Articles

Structure−Activity Relationships of *N*-Hydroxyurea 5-Lipoxygenase Inhibitors

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The discovery of second generation *N*-hydroxyurea 5-lipoxygenase inhibitors was accomplished through the development of a broad structure−activity relationship (SAR) study. This study identified requirements for improving potency and also extending duration by limiting metabolism. Potency could be maintained by the incorporation of heterocyclic templates substituted with selected lipophilic substituents. Duration of inhibition after oral administration was optimized by identification of structural features in the proximity of the *N*-hydroxyurea which correlated to low *in vitro* glucuronidation rates. Furthermore, the rate of *in vitro* glucuronidation was shown to be stereoselective for certain analogs. (*R*)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**17c**) was identified and selected for clinical development.

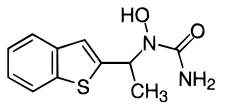
Introduction

The challenge to define the clinical relevance of leukotrienes in various human diseases is being addressed by clinical trials with investigational drugs which inhibit leukotriene biosynthesis or block leukotriene receptor activation.[1] The enzyme 5-lipoxygenase (5-LO) catalyzes the first step in the leukotriene biosynthetic pathway.[2] Selective inhibition of this enzyme provides a definitive means to limit the effects of all leukotrienes.[3] The realization of therapeutically useful 5-LO inhibitors with satisfactory oral bioavailability and duration of action and minimal toxicity has proven to be quite challenging as measured by the extent of research and elapsed time since the elucidation of the leukotriene structures in 1979.[4]

5-LO catalyzes two reactions, an oxidation of arachidonic acid to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) followed by dehydration of 5-HPETE to the reactive 5,6-epoxide, leukotriene A_4.[5] Both the 5- and 15-LO are known to contain a non-heme iron atom.[6] No structure determination has been accomplished for 5-LO. Due to the lack of structural information for the enzyme, inhibitor design involved intuitive medicinal chemistry guided by biological evaluation in various leukotriene inhibition assays. Numerous inhibitors of 5-LO have been reported in the scientific and patent literature since the discovery of this enzyme.[7] Very few 5-LO inhibitors progressed to clinical trials due to insufficient oral bioavailability or toxicity problems.

Corey and co-workers[8] first demonstrated a rational inhibitor design strategy involving a hydroxamate functionality that could hypothetically bind to the Fe^{3+} atom in the active site of 5-LO and interfere with oxidative catalysis. Several arylhydroxamates were synthesized and found to be potent inhibitors of 5-LO activity *in vitro* but suffered rapid hydrolysis *in vivo*.[9] Further studies resulted in hydroxamates with improved pharmacological properties, but clinical studies with the leading agents were eventually dropped.[10]

Bioisosteres of the hydroxamate pharmacophore were examined that might provide potent 5-LO inhibitors with improved oral bioavailability and duration of action by offering greater resistance against hydrolytic, oxidative and conjugative metabolism. One of the first *N*-hydroxyurea congeners synthesized was *N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea (zileuton, **1a**),[11] which was subsequently selected for clinical development.[12]



1a

The primary route of metabolism of **1a** in humans was *O*-glucuronidation of the *N*-hydroxyurea group, and the estimated plasma half-life after oral dosing was approximately 3 h.[13] Clinical trials with **1a** in asthmatics demonstrated efficacy with oral administration of 600 mg four times daily.[14] This validation of the therapeutic application of 5-LO inhibitors in asthma provided impetus to identify an optimized compound with greater potency and longer duration of action that would in turn provide a lower and less frequent dose of drug. The structure−activity relationships of *N*-hydroxyurea−containing 5-LO inhibitors providing the foundation for the identification of optimized 5-LO inhibitors are described in this report.

Chemistry

The majority of *N*-hydroxyureas investigated were *N*-hydroxy-*N*-methylaryl derivatives, and incipient syn-

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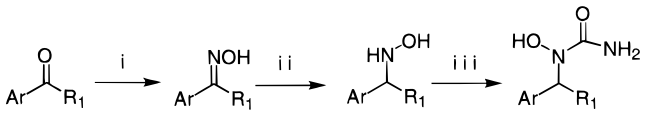
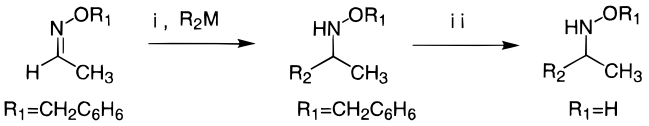
Scheme 1[a]



[a] Reagents: (i) NH_2OH; (ii) BH_3·PY, EtOH, 6 N HCl; (iii) TMSNCO or KOCN.

Scheme 2[a]



[a] Reagents: (i) BF_3·Et_2O; (ii) EtSH, $AlCl_3$.

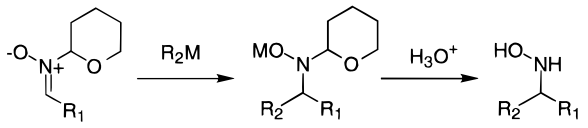
thetic strategies were based on the conversion of *N*-alkylhydroxylamines to *N*-hydroxyureas. Reduction of oximes derived from aryl ketones or aldehydes proved to be an efficient route to the desired *N*-arylmethyl-substituted *N*-hydroxylamines (Scheme 1).

Excess borane pyridine in the presence of 6 N HCl proved to be the most general method for this transformation.[15] Conversion of the mono-*N*-alkyl-substituted hydroxylamine to the primary *N*-hydroxyurea was conveniently accomplished on a research scale using trimethylsilyl isocyanate, and KCNO was useful for large-scale applications. These methods were used to prepare a majority of the analogs described including **2**, **1b**,**c**,**e**,**g**−**i**, **4i**,**l**−**t**, **7a**−**f**,**h**−**u**, **8a**−**h**,**j**,**l**−**m**, **9a**−**d**, **10a**−**c**, **11a**−**g**, **12a**−**c**,**e**−**g**, **13a**, **14a**−**f**, **16a**, and **17f**.

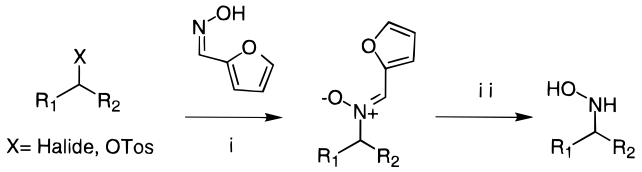
The thiourea analog **6** was also prepared by this procedure, substituting trimethylsilyl isothiocyanate for trimethylsilyl isocyanate. Alternative borane reducing reagents were employed in several cases where milder acidic conditions were appropriate. Oxime reduction with sodium cyanoborohydride[16] was accomplished for analogs **9**, **12d**, and **13b**. For some unsaturated oximes such as **12d** and **13b**, this milder method suppressed the 1,4 overreduction side products formed with borane−pyridine. Substituted ureas were prepared by sequential reaction of the hydroxylamine with phosgene followed by addition of the requisite amine. This method was used to prepare *N*′-hydroxyureas **5i** and **5z**. *N*-Alkylhydroxylamines were also reacted with substituted isocyanates and other acylating agents to synthesize *N*′-substituted ureas and alternative pharmacophore analogs including **5a**,**g**,**5h**,**j**−**z**.

An alternative strategy was envisioned to synthesize N-alkylated hydroxylamines involving addition of organometallics to acetaldehyde oxime derivatives. Reaction of organolithiums with the *O*-benzyloxime of acetaldehyde, in the presence of boron trifluoride etherate provided the desired (benzyloxy)amines[17] (Scheme 2). Selective hydrogenation of the benzyl group proved difficult without prior stabilization of the NO bond by acylation of the nitrogen. These intermediates were debenzylated with ethanethiol and $AlCl_3$, resulting in the desired hydroxylamine derivatives. The procedure shown in Scheme 2 was used for the preparation of analogs **1d** and **4k**. It was observed that in tetrahydrofuran the *Z*-oxime reacted preferentially and the *E*-isomer remained unreactive under these reaction conditions, thus limiting the yield to that of the *Z*/*E* ratio of the starting oxime. A subsequent report indicated that changing the solvent to toluene provided conversion of both oxime isomers, resulting in improved yields.[18]

Scheme 3

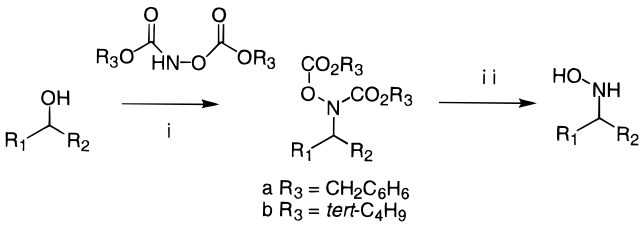


Scheme 4[a]



[a] Reagents: (i) EtONa, EtOH; (ii) NH_2OH, MeOH.

Scheme 5



[a] Reagents: (i) DEAD, Ph_3P, THF; (ii) for a, TMSI; for b, TFA.

A variation of the oxime addition reaction was developed in which the alkylated hydroxylamine could be "released" under milder conditions. Addition of organolithium reagents to the *N*-tetrahydropyranylnitrone obtained from the reaction of 5-hydroxypentanal oxime with acetaldehyde provided the *N*-tetrahydropyranylhydroxylamine adduct.[19] These intermediates were readily hydrolyzed to the desired substituted hydroxylamines (Scheme 3). Analogs **8i** and **8k** were prepared by this method.

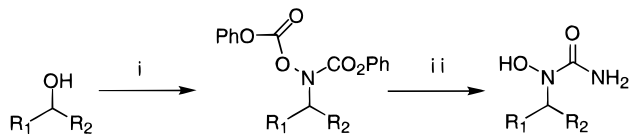
A report by Goto[20] described N-alkylation of the *Z*-oxime of furfural to provide a nitrone intermediate that after hydrolysis provided the desired substituted hydroxylamine (Scheme 4). This method was used to prepare analog **4u**.

A useful method involved reaction of N,O-substituted hydroxylamine derivatives with alcohols under Mitsunobu conditions.[21] Both *N*,*O*-bis(benzyloxycarbonyl)-hydroxylamine and *N*,*O*-bis(*tert*-butyloxycarbonyl)hydroxylamine would react smoothly with a variety of alcohols under these conditions (Scheme 5). The adducts obtained required deprotection under strongly acidic or Lewis acid conditions. Analog **13a** was prepared using *N*,*O*-bis(benzyloxycarbonyl)hydroxylamine and trimethylsilyl iodide deprotection. The *N*,*O*-bis(*tert*-butyloxycarbonyl)hydroxylamine reagent and trifluoroacetic acid deprotection was a more general method and was used to prepare analog **4j** and the early acetylene analogs **15a**−**f**.

A more general, milder method was realized using *N*,*O*-bis(phenoxycarbonyl)hydroxylamine[22] under Mitsunobu conditions (Scheme 6). Treatment of these adducts with ammonia provided the desired *N*-hydroxyureas under mild nonacidic conditions and circumvented isolation of hydroxylamine intermediates. This methodology was applied to several analogs including **7g**, **10d**, **17h**, **17j**, and the acid sensitive acetylenic furan analogs **17e** and **17f**.

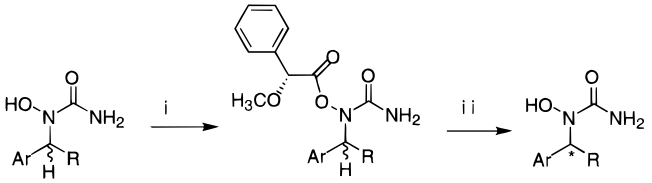
Racemates containing a stereogenic center α to the hydroxylated nitrogen of the *N*-hydroxyurea were re-

Scheme 6[a]



[a] Reagents: (i) $PhO_2CONHCO_2Ph$, $(Ph)_3P$, DIAD; (ii) NH_3.

Scheme 7[a]



[a] Reagents: (i) DMF, MeCN, oxalyl chloride, (*R*)-methoxymandelic acid, pyridine; (ii) K_2CO_3, MeOH.

solved by O-acylation with a chiral amino acid derivative[23] or *O*-methylmandelate auxiliaries[24] (Scheme 7). Separation of diastereomers and cleavage of the chiral auxiliary provided the individual enantiomers for evaluation. Compound **1a** was resolved using the diastereomeric *O*-mandelate derivatives to provide **16b** and **16c**.

Results and Discussion

A simple descriptor model, template−link−*N*-hydroxyurea, was used to define the variable components of the structure−activity relationship (SAR) investigation. The template was considered the surrogate for the lipid enzyme substrate, arachidonate. The link group was originally designed to insulate the hydroxylamine-derived pharmacophore from aryl templates to preclude the formation of arylhydroxylamine metabolites. The nature of the link group also proved to be important for limiting metabolism of *N*-hydroxyurea compounds. In actuality the template and link group could not be optimized in isolation as they mutually defined the *N*-hydroxyurea orientation. Optimization of inhibitor potency involved matching both components to maximize van der Waals interactions in the inhibitor−enzyme complex and position the potential iron-binding *N*-hydroxyurea for maximum interaction.

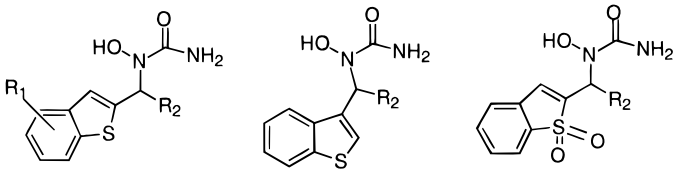
Zileuton (**1a**) was the starting point for the SAR optimization of inhibitory potency. Two leukotriene inhibition assays were used to evaluate inhibitory activity. A supernatant from rat basophilic leukemia cells with 5-LO activity converted exogenous arachidonic acid (AA) into 5-hydroperoxyeicosatetraenoic acid (5-HPETE). This assay provided a means to measure direct inhibition of 5-LO catalysis. A second method measured inhibition of LTB_4 biosynthesis in whole blood stimulated by calcium ionophore A23187. This assay provided evaluation of inhibitory activity of test compounds in a complex setting of intact cells and blood components. Structure−activity relationships of oral bioavailability were surveyed by measuring drug plasma levels of test compounds in rats. *In vivo* activity was examined in a rat anaphylaxis model.[25]

Template SAR

Substitution on the benzo[*b*]thiophene template was examined (Table 1). The 3-substituted regioisomer **2** and the sulfone **3** were less active than **1a**; however the majority of analogs studied showed only minor differences in inhibitory potency. Electron-withdrawing (**1a**−**e**), electron-donating (**1f**), bulky alkyl substituent (**1g**), 3-thioalkyl (**1h**), and 3-thioaryl (**1i**) substitution had little effect on potency. Substitution on the benzo[*b*]-thiophene added synthetic complexity with no significant gains in inhibitory activity over the unsubstituted parent **1a**.

Alternative benzofused heterocyclic systems and other aryl templates were examined with the *N*-hydroxyurea pharmacophore (Table 2). A variety of substituted phenyl analogs (**4b**−**h**) displayed similar potencies with IC_{50} values in the 0.3−1.0 μM range in both the broken cell and whole blood assays. The parent phenyl analog **4a** was significantly less potent. Benzofused heteroaryl templates provided a greater range of *in vitro* activity.

Table 1. Physical and Biological Data for Benzo[*b*]thiophene Template Analogs

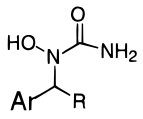


1a-i 2 3

					IC_{50}, μM	
compd	R_1	R_2	mp, °C	formula[a]	RBL-1[b]	HWBL[c]
1a	H	CH_3	ref [23]		0.5	0.74
2		CH_3	180(dec)	$C_{11}H_{12}N_2O_2S$	1.30	3.2
3		CH_3	foam	$C_{11}H_{12}N_2O_4S$	14	nt[d]
1b	3-chloro	CH_3	155−156	$C_{11}H_{11}ClN_2O_2S$	0.3	3.7
1c	4-chloro	CH_3	157.5−158.5	$C_{11}H_{11}ClN_2O_2S$	0.2	2.5
1d	5-fluoro	CH_3	149−153	$C_{11}H_{11}FN_2O_2S$*	0.5	nt[d]
1e	7-chloro	CH_3	157−159	$C_{11}H_{11}ClN_2O_2S$	89% at 0.195	1.5
1f	3-methoxy	CH_3	158(dec)	$C_{12}H_{14}N_2O_3S$	0.66	1.3
1g	5-*tert*-butyl	CH_3	151−153	$C_{15}H_{20}N_2O_2S$	0.71	nt[d]
1h	3-thio-*tert*-butyl	H	167(dec)	$C_{14}H_{18}N_2O_2S_2$	53% at 0.39	31% at 6.25
1i	3-thiophenoxy	H	165−167	$C_{16}H_{14}N_2O_2S_2$	69% at 0.39	0.64

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*); see Experimental Section for these values. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] nt indicates not tested.

Table 2. Physical and Biological Data for Aromatic Template Analogs



4a-u

compd	Ar	R	mp, °C	formula[a]	IC_{50}, μM RBL-1[b]	IC_{50}, μM HWBL[c]
4a	C_6H_5	CH_3	ref.[22]		3.0	2.7
4b	4-methylphenyl	CH_3	134−135	$C_{10}H_{14}N_2O_2 \cdot 0.25H_2O$	0.3	1.0
4c	4-phenylphenyl	CH_3	157−158	$C_{15}H_{16}N_2O_{2*}$	0.57	nt[d]
4d	4-bromophenyl	CH_3	138−140	$C_9H_{11}BrN_2O_2$	0.35	0.42
4e	4-phenylmethoxyphenyl	CH_3	140−141	$C_{16}H_{18}N_2O_3 \cdot 1.0H_2O$	0.36	0.59
4f	4-(2-phenylethoxy)phenyl	CH_3	126−128	$C_{10}H_{14}N_2O_2 \cdot 1.0H_2O$	0.33	nt[d]
4g	4-(1-phenylethoxy)phenyl	CH_3	125−130	$C_{17}H_{20}N_2O_3$	0.48	0.19
4h	3-phenoxyphenyl	CH_3	96−98	$C_{15}H_{16}N_2O_3$	50% at 0.39	0.14
4i	2-naphthyl	CH_3	140−142	$C_{13}H_{14}N_2O_2 \cdot 0.75H_2O$	0.87	1.4
4j	thieno[2,3-*b*]pyrid-2-yl	CH_3	180−182	$C_{10}H_{11}N_3O_2S$	2.40	0.86
4k	benzothiazol-2-yl	CH_3	159−160	$C_{10}H_{11}N_3O_2S$	1.9	0.52
4l	indol-2-yl	CH_3	153−154	$C_{11}H_{13}N_3O_2$	2.9	1.5
4m	1-methylindol-2-yl	CH_3	145−146	$C_{12}H_{15}N_3O_2$	1.6	nt[d]
4n	1-methylindol-3-yl	CH_3	149−150	$C_{12}H_{15}N_3O_2$	2.4	nt[d]
4o	*N*-methylbenzimidazole	H	145−147	$C_{11}H_{14}N_4O_2 \cdot 1.0H_2O$	85	nt[d]
4p	benzo[*b*]fur-2-yl	CH_3	147−150	$C_{11}H_{12}N_2O_2$	2.7	nt[d]
4q	5-chlorobenzo[*b*]fur-2-yl	CH_3	149−150	$C_{11}H_{11}ClN_2O_2$	0.81	1.6
4r	5-methoxybenzo[*b*]fur-2-yl	CH_3	151−153	$C_{12}H_{14}N_2O_3$	1.7	nt[d]
4s	dibenzofur-4-yl	H	171−173	$C_{15}H_{14}N_2O_3$	0.32	3.6
4t	4,5-thienylthien-2-yl	CH_3	167 dec	$C_9H_{10}N_2O_2S_2$	0.9	1.2
4u	quinol-2-yl	H	165−167	$C_{11}H_{11}N_3O_2$	15	nt[d]

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*), see Experimental Section for these values. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] nt indicates not tested.

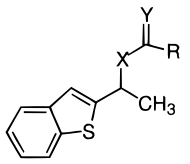
Some nitrogen-containing analogs (**4o**, **4u**) suffered significant loss of inhibitory activity. The benzofuran analogs (**4p**−**r**) resulted in a smaller loss of potency. The naphthyl **4i**, dibenzofuran **4s**, and thienylthiophene **4t** analogs had activity similar to that of **1a**. These common variations of the template did not reveal any significant advances to increasing inhibitory activity.

Pharmacophore Studies

The *N*-hydroxyureas consistently achieved higher plasma levels and exhibited greater *in vivo* activity in the rat anaphylaxis model than the related acetohydroxamate analogs previously reported.[10] These results were primarily due to improved oral bioavailability related to reduced metabolism associated with the pharmacophore. For example, **1a** exhibited greater *in vivo* potency and higher plasma levels than the *N*-methylhydroxamate (**5b**) or the "type B" acetohydroxamate (**5a**). Using **1a** as a reference standard, additional modifications of the *N*-hydroxyurea pharmacophore were studied. The activity of the congeners was evaluated *in vivo*, and drug plasma levels were measured after oral administration in rat. The plasma levels at 1 h (Table 3) were found to be a good approximation of the peak drug concentration (C_{max}) for a wide range of *N*-hydroxyurea congeners. The requirement of the N-OH function for inhibitory activity was confirmed by the inactivity of N-OR analogs **5c**−**e**. An analog (**5f**) having the *N*-hydroxy moiety blocked as a hydroxyethyl ether was also prepared. This homologated hydroxyl analog was also inactive. The regioisomeric *N*-hydroxyurea (**5h**) showed *in vitro* activity but was inactive *in vivo*, probably suffering from rapid metabolism. The dihydroxylated urea (**5z**) had much weaker activity than either isomeric *N*-hydroxyurea (**1a** or **5h**). The trifluoroacetohydroxamate (**5g**) had substantially lower activity and drug plasma concentrations. The formyl analog (**5i**) and the thioethyl ester (**5j**) had activity against the isolated enzyme but were devoid of *in vivo* activity and gave low (**5i**) to undetectable (**5j**) drug plasma levels in the rat at 1 h after oral dosing. The *N*-hydroxysemicarbazide (**5m**) was a weaker inhibitor *in vitro* and could not be detected in rat plasma after oral dosing.

A series of terminal *N*-alkyl substituents were examined (**5n**−**u**). These analogs showed good *in vitro* potencies, some up to 5-fold more potent than **1a** (compare **5s** and **5t** to **1a**). This observation was consistent with the previous hydroxamate research[10] where adding lipophilicity resulted in increased *in vitro* 5-LO inhibitory potency. However, when plasma levels are compared, an inverse relationship developed with unsubstituted ureas and smaller alkyl substituents showing higher plasma concentrations that translated to better oral activity *in vivo*. This phenomena, that ClogP values needed for good *in vitro* potencies were incompatible with good bioavailability, had been observed by others working within this series.[26] Addition of a hydroxyl group to a small terminal alkyl chain in the hydroxyethyl analog (**5v**) provided only moderate potency but excellent drug plasma levels in the rat. An attempt to restore potency with the terminal hydroxypentyl (**5w**) analog improved *in vitro* potency, but drug plasma levels were not detectable in the rat. Incorporation of polar substituents on the terminal nitrogen were investigated. The glycine analog (**5y**) had good plasma concentrations in the rat but was a weak inhibitor. The more potent amide analog (**5k**) had low plasma levels. Acylation of the terminal nitrogen provided an analog

Table 3. Physical and Biological Data for Benzo[*b*]thiophene Template Analogs



5a-z, 6

compd	X	Y	R	mp, °C	formula[a]	IC_{50}, μM RBL-1[b]	*in vivo* rat[c] ED_{50}, mg/kg (*n*), or [% I at 200 μmol/kg]	plasma[d] levels, μM
1a	NOH	O	NH_2			0.5	4.4 (6)	127
5a	NOH	O	CH_3	108−110	$C_{12}H_{13}NO_2S$	0.78	15 (1)	66
5b		O	$N(OH)CH_3$	oil	$C_{12}H_{12}NO_2S$	0.68	[30]	4.8
5c	NH	O	NH_2	205−206	$C_{11}H_{12}N_2OS$	−2% at 32	nt[e]	nt[e]
5d	$N(OCH_3)$	O	NH_2	162 dec	$C_{12}H_{14}N_2O_2S$	−2% at 32	nt[e]	nt[e]
5e	N(OBn)	O	NH_2	123−125	$C_{18}H_{18}N_2O_2S$	−10% at 32	nt[e]	−0.4
5f	$N(OCH_2CH_2OH)$	O	NH_2	182−183	$C_{12}H_{14}N_2O_3S$	−14% at 32	nt[e]	13.7
5g	NOH	O	CF_3	137−140	$C_{12}H_{10}F_3NO_2S$	13.0	[18]	7.9
5h	NH	O	$N(OH)CH_3$	111−112	$C_{12}H_{14}N_2O_2S$	0.4	28 (1)	6.1
5i	NOH	O	H	115−116.5	$C_{11}H_{12}NO_2S$	3.0	[−3]	−1.0
5j	NOH	O	SCH_2CH_3	117−119	$C_{13}H_{15}NO_2S_2$	58% at 0.1	[−76]	4.0
5k	NOH	O	$NHCH_2CONH_2$	165 dec	$C_{13}H_{15}N_3O_3S\cdot1.0H_2O$	3.0	nt[e]	14.4
5l	NOH	O	$NHCONH_2$	180 dec	$C_{12}H_{13}N_3O_3S\cdot0.25H_2O$	1.0	nt[e]	4.6
5m	NOH	O	$NHNH_2$	150−152	$C_{11}H_{13}N_3O_2S$	3.30	nt[e]	−0.5
5n	NOH	O	$NHCH_3$	149−150	$C_{12}H_{14}N_2O_2S$	0.65	13 (1)	31
5o	NOH	O	$NHCH_2CH_3$	138−139	$C_{13}H_{16}N_2O_2S$	0.51	39 (1)	21.7
5p	NOH	O	$NHCH_2CHCH_2$	123−125	$C_{14}H_{16}N_2O_2S$	0.38	28 (1)	12.1
5q	NOH	O	$NH(CH_3)_2$	138−141	$C_{13}H_{16}N_2O_2S$	0.54	46 (1)	10.6
5r	NOH	O	$NH(C_4H_9)$	108−110	$C_{15}H_{20}N_2O_2S$	0.15	[46]	1.7
5s	NOH	O	$NH(C_5H_{11})$	98−100	$C_{16}H_{22}N_2O_2S$	0.10	[47]	2.30
5t	NOH	O	$NH(C_6H_5)$	158−160	$C_{17}H_{16}N_2O_2S$	85% at 0.25	[19]	0.24
5u	NOH	O	$NHCH_2Ph$	175−176	$C_{18}H_{18}N_2O_2S$	0.12	[−38]	2.1
5v	NOH	O	$NHCH_2CH_2OH$	133−135	$C_{13}H_{16}N_2O_3S$	1.80	11 (1)	177
5w	NOH	O	$NH(C_5H_{10})OH$	130−132	$C_{16}H_{22}N_2O_3S$	0.42	nt[e]	−0.2
5x	NOH	O	$NHCOCH_3$	134−137	$C_{13}H_{14}N_2O_3S$	1.50	[32]	−0.2
5y	NOH	O	$NHCH_2CO_2H$	158 dec	$C_{13}H_{14}N_2O_4S$	39% at 32	[−26]	122
5z	NOH	O	NHOH	132−134	$C_{11}H_{12}N_2O_3S$	1.80	nt[e]	15.9
6	NOH	S	NH_2	165 dec	$C_{11}H_{12}N_2OS_2$	0.19	10 (1)	52

[a] All compounds had CHN analyses ±0.4% of the theoretical. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Rat anaphylaxis leukotriene formation, an oral dose of 200 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [d] Measured at 1 h after a 200 μmol/kg oral dose in rat. [e] nt indicates not tested.

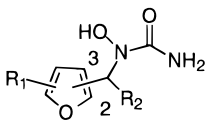
(**5x**) with moderate potency but no detectable plasma levels. The thiourea (**6**) had good *in vitro* activity but lower plasma concentrations and weaker *in vivo* activity than that of **1a**. The terminally unsubstituted *N*-hydroxyurea provided the best combination of potency and oral bioavailability and was therefore selected as the pharmacophore for all the remaining SAR studies.

Furan and Thiophene Templates

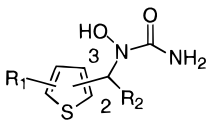
The dilemma that lipophilic substituents provided potency at the expense of bioavailability presented a formidable obstacle. This problem had been partially solved using both the heterocyclic benzo[*b*]thiophene template and the unsubstituted terminal urea nitrogen. The template study progressed further into the realm of smaller less lipophilic heterocycles and superficially seemed to perpetuate this dogma. Smaller hydrophilic heterocyclic templates led to a dramatic loss in inhibitory potency. For example, the furan **7a** (Table 4) had a 40-fold loss in potency against the isolated enzyme compared to the benzo[*b*]thiophene **1a**. The isomeric 3-substituted furan (**7b**) was also a relatively weak inhibitor. The ethyl link did not show significant differences relative to the methylene-linked analogs with the furan template. The *in vivo* activity and the plasma levels were similar in direct comparisons (**7a** to **7c** and **7b** to **7d**). This was contrary to the results observed for the ethyl link with substituted phenyl or benzofused templates having superior *in vivo* activity over the methylene link analogs. This observation supported the likelihood of different routes of metabolism for these analogs influenced by the lipophilicity of the template. The thiophene analogs generally provided at least a 2-fold boost in potency compared to the corresponding furan (for example, compare **7a** to **8a** and **7b** to **8b**). Alkyl substituents on the furan and thiophene templates (**7e**−**j**, **8e**−**f**) did improve inhibitory activity against the isolated enzyme assay. Incorporation of more lipophilic substituents such as *n*-butyl (**7p**), aryl (**7k**−**m**, **8j**), and benzyl (**8j**) gave predictably substantial increases in *in vitro* potency. Similar to previous SAR studies, these compounds achieved only a fraction of the observed plasma levels relative to the more hydrophilic congeners (compare **7a** to **7k** and **7m** or compare **8a** to **8j**).

A variety of substituents provided greater *in vitro* potency. The 5-phenethylene-substituted analogs (**7o**, **8l**) proved very potent against the isolated enzyme but suffered poor oral bioavailability. The analogous pyridyl-substituted compounds **7n** and **8h** were found to

Table 4. Physical and Biological Data for Substituted Furan/Thiophene Template Analogs



7a-u



8a-m

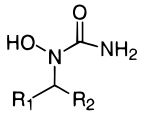
compd	isomer	R_1	R_2	mp, °C	formula[a]	IC_{50}, μM RBL-1[b]	IC_{50}, μM HWBL[c]	*in vivo* rat[d] ED_{50}, mg/kg (*n*), or [% I at 200 μmol/kg]	plasma[e] levels, μM
7a	2	H	CH_3	143−144	$C_7H_{10}N_2O_3$	24	1.95	12 (1)	120
7b	3	H	CH_3	128−130	$C_7H_{10}N_2O_3$	15	2.1	5 (3)	149
7c	2	H	H	128−131	$C_6H_8N_2O_3$	30	1.8	13 (1)	134
7d	3	H	H	124−126	$C_6H_8N_2O_3$	19	0.74	4 (2)	148
7e	3	2,5-dimethyl	CH_3	145−146	$C_9H_{14}N_2O_3$	2.9	1.8	17 (1)	33.5
7f	3	2,5-dimethyl	H	115−117	$C_8H_{12}N_2O_3$	4.0	0.38	[94]	67.3
7g	3	2-methyl	H	97−98	$C_7H_{10}N_2O_3$	19	0.46	3 (1)	38.7
7h	3	5-methyl	CH_3	131−133	$C_8H_{12}N_2O_3$	4.3	0.48	nt[f]	nt[f]
7i	2	5-methyl	H	105 dec	$C_7H_{10}N_2O_3$	11	2.1	13 (1)	221
7j	2	5-methyl	CH_3	116−118	$C_8H_{12}N_2O_3$	12	0.94	9 (4)	78
7k	2	5-phenyl	CH_3	133−136	$C_{13}H_{14}N_2O_3$	0.54	1.4	6 (2)	70
7l	2	5-phenyl	H	148 dec	$C_{12}H_{12}N_2O_3$	0.45	0.6	11 (2)	113
7m	2	5-(2,4,6-trimethylphenyl)	CH_3	139−141	$C_{16}H_{20}N_2O_3$	0.3	1.6	18 (1)	28
7n	2	5-(pyridy-2-yl)	CH_3	185−187	$C_{12}H_{13}N_3O_3S \cdot 0.5H_20$*	6.4	3.4	15(1)	69
7o	2	5-phenethylene	CH_3	140−143	$C_{15}H_{16}N_2O_3 \cdot 1.0H_2O$	0.3	nt[f]	17 (1)	19
7p	2	5-*n*-butyl	CH_3	105−107.5	$C_{11}H_{18}N_2O_3$	1.2	0.2	7.5(1)	34
7q	2	5-hydroxymethyl	H	122 dec	$C_7H_{10}N_2O_4 \cdot 0.25H_20$	12% at 32	nt[f]	nt[f]	nt[f]
7r	2	5-ethoxymethyl	CH_3	81 dec	$C_9H_{14}N_2O_4$	36	3.70	6.7 (1)	161
7s	2	5-(benzyloxy)methyl	CH_3	121 dec	$C_{15}H_{18}N_2O_4 \cdot 0.25H_20$	1.1	0.1	15 (2)	45
7t	2	5-carboxylic acid	CH_3	171−172	$C_8H_{10}N_2O_5 \cdot 0.5H_20$	−6% at 32	nt[f]	nt[f]	39
7u	2	5-(*N*-benzylacetamide)	CH_3	165 dec	$C_{15}H_{17}N_3O_4 \cdot 0.25H_20$	7.1	5.0	[42]	33
8a	2	H	CH_3	131 dec	$C_7H_{10}N_2O_2S$	3.7	1.8	7 (1)	59
8b	3	H	CH_3	138.5−140	$C_7H_{10}N_2O_2S$	3.5	2.4	[66]	105
8c	3	2,5-dimethyl	CH_3	146−148	$C_9H_{14}N_2O_2S$	1.5	1.7	[46]	nt[f]
8d	2	3-methyl	CH_3	131 dec	$C_8H_{12}N_2O_2S$	2.1	4.1	[64]	34
8e	2	3-methyl	H	110 dec	$C_7H_{10}N_2O_2S$	3.1	1.1	6 (2)	107
8f	2	5-methyl	CH_3	130−131	$C_8H_{12}N_2O_2S$	2.8	0.43	8 (1)	78
8g	2	5-phenyl	CH_3	150−151	$C_{13}H_{14}N_2O_2S \cdot 1.0H_20$	0.40	1.4	13 (1)	43
8h	2	5-(pyridy-2-yl)	CH_3	foam	$C_{12}H_{13}N_3O_2S \cdot 0.5H_20$	1.7	4.0	15 (1)	78
8i	2	5-(thien-2-yl)	CH_3	154−155	$C_{11}H_{12}N_2O_2S_2$	0.2	3.1	nt[f]	6
8j	2	5-phenylmethyl	CH_3	117−120	$C_{14}H_{16}N_2O_2S$	0.42	0.68	[57]	11
8k	2	5-(thien-2-ylmethyl)	CH_3	113−115	$C_{12}H_{14}N_2O_2S_2 \cdot 1.0H_20$*	44% at 0.195	39% at 1.56	[57]	19
8l	2	5-phenethylene	CH_3	160.5−163	$C_{15}H_{16}N_2O_2S$	0.15	0.71	[48]	4
8m	2	5-(thien-2-ylethylene	H	164−167	$C_{12}H_{12}N_2O_2S$	0.2	0.83	nt[f]	2.2

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*), see Experimental Sectionfor these values. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] Rat anaphylaxis leukotriene formation, an oral dose of 200 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [e] Measured at 1 h after a 200 μmol/kg oral dose in rat. [f] nt indicates not tested.

be less potent inhibitors. Other attempts to introduce heteroatoms included the 5-(thien-2yl)- (**8i**), 5-(thien-2ylmethyl)- (**8k**) and 5-(thien-2ylethylene)-substituted thiophenes (**8m**). These compounds were potent inhibitors *in vitro* but did not have improved oral plasma levels. A related strategy introduced 5-ethoxymethyl (**7r**) and 5-(benzyloxy)methyl (**7s**) substitution on the furan template. The benzyloxy substituent provided sufficient potency but at the expense of plasma concentration. The activity was severely diminished with a 5-hydroxymethyl substituent (**7q**) and completely abolished with introduction of a carboxylic acid (**7t**) as had been observed in the pharmacophore study. Introduction of a *N*-benzylacetamide substituent (**7u**) in the 5-position returned moderate potency but had accompanying low plasma concentrations.

The high plasma concentrations of the unsubstituted furans continued to suggest that incorporation of a heterocyclic template could provide a manifold to reduce metabolism. Despite relatively low potencies *in vitro*, the hydrophilic furan analogs displayed excellent activity in the rat anaphylaxis model. The furan analog **7b** was similar in potency in this model to **1a** even though it was 30-fold less active against the isolated enzyme. As an alternative measure of biochemical potency, compounds were evaluated for their ability to inhibit LTB_4 formation in calcium ionophore-stimulated human whole blood. The substituted phenyl and benzothiophene analogs exhibited potencies that correlated well between the rat broken cell assay and the human whole blood assay. The furan analogs exhibited greater potency in the stimulated human whole blood assay than against the rat broken cell enzyme assay. These differences were often substantial with 10−20-fold higher relative potencies in the whole blood assay (see **7a**,**c**,**d**,**j**). For this hydrophilic furan series, the whole blood assay gave a much better correlation to the *in vivo* activity observed. The structural similarity and the common pharmacophore seemed to rule out a change in mechanism, and we still considered these compounds to be direct 5-LO inhibitors. At this point we began to use the human whole blood (HWBL) assay as our primary method to evaluate inhibitors and guide the SAR studies.

Table 5. Physical and Biological Data for Small Aliphatic Template analogs

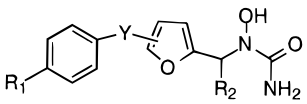


9a-e

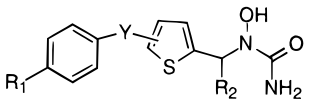
					IC$_{50}$, μM		
compd	R_1	R_2	mp, °C	formula[a]	RBL-1[b]	HWBL[c]	*in vivo* rat[d] ED_{50}, mg/kg (*n*)
9a	cyclopropyl	H	108−109	$C_5H_{10}N_2O_2$	11.6	1.0	5
9b	cyclopropyl	CH_3	94−97	$C_6H_{12}N_2O_2$	21.9	1.7	nt[e]
9c	cyclobutyl	H	112−113	$C_6H_{12}N_2O_2$	11	0.9	3
9d	cyclopentyl	H	115−116	$C_7H_{14}N_2O_2$	5.5	0.7	4
9e	cyclohexyl	H	143−144.5	$C_8H_{16}N_2O_2$	5.7	0.9	nt[e]

[a] All compounds had CHN analyses ±0.4% of the theoretical. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] Rat anaphylaxis leukotriene formation. A 1 h oral inhibitor pretreatment was used. [e] nt indicates not tested.

Table 6. Physical and Biological Data for Thiophenoxy/Phenoxy Substituted Furan/Thiophene Template



10a-d



11a-g

compd	R_1	Y	R_2	position	mp, °C	formula[a]	IC$_{50}$, μM HWBL[b]	*in vivo* rat[c] ED_{50}, mg/kg (*n*), or [% I at (dose, μmol/kg)]
10a	H	S	H	5	118−120	$C_{12}H_{12}N_2O_3S$	0.21	3 (1)
10b	H	O	H	5	118−120	$C_{12}H_{12}N_2O_4$	0.13	3 (1)
10c	F	O	H	5	125−126	$C_{12}H_{11}FN_2O_4$	93% at 0.2	nt[d]
10d	F	O	CH_3	5	ref. [23]		58% at 0.2	0% I at 30
11a	H	S	H	3	99−101	$C_{12}H_{12}N_2O_2S_2$	0.64	nt[d]
11b	H	S	H	4	111−113	$C_{12}H_{12}N_2O_2S_2$	3.2	11 (1)
11c	H	S	H	5	126−128	$C_{12}H_{12}N_2O_2S_2$	5.0	nt[d]
11d	H	O	H	3	132−133.5	$C_{12}H_{12}N_2O_3S{\cdot}0.25H_2O$	1.8	nt[d]
11e	H	O	H	4	120−122	$C_{12}H_{12}N_2O_3S$	1.2	66% I at 100
11f	H	O	H	5	114−116	$C_{12}H_{12}N_2O_3S$	0.57	92% I at 100
11g	Cl	S	H	5	155−160 dec	$C_{12}H_{11}ClN_2O_2S_2{\cdot}0.5H_2O$	0.26	79% I at 30

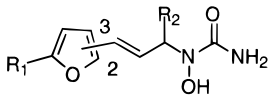
[a] All compounds had CHN analyses ±0.4% of the theoretical. [b] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [c] Rat anaphylaxis leukotriene formation, an oral dose of 30 or 100 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [d] nt indicates 177408n page.

Increases in potency in the HWBL assay of 2−3-fold were achieved by addition of methyl substituents on the furan template (compare **7f**, **7g**, and **7h** to **7b**). Many furan and thiophene analogs were prepared with methyl groups positioned to influence both potency and metabolism (**7e**−**j**, **8c**−**f**). The thiophene analog **8e**, with a methyl substituent in the 3-position, was designed to have a moderate *in vitro* potency expected of a thiophene template and a low molecular weight or minimal lipophilicity sufficient to attenuate metabolic oxidation or glucuronidation. This analog (**8e**) had high plasma levels in rat at 1 h after oral administration and good *in vivo* activity. The stereogenic center of **1a** had been eliminated, but like the majority of our best furan and thiophene derivatives no significant improvement in *in vivo* activity (ED_{50} < 3 mg/kg) was realized.

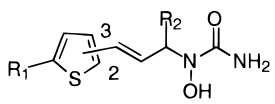
With the observation of simple furan and thiophene analogs providing similar *in vivo* activity to that of **1a**, we were interested in pursuing a better understanding of the relationship between *in vitro* inhibitory activity versus oral activity in the rat anaphylaxis model. One objective was to evaluate the minimum structural requirements for the template. A series of analogs with small alkyl templates (**9a**−**e**) were prepared (Table 5). The cyclopropane analog **9a** proved to have activity similar to that of the simple furan analogs both in *in vitro* assays and in the rat *in vivo* model. Even with modest *in vitro* inhibitory potencies, these compounds were quite effective *in vivo* with ED_{50} values of 3−5 mg/kg. As seen previously, increased lipophilic substitution resulted in increased inhibitory potency in the broken cell assay but did not result in improved oral *in vivo* activity due to a contrary trend of reduced bioavailability with increasing lipophilicity.

Template modification shifted to a closer examination of substituted thiophenes and furans. The thiophenoxy-substituted benzothiophene **1i** was an interesting lead. The whole blood potency of this compound was also superior to other substituted benzothiophene analogs. Disconnection of the fused phenyl ring of the benzo[*b*]-thiophene was envisioned to provide a new phenyl-substituted thiophene analog that might be expected to be an effective inhibitor. The presence of the second heteroatom (S or O) to link the phenyl substituent might improve oral bioavailability. All three isomeric thiophenoxy-substituted thiophene derivatives (**11a**−**c**) (Table

Table 7. Physical and Biological Data for Olefin-Linked Furan and Thiophene Analogs



12a-g

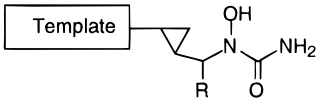


13a-b

						IC$_{50}$, μM			
compd	R_1	R_2	isomer	mp, °C	formula[a]	RBL-1[b]	HWBL[c]	*in vivo* rat[d] ED_{50}, mg/kg (*n*)	rat[e] plasma levels, μM
12a	H	H	2	118−121	$C_8H_{10}N_2O_3$	14.0	55% at 0.2	7 (1)	166
12b	H	CH_3	2	123−124	$C_9H_{12}N_2O_3$	2.1	0.76	nt[f]	53 (100 po)
12c	H	H	3	136−137	$C_8H_{10}N_2O_3$	4.5	0.44	2.25 (2)	129 (100 po)
12d	H	CH_3	3	122−123	$C_9H_{12}N_2O_3$	4.2	1.20	nt[f]	62 (100 po)
12e	CH_3	H	2	131−133	$C_9H_{12}N_2O_3$	2.0	0.16	3.25 (2)	141
12f	CH_3	CH_3	2	122−124.5	$C_{10}H_{14}N_2O_3 \cdot 0.25H_2O$*	1.3	0.88	nt[f]	55
12g	C_6H_6	H	2	158−159	$C_{14}H_{14}N_2O_3 \cdot 0.25H_2O$	0.19	0.12	8.0 (1)	4.5
13a	H	H	3	153 dec	$C_8H_{10}N_2O_2S$	1.90	0.55	2.8 (2)	106
13b	H	CH_3	3	133−134	$C_9H_{12}N_2O_2S$	1.8	0.85	4 (1)	58 (100 po)

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*); see Experimental Section for these values. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] Rat anaphylaxis leukotriene formation, an oral dose of 30 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [e] Measured at 1 h after a 200 μmol/kg oral dose in rat, or at dose indicated (μmol/kg). [f] nt indicates not tested.

Table 8. Physical and Biological Data for Cyclopropyl-Linked Analogs



14a-f

					IC$_{50}$, μM		*in vivo* rat[d] ED_{50}, mg/kg (*n*), or	
compd	template	R	mp, °C	formula[a]	RBL-1[b]	HWBL[c]	[% I at 100 μmol/kg]	rat[e] plasma levels, μM
14a	fur-2yl	H	108−110	$C_9H_{12}N_2O_3$	4.5	0.97	[90]	249
14b	fur-2yl	CH_3	oil	$C_{14}H_{14}N_2O_3 \cdot 0.25H_2O$	3.6	0.9	[65]	86
14c	fur-3yl	H	108.5−110.5	$C_9H_{12}N_2O_3$	5.2	0.23	4 (1)	108 (100 po)
14d	fur-3yl	CH_3	128−130	$C_{14}H_{14}N_2O_3$	5.1	1.20	[71]	42 (100 po)
14e	3-phenoxyphenyl	H	81.5−83	$C_{17}H_{18}N_2O_3$	0.1	0.15	6.5 (2)	49.5
14f	3-phenoxyphenyl	CH_3	132−134	$C_{18}H_{20}N_2O_3$	91% at 0.39	1.10	nt[f]	10

[a] All compounds had CHN analyses ±0.4% of the theoretical. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] Rat anaphylaxis leukotriene formation, an oral dose of 100 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [e] Measured at 1 h after a 200 μmol/kg oral dose in rat, or at dose indicated (μmol/kg). [f] nt indicates not tested.

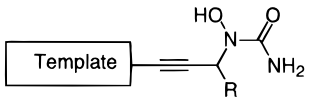
6) with the *N*-hydroxyurea in the 2-position were evaluated. The three corresponding phenoxy-substituted thiophenes (**11d**−**f**) , the 5-thiophenoxyfuran (**10a**), and the 5-phenoxyfuran (**10b**) were also examined. Several potent inhibitors were identified. The 5-phenoxyfuran (**10b**) proved to be the most potent inhibitor in both the whole blood and *in vivo* rat assay. Additional analogs with halogen substituents in the 4-position of the phenyl ring (**11g** and **10c**) also had good inhibitory activity. This study had identified that the phenoxy-substituted furan template provided a simple *N*-hydroxyurea with a significant boost in HWBL potency and one which performed well *in vivo*. This had been achieved through a lipophilic substituent and an additional heteroatom connected in a way that still provided some flexibility to the new bicyclic system.

Link Studies

The ethyl link was widely used in the early template SAR studies based on preliminary pharmacokinetic data that suggested introduction of a methyl substituent reduced oxidative metabolism of the carbon adjacent to the hydroxyurea function. Furan analogs with an olefin link[27] were evaluated (Table 7). The furan provided enough hydrophilicity to prevent induction of rapid metabolism as indicated by good oral drug plasma levels in the rat (see **12a**,**c**,**e**). The addition of the phenyl ring in **12g** increased *in vitro* potency but had low plasma concentrations. The methyl substituent α to the *N*-hydroxyurea did not seem to offer advantages in this olefin-linked series (compound **12a** vs **12b**, **12c** vs **12d**, and **12e** vs **12f**). The thiophene analogs (**13a**,**b**) had activity similar to that of the corresponding furan analogs (**12a**,**b**). The concept of an extended and/or conjugated link was ready to be more fully explored.

Using the furyl and 3-phenoxyphenyl templates, the *trans*-cyclopropyl link was examined as an olefin mimic (Table 8). These analogs had very comparable potencies both *in vitro* and *in vivo* to the olefin analogs (**14a** vs **12a** and **14b** vs **12b**). The addition of a methyl branch

Table 9. Physical and Biological Data for Acetylene-Linked *N*-Hydroxyureas



15a-f

compd	template	R	mp, °C	formula[a]	IC50, μM RBL-1[b]	IC50, μM HWBL[c]	*in vivo* rat[d] ED50, mg/kg (*n*), or [% I at 30 μmol/kg]	rat[e] plasma levels, μM
15a	3-phenoxyphenyl	H	139−141	$C_{16}H_{14}N_2O_3$	0.5	0.06	1.6 (2)	11.5
15b	3-phenoxyphenyl	CH_3	110−115	$C_{17}H_{16}N_2O_3 \cdot 0.25H_2O$	0.15	0.16	1.2 (1)	nt[f]
15c	3-(4-fluorophenoxy)phenyl	CH_3	139−140	$C_{17}H_{15}FN_2O_3$	0.3	0.10	2 (1)	12.7
15d	fur-3yl	CH_3	152−154	$C_9H_{10}N_2O_3 \cdot 0.25H_2O$*	7.0	2.0	4 (2)	107
15e	fur-2yl	CH_3	141−142	$C_9H_{10}N_2O_3$	6.2	0.9	[74]	86
15f	2-benzo[*b*]thiophene	CH_3	170.5−172	$C_{13}H_{12}N_2O_2S \cdot 0.25H_2O$	0.1	1.7	[0]	nt[f]

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*), see Experimental Section for these values. [b] Rat basophil leukemia (RBL-1) cell (2H3 subline) lysate 5-LO inhibition measured by 5-HETE production. The 95% confidence limits were ±<20% of the mean value. [c] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [d] Rat anaphylaxis leukotriene formation, an oral dose of 30 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [e] Measured at 1 h after a 100 μmol/kg oral dose in rat. [f] nt indicates not tested.

α to the *N*-hydroxyurea, in conjunction with the 3-furyl or the 3-phenoxyphenyl template, resulted in a 2−5-fold loss of whole blood potency compared to the that of methylene analog (compare **14c** to **14d** and **14e** to **14f**).

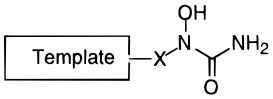
The acetylene link offered a unique geometry with respect to positioning the *N*-hydroxyurea relative to the lipophilic template. Acetylene-containing analogs (Table 9) with the 3-phenoxyphenyl template were potent inhibitors in the whole blood assay. The 3-phenoxyphenyl analog **15a** was a promising lead with an IC_{50} of 60 nM in whole blood and an ED_{50} of 1.6 mg/kg ($n = 2$) in the rat anaphylaxis assay. Addition of the methyl branch to provide the butynyl analog **15b** maintained a similar activity profile. Addition of a 4-fluoro substituent led to analog **15c** that had excellent potency in both *in vitro* assays. Two simple acetylenic furyl analogs (**15d**,**e**) also showed reasonable potency and good plasma levels. The benzothiophene analog (**15f**) was relatively weak in the whole blood assay and had little activity in the rat. Creating an extended π system via olefin or acetylene links to the aryl template appeared to improve *in vitro* potencies in the whole blood for several analogs. The phenoxyphenyl template in conjunction with the propynyl (**15a**) or butynyl link (**15b**) had superior activity in the rat *in vivo* model.

Glucuronidation Rates of *N*-Hydroxyureas: Limiting Metabolism

Although most of our pharmacokinetic studies were routinely done with the rat, improved duration in humans was the real target in the design of a second generation inhibitor. Clinical data from **1a** identified the major route of metabolism as glucuronidation of the *N*-hydroxyurea.[13b] Pharmacokinetic studies done in cynomolgus monkey indicated that many *N*-hydroxyureas had half-lives of less than 1 h. The lower molecular weight furan analogs such as **7b** exhibited a longer duration of about 3 h. The elimination half-life for the butynyl analog **15c** after iv administration in monkey was found to be 5.5 h, one of the longest we had measured up to this point for the *N*-hydroxyurea series. An *in vitro* assay using microsomes from monkey liver was developed to provide routine evaluation of glucuronidation rates for *N*-hydroxyurea inhibitors. The relative rate of *in vitro* glucuronidation inversely correlated to the elimination half-life in monkey, for example, the glucuronidation (GT) rate for zileuton (**1a**) of 0.1 (nmol/min)/mg of protein corresponded to a elimination $T_{1/2}$ of 0.4 h. In contrast, compound **15c** had a GT rate and $T_{1/2}$ of 0.05 (nmol/min)/mg of protein and 5.5 h, respectively. An *in vitro* glucuronidation assay using human liver microsomes provided results similar to those found with monkey liver microsomes. Utilizing micrometabolism assays, the structure−activity relationships influencing pharmacokinetic properties could be evaluated independently of the inhibitory activity.

Reexamination of several furyl and thienyl analogs in the glucuronidation assay revealed low GT rates for the low molecular weight hydrophilic compounds (Table 10). Compounds **7j**, **7i**, and **8b** did not give detectable rates of glucuronidation. The furyl analog **7a** while having a low measurable rate of glucuronidation had the longest half-life in monkey. The addition of the methyl branched link (**7j** vs **7i**) did not affect glucuronidation rates of these furan analogs. The lack of correlation of GT rate to elimination half-life in monkey for this subgroup of analogs suggested that glucuronidation might not be the main route of metabolism for these hydrophilic compounds. Glucuronidation rates did increase for furyl analogs (for example **7k**) with lipophilic substituents. Rates of glucuronidation for the more potent benzothiophene and substituted phenyl template analogs were also examined. Although a correlation between lipophilicity and glucuronidation rate could be easily rationalized, a less intuitive pattern began to develop (Table 10). For potent inhibitors with lipophilic templates, identifiable trends in glucuronidation rates were observed to be dependent on the type of linking group. The cyclopropyl link consistently had higher rates of glucuronidation (**14c**, **14e**). Even the small hydrophilic furyl analog (**14c**) with the cyclopropyl link exhibited a rapid rate of glucuronidation. In the benzo[*b*]thienyl series the ethyl link consistently showed slower rates of glucuronidation than the corresponding methylene analogs (compare **1a** with **16a**). This observation could now help explain the previous results of better *in vivo* activity and superior plasma levels of the early ethyl link analogs over their methylene counter-

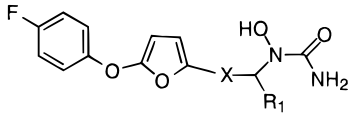
Table 10. Glucuronidation Rates for Classes of *N*-Hydroxyurea Analogs



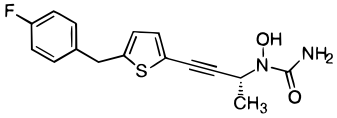
compd	template	X	GT rate[a]	monkey half-life[b] (dose)
7a	fur-3yl	$CH(CH_3)$	0.03	3.10 (118 iv)
7j	5-methylfur-2-yl	$CH(CH_3)$	0.0	0.71 (54 iv)
7i	5-methylfur-2-yl	CH_2	0.0	nt[c]
7k	5-phenylfur-3-yl	$CH(CH_3)$	1.20	1.49 (10 iv)
15d	fur-3yl	$C{\equiv}CCH(CH_3)$	0.00	nt[c]
14c	fur-3yl	*trans*-cyclopropyl	0.16	nt[c]
8b	thien-3-yl	$CH(CH_3)$	0.0	nt[c]
1a	2-benzo[*b*]thiophene	$CH(CH_3)$	0.1	0.4 (20 iv)
16a	2-benzo[*b*]thiophene	CH_2	0.5	0.2 (90 iv)
16b	2-benzo[*b*]thiophene	$CH(CH_3)$ (*R*)	0.055	0.44 (85 iv)
16c	2-benzo[*b*]thiophene	$CH(CH_3)$ (*S*)	0.175	0.32 (85 iv)
15f	2-benzo[*b*]thiophene	$C{\equiv}CCH(CH_3)$	0.09	0.66 (85 iv)
14e	3-phenoxyphenyl	*trans*-cyclopropyl	1.40	0.4 (34 iv)
15b	3-phenoxyphenyl	$C{\equiv}CCH(CH_3)$	0.015	nt[c]
15c	3-(4-fluorophenoxy)phenyl	$C{\equiv}CCH(CH_3)$	0.06	5.5 (9 iv)

[a] The rate of glucuronidation using a microsomal preparation from monkey liver tissue is expressed in units of nmol/minute/mg protein with an initial compound concentration of 100 μM. [b] Calculated $T_{1/2}$ (h) after dose indicated (μmol/kg). [c] nt indicates not tested.

Table 11. Physical and Biological Data for 5-(Fluorophenoxy)Furan Analogs



17a-k

compd	X	mp, °C	formula[a]	IC_{50}, μM HWBL[b]	*in vivo* rat[c] ED_{50}, mg/kg (*n*), or [% I at 30 μmol/kg]	GT rate[d]	monkey half-life[e] (dose)
17a	$C{\equiv}CCH(CH_3)$	ref 23		0.07 (16)	1.2 (6)	0.02	7.4 (2 po)
17b	$C{\equiv}CCH_2$	ref 23		0.06	[70]	0.07	1.5 (7 po)
17c	$C{\equiv}CCH(CH_3)$ (*R*)	ref 23		0.08	1.4 (6)	0.01	8.7 (2 po)
17d	$C{\equiv}CCH(CH_3)$ (*S*)	ref 23		0.05	1.5 (1)	0.05	1.7 (2 po)
17e	$C{\equiv}CC(CH_3)_2$	105−106	$C_{16}H_{15}FN_2O_4$	0.15	[71]	0.4	0[f] (6 po)
17f	$C{\equiv}CC(CH_2)_3$	160−161	$C_{17}H_{15}FN_2O_4{\cdot}0.25H_2O$	62 at 0.1	[48]	0.08	nt[h]
17g	$CH{=}CHCH(CH_3)$ (*E*)	ref 23		0.16	13.5 (4)	0.07	2.8 (2 po)
17h	*trans*-cyclopropyl	oil	$C_{15}H_{15}FN_2O_4$*	77 at 0.1	[50]	0.74	nt[h]
17i	$CH{=}NOCH_2CH_2$	amorphous solid	$C_{14}H_{14}FN_3O_5$	0.06	4 (1)	0.06	1.6 (6 po)
17j	$CH{=}NOCH_2CH(CH_3)$	124−125	$C_{15}H_{16}FN_3O_5$	0.11	[56]	0.03	nt[h]
17k	$CH{=}CFCH(CH_3)$ (*E*)	160−162	$C_{15}H_{14}F_2N_2O_4$	0.09	[63]	0.03	2.4 (2 po)
18		ref 23		0.16	2.8 (4)	<0.01[g]	15 (2 po)

[a] All compounds had CHN analyses ±0.4% of the theoretical, except as indicated by an asterisk (*); see Experimental Section for these values. [b] Human whole blood stimulated with calcium ionophore (A23187) to produce LTB_4 which was measured by enzyme immunoassay. The 95% confidence limits were ±<50% of the mean value. [c] Rat anaphylaxis leukotriene formation, an oral dose of 30 μmol/kg was used to screen compounds for *in vivo* activity, reported as [percent inhibition of LTE_4 from peritoneal fluids]. Dose−response studies were conducted for the more promising compounds, and ED_{50} values (mg/kg) are reported as the mean of separate dose−response determinations (*n*). A 1 h oral inhibitor pretreatment was used. [d] The rate of glucuronidation using a microsomal preparation from monkey liver tissue is expressed in units of (nmol/min)/mg protein with an initial compound concentration of 100 μM. [e] Estimated oral half-life (h) after oral dosing at dose indicated (μmol/kg). [f] No detectable concentration. [g] The compound concentration was increased to 500 μM to facilitate the detection of a glucuronidation rate. [h] nt indicates not tested.

parts. The stereogenic center created by the ethyl link was investigated through resolution of **1a**. Stereoselective glucuronidation was observed *in vitro* for the *R* (**16b**) and *S* (**16c**) enantiomers; however, only a slightly higher glucuronidation rate was found for the *S* isomer and a similar small difference was found for the elimination half-lives in the monkey (Table 10).

The phenoxyphenyl acetylene analog (**15b**) had a slow glucuronidation rate similar to the hydrophilic furyl analog (**7a**). The *N*-(arylbutynyl)-*N*-hydroxyureas offered an opportunity to reduce the glucuronidation rate of the *N*-hydroxyurea function while maintaining high inhibitory potency found for more lipophilic analogs.

Second Generation 5-Lipoxygenase Inhibitors

The phenoxyphenyl-substituted template in combination with the butynyl link proved to be a compatible match that enhanced inhibitory potency and provided resistance to glucuronidative metabolism. A detailed study of the acetylene link and the 5-(4-fluorophenoxy)-furan template is shown in Table 11. *N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxy-

urea (**17a**, A-78773) was at least 6 times more potent in the whole blood assay than **1a**. With an ED_{50} of 1.2 mg/kg in the rat, **17a** was the one of the most potent inhibitors *in vivo*, at least 4 times more potent than **1a**. Pharmacokinetic studies in the monkey revealed an estimated oral plasma half-life of 7 h and elimination half-life of 4.7 h. This combination of inhibitory activity and duration in monkey led to the clinical evaluation of **17a**.[28] Human half-life of **17a** was determined to be 5.9 h compared to 2.3 h for **1a** and was attributed primarily to a slower rate of glucuronidation.

The stereogenic butynyl link proved to have a dramatic role on pharmacokinetic properties unlike the simple ethyl link as seen for the enantiomers of **1a** (Table 10). The propynyl compound (**17b**) showed similar inhibitory activity as **17a** in the whole blood assay but proved weaker *in vivo* and had a much faster *in vitro* glucuronidation rate. The enantiomers of **17a** were resolved and evaluated. The *R* stereoisomer (**17c**) had a 5-fold slower rate of glucuronidation than the *S* (**17d**). The elimination half-lives in monkey were 7.4 h for the racemate **17a**, 8.7 h for *R*-**17c**, and 1.7 h for *S*-**17d**.

In an attempt to eliminate the stereogenic center, the dimethyl-substituted analog **17e** was prepared. This analog could be prepared with the Mitsunobu methodology since tertiary propargyl alcohols react under these conditions. This achiral analog had an *in vitro* glucuronidation rate higher than that of the unsubstituted propynyl **17b**, indicating that a sterically hindered environment was not sufficient to block conjugation metabolism. The spiro cyclobutyl analog (**17f**) had good inhibitory potency *in vitro* but performed poorly *in vivo* and exhibited a glucuronidation rate similar to that of the propynyl analog (**17b**).

Several other link groups examined with the 5-[(4-fluorophenoxy)methyl]-2-furyl template were evaluated and the results are shown in Table 11. The cyclopropyl analog (**17h**) had, as expected, rapid glucuronidation. The olefin analog (**17g**) was found to be a less potent inhibitor. Other link groups such as the fluoroalkene (**17k**) and oxime (**17i**,**j**) had faster rates of *in vitro* conjugation, shorter half-lives in the monkey, and were less active *in vivo*.

On the basis of the pharmacokinetic data in monkey and the favorable *in vitro* glucuronidation rates, the *R* antipode, **17c** (A-79175) was selected to replace the racemate (**17a**) in clinical development. Data from phase 1 clinical studies provided an estimated oral half-life of 7 h for **17c**.[29] Continued optimization ultimately revealed a close congener **18** as a follow-up clinical candidate. The preferential attributes of ABT-761 (**18**) led to its selection to replace **17c** in clinical development.[23] ABT-761 is currently in phase 3 clinical evaluation.

Conclusions

(*R*)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl-1-methyl]-2-propynyl]-*N*-hydroxyurea (**17c**) was identified as a potent 5-lipoxygenase inhibitor that was more resistant to metabolic glucuronidation than **1a**. This resistance to glucuronidative metabolism translated to an orally active agent with a significantly prolonged half-life in humans. Second generation *N*-hydroxyurea 5-lipoxygenase inhibitors **17c** and **18** were identified through an extensive SAR study. The focus of these studies involved both potency and duration of action in multiple species. The dual optimization of these parameters provided effective new agents for evaluation of the role of 5-lipoxygenase in disease processes.

Experimental Section

Chemistry General. Melting points were determined in open glass capillaries and are uncorrected. ^{1}H NMR spectra were recorded on a GE QE300 spectrometer, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan MAT SSQ700 instrument. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter with a continuous Na lamp (569 nm). Elemental analyses (C, H, N) were performed by Abbott Laboratories Pharmaceutical Products Division, Structural Chemistry Department, or Robertson Microlit Laboratories, Inc. Madison, NJ. Silica gel 60 (E. Merck, 230−400 mesh) was used for preparative column chromatography. THF was freshly distilled from sodium benzophenone ketyl. Other solvents were HPLC grade. Reagents were obtained commercially and used without further purification. Chemical yields reported are unoptimized specific examples of one preparation. Analytical TLC using E. Merck F254 commercial plates was used to follow the course of reactions.

Representative Experimental Procedures: Method 1a. Procedure for oxime formation, reduction, and reaction of N-substituted hydroxylamine derivatives with trimethylsilyl isocyanate.

***N*-Hydroxy-*N*-[1-[4-(2-phenylethoxy)phenyl]ethyl]-urea (4f).** To a stirred solution of 4-hydroxyacetophenone (13.62 g, 100 mmol) in 2-butanone (100 mL) was added K_2CO_3 (15.2 g, 110 mmol). The reaction mixture was stirred for 20 min, and (2-bromoethyl)benzene (20.35 g, 110 mmol) was added. The reaction mixture was heated to reflux, stirred for 48 h, cooled, and diluted with water and ethyl acetate. The aqueous layer was extracted with ethyl acetate, and the organic extracts were combined, dried ($MgSO_4$), and concentrated. The residue was purified by flash column (SiO_2, eluting with 25% ethyl acetate/hexanes) to give 7.9 g (33%) of 4-(2-phenylethoxy)acetophenone as a white crystalline solid.

To a stirred solution of 4-(2-phenylethoxy)acetophenone (7.88 g, 32.8 mmol) in ethanol (50 mL) and pyridine (50 mL) was added hydroxylamine hydrochloride (4.11 g, 59 mmol). The mixture was stirred overnight at room temperature and concentrated and the residue dissolved in ether. The ether solution was washed with 3 N HCl, dried ($MgSO_4$), and concentrated to give 7.8 g (93%) of 4-(2-phenylethoxy)acetophenone oxime as a white crystalline solid.

To a stirred 0 °C solution of 4-(2-phenylethoxy)acetophenone oxime (7.78 g, 30.5 mmol) in ethanol (100 mL) was added borane−pyridine (6.24 g, 67.1 mmol). The mixture was stirred for 10 min, and 6 N HCl (35 mL) was added rapidly dropwise. The reaction mixture was stirred for 1 h at room temperature, and the pH was adjusted to 9 with 2 N NaOH. The mixture was concentrated and extracted with ethyl acetate, and the combined extracts were dried ($MgSO_4$). Concentration gave 6.08 g (78%) of (1-(4-(2-phenylethoxy)phenyl)ethyl)hydroxylamine that was used without further purification.

To a stirred solution of the crude hydroxylamine derivative (2.22 g, 8.64 mmol) in dioxane (30 mL) and THF (10 mL) was added trimethylsilyl isocyanate (1.19 g, 10.37 mmol). The mixture was heated to 90 °C and stirred for 1 h. The reaction was cooled, poured into saturated NH_4Cl/ice, and extracted with ethyl acetate. The combined ethyl acetate extracts were washed with brine and dried ($MgSO_4$). Concentration gave a white solid that was washed with 1:1 ether/hexanes and dried to give 1.3 g (50%) of **4f** as a white solid: mp 126−128 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (d, J = 7 Hz, 3H), 3.02 (d, J = 7 Hz, 2H), 4.16 (d, 2H), 5.23 (q, J = 7 Hz, 1H), 6.25 (bs, 2H), 6.84 (m, 2H), 7.23 (m, 2H), 7.32 (m, 5H), 8.98 (s, 1H); MS (EI) m/z 283 $(M - OH)^+$. Anal. ($C_{17}H_{20}N_2O_3 \cdot 1.0H_2O$) C, H, N.

Method 1b. Alternative phosgene procedure for conversion of hydroxylamine derivatives to *N*-hydroxyureas.

The N-substituted hydroxylamine prepared by oxime reduction (10 mmol) was dissolved in toluene (100 mL), and HCl gas was bubbled through the mixture for about 4 min. The solution was then heated to reflux, and phosgene was bubbled through for 4 min. The heating was continued for 1 h, and the mixture was cooled to room temperature and poured into a cold NH_4OH solution. The precipitate was collected and recrystallized or purified by flash column chromatography.

Method 2. Addition of organolithium reagents to *N*-THP nitrone derived from acetaldehyde.

***N*-Hydroxy-*N*-[1-[5-(thien-2-ylmethyl)thien-2-yl]ethyl]-urea (8k).** To a −78 °C stirred solution of 2,2′-methylenedithiophene (Lancaster) (4.5g, 25 mmol) in THF (50 mL) under argon was added *n*-butyllithium (10 mL, 2.5 M in hexanes, 25 mmol) and the mixture stirred for 0.5 h. To this cold stirred solution was added a solution of the nitrone prepared as follows: acetaldehyde (4.0 mL, 72 mmol) was added to a 0 °C stirred suspension of 5-hydroxypentanal oxime (3.5 g, 30 mmol) and $CaCl_2$ (15 g, 135 mmol) in CH_2Cl_2; the mixture was stirred for 6 h, filtered, evaporated to dryness, redissolved in THF (50 mL), and cooled to 0 °C. The −78 °C bath was removed and the reaction allowed to stir for 0.5 h. Ethanol (50 mL) and 6 N HCl (5 mL) were added, and the mixture was stirred for 1.5 h at 40 °C and room temperature overnight. The mixture was concentrated, diluted with water (150 mL), made basic with sodium carbonate, and extracted with ether. The combined ether extracts were dried ($MgSO_4$) and concentrated to give 4.25 g (71%) of crude hydroxylamine derivative.

The crude hydroxylamine was converted to **8k** using trimethylsilyl isocyanate (10%) as described in method 1a: mp 113−115 °C; ^{1}H NMR (DMSO-d_6) 1.38 (d, J = 7 Hz, 3H), 4.29 (s, 2H), 5.41 (q, J = 7 Hz, 1H), 6.34 (bs, 2H), 6.73 (m, 2H), 6.95 (m, 2H), 7.36 (dd, J = 5 Hz, J = 1 Hz, 1H), 9.11 (s, 1H); MS (DCI-NH_3) m/z 300 (M + NH_4)$^+$, 283 (M + H)$^+$. Anal. ($C_{12}H_{14}N_2O_2S_2 \cdot H_2O$) C, N; H: calcd, 4.63; found, 5.36.

Method 3. Addition of organolithium reagents to *O*-benzyloxime of acetaldehyde.

***N*-(1-Benzo[*b*]thiazol-2-ylethyl)-*N*-hydroxyurea (4k).** To a stirred −78 °C solution of benzothiazole (3.7g, 27.4 mmol) in THF (200 mL) was added *n*-butyllithium (11.5 mL, 28.7 mmol, 2.5 M solution in hexanes). The mixture was stirred for 0.5 h, and boron trifluoride etherate (4.1g, 38.7 mmol) was added, followed immediately by the addition of *O*-benzyl acetaldehyde oxime (4.5g, 38.7 mmol). The reaction mixture was stirred for 1 h at −78 °C, saturated NH_4Cl (20 mL) was added, and the ice bath was removed. After being warmed to room temperature, the mixture was concentrated and diluted with ether and water. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography (SiO_2, eluting with 15% ethyl acetate/hexanes) to give 2.8 g (36%) of *N*-(1-benzo[*b*]thiazol-2-ylethyl)-*N*-(benzyloxy)amine as a yellow oil that was used without further purification.

Ethanethiol (30 mL) was cooled to 0 °C, and aluminum chloride (9.23 g, 69.3 mmol) was added in three portions while stirring. The mixture was stirred for 10 min at 0 °C. To this 0 °C stirred mixture was added dropwise a CH_2Cl_2 (10 mL) solution of the (benzyloxy)amine (2.8g, 9.9 mmol) prepared above. The ice bath was removed, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured onto ice (100 g) and extracted with ethyl acetate. The aqueous layer was neutralized with 3 N NaOH and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined with the ethyl acetate layer from above, dried ($MgSO_4$), and concentrated. The residue was recrystallized from ethyl acetate/hexanes to give 0.94 g of a white solid. The mother liquor was concentrated and the residue purified by flash column (SiO_2, eluting with 50% ethyl acetate/hexanes) to give an additional 0.6 g of *N*-(1-(benzo[*b*]thiazol-2-yl)ethyl)-*N*-hydroxylamine. The combined yield was 1.54 g (81%).

The hydroxylamine was converted to **4k** using trimethylsilyl isocyanate (69%) as described in method 1a: mp 159−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.58 (d, J = 7.5 Hz, 3H), 5.28 (q, J = 7.5 Hz, 1H), 6.64 (bs, 2H), 7.45 (m, 2H), 7.96 (m 1H), 8.08 (m, 1H), 9.46 (s, 1H); MS (DCI-NH_3) m/z 238 (M+H)$^+$. Anal. ($C_{10}H_{11}N_3O_2S$) C, H, N.

Method 4. Preparation of N-substituted hydroxylamines by substitution reaction of (*Z*)-furfuraldoxime and hydrolysis of the resulting nitrone.

***N*-Hydroxy-*N*-[1-(quinolin-2-yl)methyl]urea (4u).** To a 0 °C stirred solution of 2-(chloromethyl)quinoline hydrochloride (8.73 g, 40.8 mmol) and the Z-oxime of 2-furaldehyde[20] (6.8 g, 61.3 mmol) in ethanol (50 mL) was added a 1 N ethanol solution of sodium ethoxide (100 mL, 100 mmol). The ice bath was removed and the reaction mixture allowed to stir at room temperature for 1 h. The mixture was concentrated to dryness, and the residue was diluted with ethyl acetate (200 mL) and water (250 mL). The aqueous layer was washed with ethyl acetate, and the organic layers were combined, washed with brine, and dried ($MgSO_4$). Concentration gave a tan solid that was washed with 1:1 ether/hexane and dried to afford the nitrone as a tan solid (5.8 g, 56%).

The nitrone (2.52 g, 10 mmol) obtained above was dissolved in a 1 N methanol solution (80 mL) of hydroxylamine (prepared by addition of hydroxylamine hydrochloride to a methanol/sodium methoxide solution followed by filtration of NaCl). The reaction mixture was stirred at 40 °C for 1 h, cooled, and concentrated. The resulting residue was purified by flash column chromatography (SiO_2, eluting with 7% MeOH/CH_2Cl_2) to give a solid that was washed with 1:1 ether/hexane and dried to afford *N*-[1-(quinolin-2-yl)methyl]hydroxylamine as a tan solid (0.826 g, 47%).

The hydroxylamine derivative was converted to **4u** using trimethylsilyl isocyanate (69%) as described in method 1a: mp 163−168 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.80 (s, 2H), 6.52 (bs, 2H), 7.48 (d, J = 9 Hz, 1H), 7.57 (m, 1H), 7.73 (m, 1H), 7.95 (m, 1H), 8.34 (d, J = 9 Hz, 1H), 9.54 (s, 1H); MS (DCI-NH_3) m/z 218 (M + H)$^+$. Anal. ($C_{11}H_{11}N_3O_2$) C, H, N.

Method 5. Preparation of *N*-substituted hydroxylamines by Mitsunobu alkylation of *N*,*O*-bis(benzyloxycarbonyl)hydroxylamine and reaction with TMSI.

***N*-Hydroxy-*N*-[3-(thien-3-yl)prop-2-enyl]urea (13a).** To a −4 °C stirred solution of 3-(thien-3-yl)prop-2-en-1-ol (7.45 g, 53.2 mmol) (prepared by malonic acid condensation of 3-thiophene carboxaldehyde, esterification, and DIBAL reduction), *N*,*O*-bis(benzyloxycarbonyl)hydroxylamine[30] (17.26 g, 58.5 mmol), and triphenylphosphine (12.91 g, 63.86 mmol) in THF (150 mL) was added a THF (50 mL) solution of diisopropyl azodicarboxylate (16.75 g, 63.86 mmol). The mixture was stirred 0.5 h at room temperature and concentrated. The residue was purified by flash column chromatography (SiO_2, eluting with 20% ether/pentane) to give 18.72 g (81%) as 3:1 mixture of the desired *N*,*O*-bis(benzyloxycarbonyl)-*N*-(3-(thien-3-yl)prop-2-enyl)hydroxylamine and the S_N2' product *N*,*O*-bis-(benzyloxycarbonyl)-*N*-(1-(thien-3-yl)prop-2-enyl)hydroxylamine. A second flash column (SiO_2, eluting with 20% ether/pentane) gave 12.72 g of the desired *N*,*O*-bis(benzyloxycarbonyl)-*N*-(3-(thien-3-yl)prop-2-enyl)hydroxylamine.

To a 0 °C stirred solution of *N*,*O*-bis(benzyloxycarbonyl)-*N*-(3-(thien-3-yl)prop-2-enyl)hydroxylamine (0.95 g, 2.24 mmol) in CH_2Cl_2 was added TMSI (1.35g, 6.73 mmol). The mixture was stirred for 1 h at 0 °C and for 1 h at room temperature. The mixture was poured into water (200 mL) and extracted with hexanes (3 × 100 mL). The aqueous layer was made basic with solid $NaHCO_3$ and extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and concentrated to give 0.26 g (75%) of the desired N-substituted hydroxylamine as a yellow oil that solidified upon standing.

The hydroxylamine was converted to **13a** using trimethylsilyl isocyanate as described in method 1a: mp 153 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.05 (m, 2H), 6.10 (dt, 1H), 6.34 (bs, 2H), 6.55 (d, J = 16 Hz, 1H), 7.30 (m, 1H), 7.42 (m, 1H), 7.50 (m, 1H), 9.29 (s, 1H); MS (DCI-NH_3) m/z 216 (M + NH_4)$^+$, 199 (M + H)$^+$. Anal. ($C_8H_{10}N_2O_2S$) C, H, N.

Method 6. Preparation of N-substituted hydroxylamines by Mitsunobu alkylation of *N*,*O*-bis(*tert*-butyloxycarbonyl)-hydroxylamine and reaction with TFA.

***N*-Hydroxy-*N*-[1-(thieno[2,3-*b*]pyrid-2-yl)ethyl]urea (4j).** 2-(1-Hydroxyethyl)thieno[2,3-*b*]pyridine was prepared in 90%

yield by metalation of thieno[2,3-*b*]pyridine[31] with *n*-butyllithium (1.1 equiv, 2.5 M in hexanes) in THF at −78 °C and reaction with acetaldehyde. 2-(1-Hydroxyethyl)thieno[2,3-*b*]-pyridine was converted to *N*,*O*-bis(*tert*-butyloxycarbonyl)-*N*-[1-(thieno[2,3-*b*]pyrid-2-yl)ethyl]hydroxylamine using *N*,*O*-bis-(*tert*-butyloxycarbonyl)hydroxylamine and the procedure described in method 5. The final chromatographic purification was done using a gradient elution of 10−25% ethyl acetate and gave a 33% yield of the desired hydroxylamine derivative as an oil.

To a stirred room temperature solution of *N*,*O*-bis(*tert*-butyloxycarbonyl)-*N*-(1-(thieno[2,3-*b*]pyrid-2-yl)ethyl)hydroxylamine (2.75 g, 6.97 mmol) in CH_2Cl_2 (3 mL) was added TFA (3 mL). The mixture was stirred for 0.5 h at room temperature and evaporated to dryness. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated to give 1.11 g (82%) of *N*-[1-(thieno[2,3-*b*]pyrid-2-yl)ethyl]hydroxylamine.

The hydroxylamine derivative was converted to **4j** using trimethylsilyl isocyanate (42%) as described in method 1a: mp 181−182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.52 (d, J = 7 Hz, 3H) 5.59 (q, J = 7 Hz, 1H), 6.51 (bs, 2H), 7.72 (d, J = 1 Hz, 1H), 7.48 (dd, J = 4 Hz, 1H), 8.17 (dd, J = 1.5 Hz, J = 7.5 Hz, 1H), 8.49 (dd, J = 1.5 Hz, J = 5 Hz, 1H), 9.31 (s, 1H); MS(DCI-NH_3) m/z 255 $(M + NH_4)^+$, 238 $(M + H)^+$. Anal. ($C_{10}H_{11}N_3O_2S$) C, H, N.

Method 7. Preparation of N-substituted *N*-hydroxyureas by Mitsunobu alkylation of *N*,*O*-bis(carbophenoxy)hydroxylamine and reaction with ammonia. This procedure was done as previously described.[22,23]

Biological Methods. Percent inhibition was computed by comparing individual values in treatment groups to the mean value of the control group. Statistical significance was determined using one way analysis of variance and Tukeys multiple comparison procedure. Linear regression was used to estimate IC_{50} and ED_{50} values.

Glucuronosyltransferase Activity in Monkey Liver Microsomes. Glucuronidation rates were obtained using microsomal pellets prepared from frozen cynomolgus monkey livers as previous described by Bell *et al.*[29] Data shown are from rates derived from duplicate incubations at the times noted.

Determination of Drug Plasma Concentrations. Compounds for oral administration were suspended in 0.2% HPMC with a Potter-Elvehjem homogenizer equipped with a Teflon-coated pestle and administered orally to cynomolgus monkeys and or rats as described by Bell *et al.*[29] Data presented are means from at least three animals.

Rat Basophil Leukemia (RBL) Cell Lysate 5-Lipoxygenase Inhibitor Potency. Adherent rat basophilic leukemia (RBL-1) cells (2H3 subline) lysate was centrifuged at 20000*g* for 20 min and the supernatant containing 5-LO activity stored frozen until used. Compounds were evaluated for 5-lipoxygenase inhibitory activity according to the method described by Carter *et al.*[11d] Data are from duplicate incubations.

Human Whole Blood Eicosanoid Formation. Aliquots of heparinized (20 USP units/mL) human blood (0.3 mL) were preincubated with drug or vehicle for 15 min at 37 °C, and ecosanoid biosynthesis was initiated by adding calcium ionophore A23187 according to the method described by Bell *et al.*[29] The amount of LTB_4 in aliquots of the extracts was analyzed by enzyme immunoassay (EIA). All results are means of at least duplicate and in most case triplicate determinations.

Rat Peritoneal Anaphylaxis Model. This *in vivo* leukotriene assay was conducted as described by Young *et al.* [25] Samples were analyzed for leukotrienes by enzyme immunoassay (EIA reagents for LTE_4, LTB_4 and thromboxane: Cayman Chemical CO., Ann Arbor, MI, and LTB_4 antibody from Advance Magnetics, Cambridge, MA, and LTE_4 tracer was prepared at Abbott).

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Supporting Information Available: Spectral data and experimental procedures for all compounds not reported in the general methods (39 pages). Ordering information is given on any current masthead page.

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