the type B reverse hydroxamic acids possessed significant oral activity. The most potent compound, orally, was **3a**, [IC_{50} = 270 nM; ED_{50} = 1.86 mg/kg], which compares favorably with the clinically useful 5-lipoxygenase inhibitor, zileuton. Unlike known hydroxamic acid inhibitors, the oral activity in this series appears to be associated with the second hydroxamic acid group. The corresponding monohydroxamic acids retained inhibitor potency, *in vitro*, with reduced oral activity in a mouse zymosan peritonitis model. Compound **4e** [IC_{50} = 7 nM], a monohydroxamic acid derivative related to **3a**, is among the most potent inhibitors of the isolated enzyme yet to be reported.

Introduction

Leukotrienes are potent biological mediators derived from arachidonic acid through the action of the enzyme 5-lipoxygenase (5-LO).[1] The peptidoleukotrienes, LTC_4, LTD_4 and LTE_4 are potent spasmogenic agents which have been implicated in the pathology of allergic diseases.[2,3] Thus, the control of leukotriene biosynthesis through the inhibition of 5-lipoxygenase represents a potential new method for treating diseases such as asthma, rheumatoid arthritis and psoriasis.[4] Among the known inhibitors of the enzyme are a variety of polyhydroxylated natural products, such as quercetin, cirsiliol, baicalein and nordihydroguaiaretic acid (NDGA, **1**).[5,6,7]



Nordihydroguaiaretic Acid (NDGA)

Despite their *in vitro* potency against 5-lipoxygenase, these natural products generally lack oral activity. In the search for novel, orally active 5-lipoxygenase inhibitors it was decided to explore analogs of NDGA by replacing the catechol functionality with appropriate groups. It has been postulated that catechol containing inhibitors function to inhibit 5-lipoxygenase activity through chelation of a prosthetic iron.[5,8] Since hydroxamic acids are known to form strong complexes with a variety of transition metals and this property has been successfully exploited to design inhibitors of 5-lipoxygenase,[9–13] it was thought to be an appropriate replacing group for the catechols of **1**. Based upon calculated hydrophobicity parameters, an extensive QSAR study of a large series of hydroxamic acids, suggested that a hydrophobic binding region within the active site of the enzyme was a major contributor to inhibitor potency.[14]

Three types of compounds were therefore synthesized to further study the binding requirements of 5-lipoxygenase inhibitors. These types include: a) type A bishydroxamic acids (**2a–f**) derived from the substituted benzoic acids, b) type B 'reversed' hydroxamic acids derived from N-acylated-N-benzylhydroxylamines (**3a–c**) and c) the corresponding monohydroxamic acids (**4a–f**). The synthesis and the biological activities of these three series of compounds are reported below.

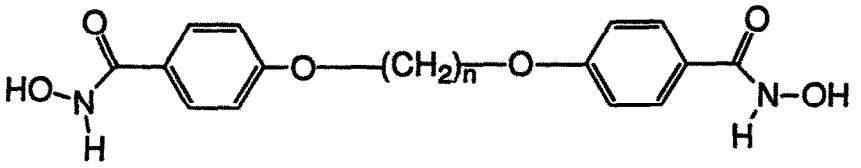
Chemistry

The synthetic routes to these compounds are shown in Schemes I, II and III. For the synthesis of the type A hydroxamic acids (**2a–f**), two equivalents of ethyl 4-hydroxybenzoate (**5**) were first reacted with one equivalent of the appropriate dibromoalkane (**6a–f**) in the presence of sodium ethoxide to give the diesters (**7a–f**), followed by reaction of **7a-f** with hydroxylamine[15] to give **2a–f**.

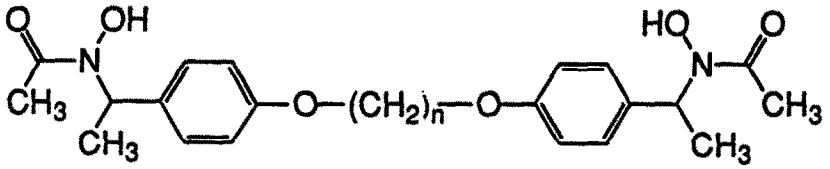
The type B hydroxamic acids **3a–c**, were synthesized by alkylation of two equivalents of 4-hydroxyacetophenone (**8**), with one equivalent of the appropriate dibromoalkane (**6c–e**) to give the diketones (**9a–c**), and reacted with hydroxylamine to give the corresponding oximes. Reduction of the oximes with sodium cyanoborohydride[16] gave the hydroxylamines (**10a–c**), and acylation with two equivalents of acetyl chloride gave the final compounds.

For the synthesis of the monohydroxamic acids **4a–f**, phenoxybutylbromide (**11**) was reacted with **8** to give **12**.[17] The ketone group of **12** was then converted to the hydroxylamine **13** as discussed earlier, and acylated with the appropriate acid chlorides to the final products **4a, b, d, e** and **f**. The acetyl group of **4b** was selectively hydrolyzed with lithium hydroxide in isopropanol to give **4c**.[18]

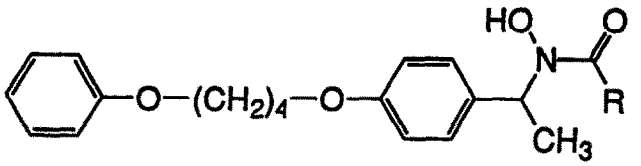
Table 1. *In vitro* and *in vivo* 5-lipoxygenase inhibitory activities



Compound	n	*In Vitro* 5-LO Inhibn[a] IC50(nM)[95% CL]	*In Vivo* LTC_4 Inhibn[b] %Inh @ 30 mg/kg+ S.E.	[ED_{50}mg/kg(95% CL)]
2a	2	5200 [2,154 - 8,804]	44.19 ± 18.63	
2b	3	3690 [2,677 - 5,151]	47.24 ± 6.16	
2c	4	1340 [1,028 - 1,787]	38.43 ± 8.77	
2d	5	990 [662 - 1,425]	17.90 ± 8.07	
2e	6	210 [193 - 220]	41.30 ± 8.38	
2f	7	80 [46 - 113]	18.25 ± 8.66	

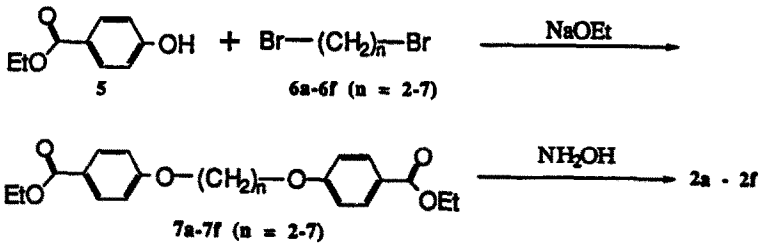


3a	4	270	[143 - 482]	1.86 [0.57 - 3.41]
3b	5	20	[12 - 29]	7.40 [5.20 - 10.38]
3c	6	10	[6 - 20]	10.95 [7.21 - 14.56]

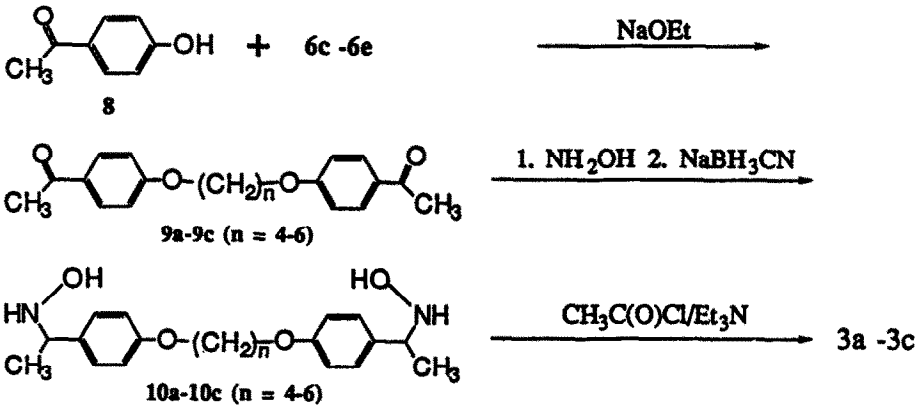


Cpd	R	*In Vitro* 5-LO Inhibn[a] IC50(nM)[95% CL]	*In Vivo* LTC_4 Inhibn[b] %Inh @ 30 mg/kg+ S.E.	[ED_{50}mg/kg(95% CL)]
4a	CH_3	80 [52 - 137]	NS	
4b	CH_2OAc	130 [87 - 194]	44.76 ± 6.53	
4c	CH_2OH	290 [141 - 533]		7.50 [4.39 - 11.17]
4d	CH_2CO_2Et	190 [30 - 478]	NS	
4e	$(CH_2)_2CO_2Et$	7 [1.0 - 20]	NS	
4f	$(CH_2)_3CO_2Me$	80 [35 - 169]	22.67 ± 13.37	
14		110,000[12]	NS	
15		900 [639 - 1,217]		8.77 [5.51 - 11.64]
Zileuton		370 [247 - 547]		2.71 [1.39 - 6.93]
NDGA		110 [83 - 143]	NS	

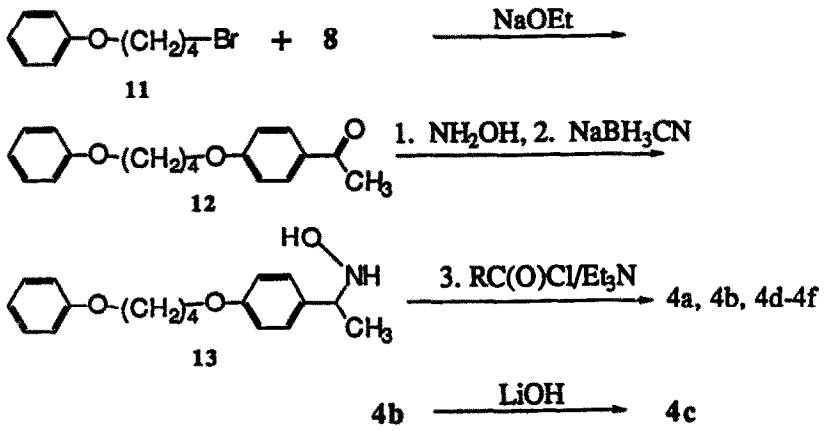
[a]IC_{50} with 95% confidence limits[20] in parentheses for the *in vitro* inhibition of 5-lipoxygenase from 9000 x *g* supernatant of RBL broken cell assay (see method).
[b]ED_{50} with 95% confidence limits[20] in parentheses or mean percent inhibition values + SEM for inhibition of LTC4 in the mouse zymosan peritonitis assay (See method).
[c]N.S. = No significant activity at 30 mg/kg.



Scheme I.



Scheme II.



Scheme III.

Design of inhibitors, results and discussions

In our study of the hydroxamic acid analogs of NDGA (**1**) it was found expedient to incorporate ether functions in the bridge linkage, both for ease of synthesis and increased polarity. The methylene linkage was varied between 2 and 7 carbons in search for optimum chain length which would take advantage of the hydrophobic binding region of the enzyme. Table 1 shows both the *in vitro* and the *in vivo* activities of the target compounds compared to **1** and zileuton against 5-lipoxygenase. The inhibitory data on the monohydroxamic acids $C_6H_5C(O)NHOH$ (**14**),[12] and $C_6H_5CH(CH_3)N(OH)Ac$ (**15**) are also included for comparison. All compounds synthesized were very potent

Table 2. Physical properties of compounds not discussed in the experimental section

#	Mp °C	Formula	Calcd.	Found
7a	104-105	$C_{20}H_{22}O_6$	C 67.03, H 6.19;	C 67.09, H 6.12
7b	108-109	$C_{21}H_{24}O_6$	C 67.73, H 6.50;	C 67.87, H 6.53
7c	97-98	$C_{22}H_{26}O_6$	C 68.38, H 6.78;	C 68.56, H 6.98
7e	129-130	$C_{24}H_{30}O_6$	C 69.54, H 7.30;	C 69.59, H 7.50
7f	93-94	$C_{25}H_{32}O_6$	C 70.07, H 7.53;	C 70.22, H 7.64
9a	140-141	$C_{20}H_{22}O_4$	C 73.60, H 6.79;	C 73.59, H 6.81
9c	119-120	$C_{22}H_{26}O_4$	C 74.55, H 7.39;	C 74.18, H 7.40
10a	150-152	$C_{20}H_{28}N_2O_4$	C 66.64, H 7.83, N 7.77;	C 66.64, H 7.61, N 8.00
10c	116-118	$C_{22}H_{32}N_2O_4$	C 68.01, H 8.30, N 7.21;	C 67.84, H 8.40, N 7.01
2a	234-235	$C_{16}H_{16}N_2O_6$ 0.30H_2O	C 56.90, H 4.95, N 8.29;	C 57.28 H 4.91, N 7.91
2b	207-208	$C_{17}H_{18}N_2O_6$	C 58.96, H 5.24, N 8.09;	C 59.22, H 5.18, N 8.02
2c	225-226	$C_{18}H_{20}N_2O_6$	C 59.99, H 5.59, N 7.77;	C 60.04, H 5.50, N 7.39
2e	213-214	$C_{20}H_{24}N_2O_6$ 0.20H_2O	C 61.28, H 6.30, N 7.11;	C 61.09, H 6.21, N 6.79
2f	184-185	$C_{21}H_{26}N_2O_6$	C 62.67, H 6.51, N 6.96;	C 62.33, H 6.60, N 6.77
3a	151-152	$C_{24}H_{32}N_2O_6$	C 64.85, H 7.26, N 6.30;	C 64.45, H 7.38, N 6.17
3c	142-143	$C_{26}H_{36}N_2O_6$ 0.20H_2O	C 65.58, H 7.70, N 5.88;	C 65.49, H 7.64, N 5.75
4b	141-143	$C_{22}H_{27}NO_6$	C 65.82, H 6.78, N 3.49;	C 66.21, H 6.71, N 3.31
4d	116-117	$C_{23}H_{29}NO_6$	C 66.49, H 7.04, N 3.37;	C 66.67, H 7.13, N 3.08
4e	107-109	$C_{24}H_{31}NO_6$	C 67.11, H 7.27, N 3.26;	C 67.22, H 7.49, N 3.09
4f	106-109	$C_{24}H_{31}NO_6$	C 67.11, H 7.27, N 3.26;	C 67.24, H 7.22, N 3.09

in vitro inhibitors of the enzymes. Within the type A hydroxamic acids, potencies increased from IC_{50} values of 5200 ηM for **2a** to 80 ηM for **2f**, as the methylene chain length between the two rings increased, leveling off between 6 and 7 carbons. The enhancement in potency observed with the increase in chain length may be related to the known lipophilic nature of the active site of the 5-lipoxygenase enzyme. All the bis-hydroxamic acids were more potent than their monohydroxamic acid analog (**14**), [IC_{50}: 110000ηM].[12] The most potent compound **2f**, was over one thousand times more potent than **14**, and equal to **1**. Due to solubility problems and also the leveling of the inhibitory activities, compounds with methylene chain lengths greater than 7 were not pursued. These type A hydroxamic acids showed low to moderate inhibition when administered orally. At 30 mg/kg, the best compound, **2b**, gave only 47% inhibition of the enzyme in the mouse zymosan peritonitis assay. Since type A hydroxamic acids are known to be prone to metabolism, the discrepancies between the *in vitro* and *in vivo* activities has been suggested to be due at least in part to metabolic inactivation. Reverse hydroxamic acids (type B) are reported to be more potent *in vivo* due to metabolic stability.[13,18]

Once the concept of hydroxamic acids as replacements for catechol groups of NDGA was established, the type B hydroxamic acids were prepared to increase the oral potency of the series. Taking into consideration both the *in vitro* and the *in vivo* activities of the compounds, **2e** was selected for further analog studies. Compounds **3a–c** with the methylene chain between the two rings ranging from 4 to 6 were therefore synthesized and studied to help determine which contribution to binding is most important, the distance between the two benzene rings or the distance between the two hydroxamic acids. Again, compounds with chain length greater than 6 were not pursued. Compounds **3a–c** were isolated as mixtures of diastereomers and no attempt was made to isolate or characterize the individual isomers. As shown in Table 1, on the isolated enzyme, the type B compounds, **3a–c**, were more potent inhibitors than the sterically closest

corresponding type A analogs, **2a–f**. In both series, an increase in methylene chain length increased the *in vitro* potency. Removal of one hydroxamic acid group, as in **4a**, led to a three-fold increase in inhibitor potency when compared to the bis acid, **3a**. The simple type B monohydroxamic acid, **15**, was eleven-fold weaker than **4a**. These modifications demonstrate an important hydrophobic contribution to binding affinity by the phenoxyalkyl substitution while the second hydroxamic acid moiety substitution does not improve inhibitor potency. Structural modifications of the hydroxamic acid of **4a** such as the O-acetyl (**4b**) and the hydroxyl (**4c**) derivatives, and the three carboxylic esters (**4d–f**), either maintained or improved the *in vitro* potencies. Compound **4e** with an IC_{50} of 7 nM is one of the most potent inhibitor of 5-lipoxygenase (leukotriene biosynthesis inhibitors) to be reported.

Unlike the type A hydroxamic acids, which showed poor oral activity, the type B analogs, **3a–c**, **4c** and **15**, demonstrated good oral potency with ED_{50}'s ranging from 1.86 mg/kg for **3a** to 11.0 mg/kg for **3c**. The most active compound, **3a**, was about four fold more active than its monohydroxamic acid derivative **15** and compared favorably with the clinical candidate zileuton [ED_{50}: 2.7 mg/kg]. The decreased oral bioavailability associated with increasing chain length or lipophilicity has been observed with other 5-lipoxygenase inhibitors.[19] Their increased lipophilicity may have affected their bioavailability through reduced solubility or by altering the ability of the compounds to cross membranes. The exact function of the second hydroxamic acid moiety in relation to the oral activity of this series of compounds is unknown, but it is accomplished without appreciable change in affinity for the enzyme, *in vitro*. Most probably, the oral potency observed is a result of decreased metabolism, physical properties of the molecules, and their inhibition potency.

Experimental Section

Melting points were determined on a Meltemp II apparatus and are uncorrected. Elemental analyses (are within 0.4% of the theoretical values unless otherwise indicated) and the mass spectral data (chemical ionization technique) were performed by the analytical group at the R.W. Johnson Pharmaceutical Research Institute. All ^1H NMR spectra were recorded on a GE-300 spectrometer, and values are reported in ppm from Me_4Si.

General procedure for the preparation of diethyl[alkanylbis(oxy)]bisbenzoates (7a–f)

The following procedure for the preparation of 4,4'-[1,5-pentanediylbis(oxy)]bisethylbenzoate (**7d**) is representative. To a solution of Na metal (2.6 g, 0.11 mol) in EtOH (75 mL) was added a solution of ethyl-4-hydroxybenzoate (18 g, 0.11 mol) in EtOH (75 mL). The mixture was allowed to stir at 25 °C for 5 min. 1,5-Dibromopentane (8.3 g, 36.1 mmol) was then added and the mixture refluxed for 10 h, cooled to 25°C and filtered. The semisolid obtained was washed with large volumes of H_2O and was homogenous by TLC. The filtrate was poured into ice/water mixture (200 mL) and the white precipitate which formed was filtered, washed with large volumes of H_2O and air dried. The solids were combined and recrystallized from hexane/EtOAc to give 9.0 g (62%) of 7d, m.p. 91–92 °C. MS (CI, CH_4) MH^+ at 401. Anal. Calcd for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 69.08; H, 7.16. ^1H NMR ($CDCl_3$): δ 1.38 (t, 6H, $CH_3CH_2CO_2$), 1.69 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.88 (m, 4H, OCH_2CH_2), 4.05 (t, 4H, OCH_2), 4.34 (q, 4H, $CH_3CH_2CO_2$), 6.90 (d, 4H, 3, 3', 5, 5'-H), 7.99 (d, 4H, 2, 2', 6, 6'-H).

General procedure for the preparation of alkanylbis(oxy)bis(N-hydroxybenzamides) (2a–f)

The following procedure for the preparation of 4,4'-[1,5-pentanediylbis(oxy)bis(*N*-hydroxybenzamide) (**2d**) is representative. To a suspension of hydroxylamine HCl (46.7 g, 0.67 mol) in methanol (240 mL) was added a solution of KOH (36 g, 0.64 mol) in methanol (100 mL). After stirring for 15 min, it was filtered and the filtrate was added to a cooled (5–10 °C) solution of **7d** (4 g, 10 mmol) in THF (100 mL). The mixture was stirred at 25 °C for 17 h. The precipitate which formed was filtered and suspended in H_2O (50 mL) and stirred for 20 min. The pH of the mixture was adjusted to 6 with acetic acid and the solid obtained was recrystallized from DMSO/H_2O to give 2.18 g (58%) of **2d**, m.p. 202–203 °C. MS (CI, CH_4) MH^+ at 375. Anal. Calcd for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.92; N, 7.48. Found: C, 60.58; H, 5.91; N, 7.17. ^1H NMR (DMSO-d_6): δ 1.56 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.79 (m, 4H, OCH_2CH_2). 4.04 (t, 4H,OCH_2), 6.99 (d, 4H, 3, 3', 5, 5'-H), 7.71 (d, 4H, 2, 2', 6, 6'-H), 8.90 (br s, 2H, N*H*, 11.06 (br s, 2H, O*H*).

General procedure for the preparation of [alkanediylbis(oxy)]bisacetophenones (9a–c)

The following procedure for the preparation of 4,4'-(1,5-pentanediylbis(oxy)]bisacetophenone (**9b**) is representative. A solution of 4-hydroxyacetophenone (25 g, 0.18 mol) in DMF (250 mL) was added sodium hydride, 60% dispersion in mineral oil (8.0 g, 0.20 mol) and stirred at 25 °C for 45 min. 1,5-Dibromopentane (21 g, 0.09 mol) was added and the mixture stirred at 25 °C for 1 h and then heated at 140 °C for another 16 h. The reaction mixture was poured into an ice/H_2O mixture (600mL). The product was filtered and recrystallized from DMSO/H_2O to give 24 g (77%) of **9b**, m.p. 90–91 °C. MS (CI, CH_4) MH^+ at 341. Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11. Found: C, 73.81; H, 7.02. ^1H NMR (DMSO-d_6): δ 1.60 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.81 (m, 4H, OCH_2CH_2), 2.51 (s, 6H, CH_3CO), 4.09 (t, 4H, OCH_2), 7.04 (d, 4H, 3, 3', 5, 5'-H), 7.92 (d, 4H, 2, 2', 6, 6'-H).

General procedure for the preparation of [alkanediylbis(oxy)]bis(1-methyl-N-hydroxybenzylamine) (10a–c)

The following procedure for the preparation of 4,4'-[1,5-pentandiylbis(oxy)]bis(1-methyl-*N*-hydroxybenzylamine)

(**10b**) is representative. A solution of **9b** (20 g, 0.059 mol), hydroxylamine HCl (30 g, 0.43 mol), NaOH (44 g, 1.1 mol) in 95% EtOH (400 mL) and H_2O (10 mL) was stirred at 25 °C for 30 min and then refluxed for 1.5 h. The reaction was then poured into 3N HCl (100 mL) and ice to give a precipitate. The precipitate was filtered and recrystallized from MeOH/THF to give 21.31 g (98%) of the oxime, m.p. 147–148 °C. MS (CI, CH_4) MH^+ at 371. Anal. Calcd for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 67.98; H, 7.03; N, 7.43. ^1H NMR (DMSO-d_6): δ 1.59 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.77 (m, 4H, OCH_2CH_2), 2.11 (s, 6H, CH_3C), 4.01 (t, 4H, OCH_2), 6.93 (d, 4H, 3, 3', 5, 5'-H), 7.57 (d, 4H, 2, 2', 6, 6'-H), 10.96 (s, 2H, O*H*).

To a solution of the oxime (20 g, 0.54 mmol) in MeOH (600 mL) and THF (400 mL) was added in one portion, $NaBH_3CN$ (10 g, 0.16 mol) and 1 mg of methyl orange. 12N HCl was added dropwise to the mixture until the color remained pink. The mixture was stirred at 25 °C for 1 h and more $NaBH_3CN$ (10 g, 0.16 mol) was added, acidified by dropwise addition of 12N HCl to change the color to pink. The reaction was continued at 25 °C for 24 h, with an occasional addition of 12N HCl to maintain the pink color. The solvent was removed under vacuum and 6N NaOH was added to adjust the pH to 9. The product was extracted with methylene chloride (3 x 250 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give an off white solid which was recrystallized from THF hexane to give 19 g (94%) of **10b**, m.p. 84–85 °C. MS (CI, CH_4) MH^+ at 375. Anal. Calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.15; H, 8.17; N, 7.43. ^1H NMR (DMSO-d_6): δ 1.18 (d, 6H, CH_3CH), 1.56 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.76 (m, 4H, OCH_2CH_2), 3.85 (q, 2H, C*H*), 3.95 (t, 4H, OCH_2), 5.64 (br s, 2H, NH, 6.84 (d, 4H, 2, 2', 6, 6'-H), 7.10 (br s, 2H, O*H*), 7.22 (d, 4H, 3, 3', 5, 5'-H).

*General procedure for the preparation of alkanediylbis(oxy)bis(N-acetyl-N-hydroxy-α-methylbenzylamines) (**3a-c**)*

The following procedure for the preparation of 4,4'-(1,5-pentanediylbis(oxy)bis(*N*-acetyl-*N*-hydroxy-α-methylbenzylamine) (**3b**) is representative. To a solution of **10b** (5.0 g, 13 mmol) in THF (100 mL) and Et_3N (2 mL) was added acetyl chloride (2.3 g, 27 mmol). The solution was stirred at 25 °C for 1 h and the solvent removed under vacuum. The residue was recrystallized from hexane/THF and few drops of acetic acid to give 3.8 g (62%) of **3b**, m.p. 99–100 °C. MS (CI, CH_4) MH^+ at 459. Anal. Calcd for $C_{25}H_{34}N_2O_6$: C, 65.48; H, 7.47; N, 6.11. Found: C, 65.20; H, 7.48; N, 5.96. ^1H NMR (DMSO-d_6): δ 1.41 (d, 6H, CH_3CH), 1.55 (m, 2H, $(CH_2)_2CH_2(CH_2)_2$), 1.76 (m, 4H, OCH_2CH_2), 1.98 (s, 6H, CH_3CO), 3.96 (t, 4H, OCH_2), 5.56 (q, 2H, C*H*), 6.87 (d, 4H, 2, 2', 6, 6'-H), 7.21 (d, 4H, 3, 3', 5, 5'-H), 9.45 (br s, 2H, O*H*).

*N-Hydroxy-α-methyl-4-(4-phenoxybutoxy)benzylamine (**13**)*

Compound **13** was prepared in a similar procedure as described for **10b** from **12**[17] (25 g, 0.088 mol), hydroxylamine HCl (25 g, 0.36 mol), and NaOH (18.0 g, 0.45 mol) in 95% EtOH (300 mL) and recrystallized from DMF/H_2O to give 19 g (72%) of the oxime m.p. 148–149 °C. MS (CI, CH_4) MH^+ at 300. Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.39; H, 7.09; N, 4.44. ^1H NMR (DMSO-d_6): δ 1.87 (m, 4H, OCH_2CH_2), 2.11 (s, 3H, CH_3C), 4.04 (m, 4H, OCH_2, 6.92 (m, 5H, 3, 5, 2', 4', 6' - Hs), 7.28 (t, 2H, 3', 5' - H), 7.58 (d, 2H, 2, 6 - H), 10.97 (s, 1H, O*H*).

The oxime (11.3 g, 0.038 mol), was reduced with $NaBH_3CN$ (2 X 9.0 g, 0.14 mol) in THF (400 mL) and MeOH (50 mL) and gave 5.7 g (50%) of **13**, m.p. 115–116 °C. MS (CI, CH_4) MH^+ at 302. Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.62; H, 7.73; N, 5.04. ^1H NMR (DMSO-d_6): δ 1.17 (d, 3H, CH_3CH), 1.87 (m, 4H, OCH_2CH_2), 3.87 (q, 1H, C*H*), 4.05 (m, 4H, OCH_2), 5.73 (s, 1H, N*H*), 6.90 (m, 5H, 2, 6, 3', 4', 5' - H), 7.13 (s, 1H, O*H*), 7.28 (m, 4H, 3, 5, 2', 6' - H).

*General procedure for the preparation of the monohydroxamic acids (**4a, b, d, e, f and 15**)*

The following procedure for the preparation of **4a** is representative. Compound **4a** was prepared in a similar procedure as that described for **3b** from a solution of **13** (1.00 g, 0.0033 mol), acetyl chloride (260 mg, 0.0033 mol) in THF (50 mL) and Et_3N (5 mL) and gave 900 mg (79%) of **4a**, m.p. 121–122 °C. MS (CI, CH_4) MH^+ at 344. Anal. Calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 69.72; H, 7.37; N, 4.14. ^1H NMR (DMSO-d_6): δ 1.41 (d, 3H, CH_3CH), 1.83 (m, 4H, OCH_2CH_2). 2.01 (s, 3H, CH_3CO), 4.01 (m, 4H, OCH_2), 5.55 (q, 1H, CH, 6.92 (m, 5H, 2, 6, 3', 4', 5' - H), 7.26 (m, 4H, 3, 5, 2', 6' - H), 9.48 (s, 1H, O*H*).

*N,2-Dihydroxy-N-[1-[4-(phenoxybutoxy)phenyl]ethyl]-acetamide (**4c**)*

To a solution of **4b** (420 mg, 1.05 mmol) in warm 2-propanol (25 mL) was added H_2O (2 mL). The mixture was allowed to cool to 25 °C and solid LiOH (396 mg, 16.5 mmol) was added. The reaction was stirred at 25 °C for 2 h. The pH of the mixture was then adjusted to 2 with 2N HCl, followed by the addition of H_2O (50 mL). The resulting precipitate was filtered and the filtrate extracted with EtOAc (2 x 150 mL). The combined organic layers was washed with H_2O (2 x 75 mL) and dried over $MgSO_4$ to give an additional crude solid. The solids were combined and chromatographed on silica gel with 40% EtOAc/Hexane to give 0.18 g (48%) of **4c**, m.p. 112–114 °C. MS (CI, CH_4) MH^+ at 360. Anal. Calcd for $C_{20}H_{25}NO_5$

$1H_2O$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.32; H 6.87; N, 3.51. ^1H NMR (DMSO-d_6): δ 1.43 (d, 3H, CH_3CH), 1.86 (m, 4H, OCH_2CH_2), 4.05 (m, 4H, OCH_2), 4.11 (t, 2H, $COCH_2OH$), 5.50 (q, 1H, CH), 6.91 (m, 5H, 2, 6, 3', 4', 5' - H), 7.26 (m, 4H, 3, 5, 2', 6' - H), 9.39 (s, 1H, NO*H*).

RBL-1 5-lipoxygenase inhibition

RBL-1 cells from the American Type Culture Collection (ATCC) were grown in suspension cultures and harvested by centrifugation at 2,000 x *g* for 5 min. Washed cells at a concentration of 5 x 10^7 cells/mL were suspended in $NaHPO_4/CaCl_2$ buffer, homogenized at 0 °C, and then centrifuged at 9,000 x *g* for 50 min. The 5-lipoxygenase activity in the 9,000 x *g* supernatant was determined radiometrically by measuring the conversion of arachidonic acid to 5-HETE. Increasing logarithmic doses of test compound were utilized in order to determine a dose–response curve for each drug. Doses were chosen such that the IC_{50} concentration of the drug fell within the linear portion of the sigmoidal dose–response curve. A mixture of 5.5 μL of test compound and 500 μL of enzyme supernatant was pre-incubated for 5 min at 37 °C. Then, 10 μL of 50 μCi/mL ^{14}C-arachidonic acid was added to each sample followed by a 20 min incubation period at 37 °C. The reaction was stopped by the addition of 1.0 mL of 2N formic acid per sample. The primary 5-LO product, 5-HETE, was isolated by chloroform extraction, followed by TLC on silica gel, and detection of radioactive emissions of product via a Bioscan imaging system plate scanner. The inhibition of 5-LO product formation is expressed as a percentage of the arachidonic acid converted to 5-HETE by the control group versus the drug treatment group. IC_{50} values with 95% confidence limits (CL) were determined by the method of Finney.[20]

Mouse zymosan peritonitis model

Mice (CD-1), 18–25 g, were dosed orally with test compound suspended in polyethylene glycol 200. One hour later, the animals were injected (ip) with 3 mg of zymosan-A suspended in 0.5 mL of 0.9% sterile saline. Fifteen min after receiving zymosan, the mice were sacrificed by CO_2 inhalation. The abdomens were injected with 2 mL of a 10 μM indomethacin solution. Subsequent to massaging the abdominal area, the skin was removed and the abdominal wall was opened. A 0.2 mL aliquot of peritoneal fluid was withdrawn and added to 1 mL of cold 95% ethanol. The solutions were incubated in an ice bath (minimum of 30 min) and then centrifuged at 28000 x *g* for 15 min at 4 °C. Supernatant fractions were decanted and evaporated under a stream of nitrogen at room temperature. The samples were capped and stored at –70 °C until assayed. Radioimmunoassays (RIAs) for LTC_4 were performed on a 1:20 dilution of original samples using [^3H] RIA kits from Advanced Magnetics, Inc. according to kit instructions. ED_{50} values (that dose calculated to cause a 50% reduction in the immunoreactive LTC_4 with 95% confidence limits (CL)) were calculated from the percentage of inhibition determined for each animal at the doses tested and then fitted to a straight line by a log-linear regression analysis according to the method of Finney.[20]

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