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(R)-(+)-N-[3-[5-[(4-Fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (ABT-761), a Second-Generation 5-Lipoxygenase Inhibitor

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Structure–activity optimization of inhibitory potency and duration of action of N-hydroxyurea containing 5-lipoxygenase inhibitors was conducted. The lipophilic heteroaryl template and the link group connnecting the template to the N-hydroxyurea pharmacophore were modified. Inhibition of 5-lipoxygenase was evaluated *in vitro* in a human whole blood assay. An *in vitro* assay using liver microsomes from monkey was used to evaluate congeners for comparative rates of glucuronidation. (3-Heteroaryl-1-methyl-2-propynyl)-N-hydroxyureas were found to be more resistant to *in vitro* glucuronidation. The promising inhibitor N-[3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-N-hydroxyurea (**6**) was found to have stereoselective glucuronidation in monkey and man. The R enantiomer **7** provided longer duration of inhibition as evaluated by an *ex vivo* whole blood assay. Further optimization of the lipophilic template led to the discovery of (R)-(+)-N-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (**11**) with more effective and prolonged inhibition of leukotriene biosynthesis than zileuton (**1**) and **7** in monkey and man. The optimized 5-lipoxygenase inhibitor **11** was selected for development as an investigational drug for leukotriene-mediated disorders.

Introduction

Several agents which block leukotriene (LT) biosynthesis or receptor activation have demonstrated efficacy in clinical trials in recent years.[1] The 5-lipoxygenase inhibitor zileuton[2] has demonstrated promising results in asthmatics.[3] Our objective was to invent a more potent and longer acting, orally administered 5-lipoxygenase (5-LO) inhibitor to enable clinical studies to determine the therapeutic outcome of more complete and sustained leukotriene inhibition. We describe in this report the discovery of (R)-(+)-N-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (**11**),[4] a 5-LO inhibitor with improved potency and duration of action.

The *in vitro* optimization of N-hydroxyurea 5-LO inhibitors was guided by two parallel structure–activity approaches. One study centered on optimization of inhibitor potency using a human whole blood assay which measured inhibition of calcium ionophore-stimulated LTB_4 biosynthesis. The second study involved extending the duration of action of these inhibitors by limiting glucuronidation of the N-hydroxyurea pharmacophore. The metabolism of zileuton in man involved predominantly glucuronidation of the N-hydroxyurea group and subsequent urinary excretion.[5] The oral half-life of zileuton in man was about 3 h. A similar but more rapid metabolism of zileuton ($t_{1/2}$ of 0.4 h) was observed in cynomolgus monkeys.[2d] An *in vitro* assay was developed to routinely measure the rate of glucuronidation of N-hydroxyurea inhibitors using microsomal preparations from monkey liver.[6] The predictive capability of *in vitro* glucuronidation rates was validated by pharmacokinetic evaluation of promising inhibitors in cynomolgus monkeys.

As a conceptual working hypothesis, the N-hydroxyurea function was conserved as the pharmacophore for inhibitory activity, and we defined two variable components, the *template* and the *link*, for structure–activity optimization (Table 1). The template was envisioned as the entity providing lipophilic binding interactions. The link group via proximity to the pharmacophore could modulate inhibition and metabolism phenomena. The link group also influenced the spatial orientation of the lipophilic template with respect to the pharmacophore and thus could have a dramatic effect on pharmacological properties.

Chemistry

The syntheses of the compounds reported are outlined as follows. Zileuton (**1**) and the congener **4** were prepared by the reduction of the corresponding oxime and treatment of the resulting hydroxylamine with trimethylsilyl isocyanate (Scheme 1). Compounds **2**, **5**, **6**, **9**, and **10** were prepared by an alternative general method which utilized N,O-bis(phenoxycarbonyl)hydroxylamine as the nucleophile in a Mitsunobu reaction for the conversion of a hydroxyl intermediate into the corresponding N-hydroxyurea (Scheme 2).[7] The requisite alkynyl hydroxy intermediates were prepared from the appropriate aldehydes by the Corey–Fuchs method[8] or reaction of the appropriate halide (Br or I) in a palladium-catalyzed Heck reaction.[9] The Z olefin **3** was prepared from the alkyne **6** by catalytic hydrogenation with Pd and $BaSO_4$ in ethanol/ethyl acetate/quinoline.

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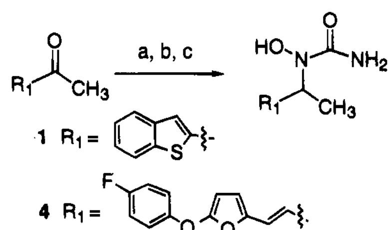
Scheme 1. Oxime Reduction Route to *N*-Hydroxyureas[a]



[a] Reagents: (a) $H_2NOH\cdot HCl$, ethanol/pyridine; (b) $BH_3\cdot$pyridine or $NaBH_3CN$, AcOH; (c) TMSNCO, THF.

The racemate **6** was resolved by acylation with the optically pure amino acid derivative *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine mediated by dicyclohexylcarbodiimide, separation of the diastereomers by column chromatography on silica gel, and then cleavage of the chiral auxilary with ammonia to provide the pure enantiomers **7** and **8**. The palladium-coupling method was also amenable to direct coupling of (*R*)- or (*S*)-butynyl-*N*-hydroxyurea to provide a direct asymmetric synthesis of enantiomers **11** and **12** (Scheme 3).[10]

Results and Discussion

Several templates were examined to replace the benzo[*b*]thienyl group of zileuton (**1**).[11] One interesting congener, **2**, with a (4-fluorophenoxy)fur-2-yl template exhibited about a 3-fold increase in inhibitory potency compared to zileuton. However, **2** showed disappointing results *in vivo* which was subsequently found to be due to rapid glucuronidation. Modifications which removed the hydroxyl site of glucuronidation in the hydroxyurea pharmacophore such as in the alkoxy [N(OR)$CONH_2$] or alkyl [N(R)$CONH_2$] urea analogs resulted in inactive compounds. The *N*-hydroxyurea or hydroxamate function had been established in previous studies by us and others to be important for 5-LO inhibitory activity.[12]

The discovery of a structural parameter that might reduce the substrate affinity to glucuronosyltransferases and yet maintain affinity for 5-LO was sought. Previous experience had shown that introducing increased lipophilicity into the link group often led to more rapid glucuronidation. The more potent inhibitor **2** was investigated further with alternative alkenyl or alkynyl link groups as in compounds **3–8**, which would have different constrained spatial orientations of the template and pharmacophore imposed by the respective unsaturated link group. For the series of compounds **2–8**, the rate of glucuronidation decreased over 100-fold for the following link groups: *cis*-1-methylpropenyl > 1-ethyl > *trans*-1-methylpropenyl > 1-methylpropynyl. These analogs were potent inhibitors of leukotrienes *in vitro* and were orally effective in a rat anaphylaxis model (Table 1).

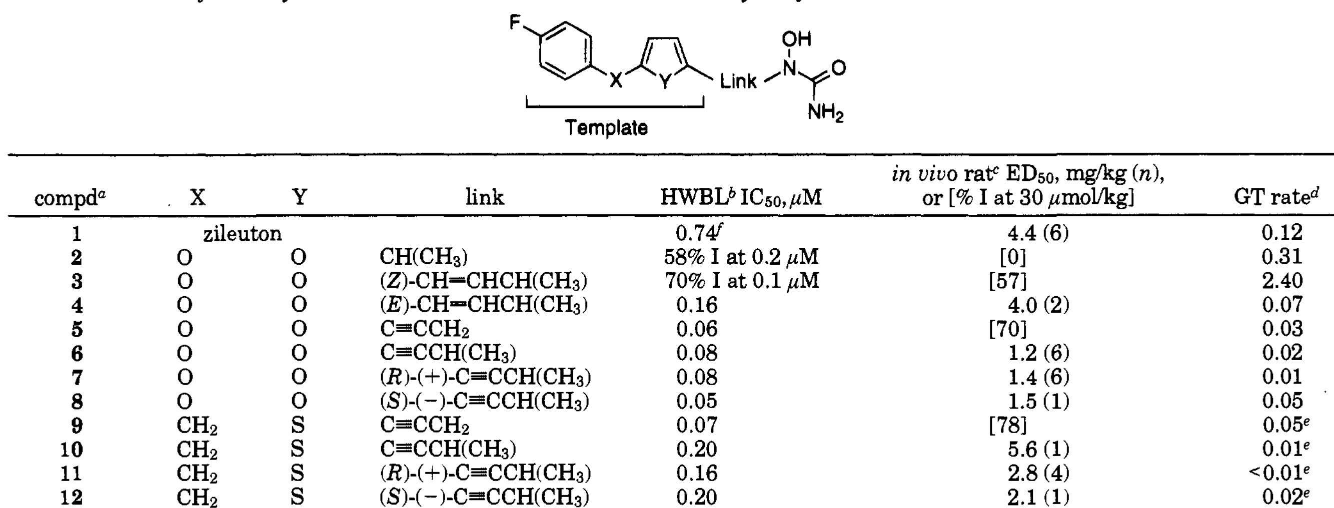
The 1-methylpropynyl link group as in **6** proved to be a promising structural modification providing potent inhibitory activity and a dramatically reduced *in vitro* glucuronidation rate in microsomes from monkey liver compared to zileuton (**1**). Resolution of the racemate **6** revealed minor differences in 5-LO inhibitory activity for the enantiomers (*R*)-(+)-**7** and (*S*)-(−)-**8** in the human whole blood assay, with IC_{50}s of 80 and 50 nM, respectively. However, a 5-fold difference in glucuronidation rate was observed in monkey microsomes with greater resistance to glucuronidation found for the (*R*)-(+)-enantiomer **7**. This stereoselective *in vitro* glucuronidation observed for these *N*-hydroxyureas correlated with the elimination half-lives determined from iv administration to monkeys as follows: 0.4 h for zileuton (**1**), 4.7 h for (±)-**6**, 9.0 h for (*R*)-(+)-**7**, and 1.8 h for (*S*)-(−)-**8**.[13] This approach to improve duration of action of *N*-hydroxyurea 5-LO inhibitors was validated by a phase I clinical study with racemate **6**. Healthly male volunteers were given a single oral dose of 400 mg of **6** (A-78773), and the time courses of plasma levels of **6** and its enantiomers **7** and **8** were analyzed by stereoselective HPLC methods.[14] The stereoselective preference for glucuronidation was confirmed in man with the *R* (+)-enantiomer **7** exhibiting an apparent elimination half-life of about 6 h compared to 2.2 h for the *S* (−)-enantiomer **8**. The *R* (+)-enantiomer **7** (A-79175) was thus selected for clinical development based on its extended duration and promising pharmacological properties.[15]

Discovery efforts continued to further optimize the *N*-hydroxyurea series. With the alkynyl link established as a breakthrough for reduced glucuronidation, we reexamined alternative templates. Of the many templates investigated, the [(4-fluorophenyl)methyl]-2-thienyl template as in **10** had comparable *in vitro* inhibitory activity and a similar glucuronidation rate to **7**. The [(4-fluorophenyl)methyl]-2-thienyl template was more amenable to large scale synthesis due to improved stability of synthetic intermediates compared to the acid labile (4-fluorophenoxy)fur-2-yl template. Examination of the enantiomers of inhibitor **10** peaked our interest further when a 500 μM solution of (*R*)-(+)-**11** showed undetectable *in vitro* glucuronidation with monkey microsomes and a marginally detectable rate (0.005) in human microsomes. Evaluation in the cynomolgus monkey revealed an exceptional elimination half-life of 16 h, one of the longest we have observed. A single 0.5 mg/kg oral dose of **11** in monkeys provided higher plasma levels (Figure 1) and greater sustained inhibition of *ex vivo* stimulated LTB_4 formation (Figure 2) than any other *N*-hydroxyurea 5-LO inhibitor we had studied.

Characterization of **11** in several 5-LO inhibition assay systems was conducted. Like zileuton (**1**) and **7**, this new congener was a direct inhibitor of 5-LO as demonstrated by potent inhibition of 5-HPETE formation in a crude enzyme preparation from sonicated rat basophil leukemia cells with an IC_{50} of 23 nM at an arachidonate concentration of 6 μM. Potency in this assay was dependent on the arachidonate concentration, for example, at 65 μM the IC_{50} was 151 nM. These results implied that **11** was a direct competitive inhibitor of arachidonate oxidation. Inhibition of LTB_4 formation in calcium ionophore-stimulated purified human neutrophils from four donors gave a mean IC_{50} of 23 nM. In the more complex medium of human whole blood, **11** was a selective inhibitor of calcium ionophore-stimulated LTB_4 formation with an IC_{50} of 150 nM (95%, cl 100–200). In the same assay an IC_{50} of 11 μM (95%, cl 8–12) was measured for 12-HETE, 50% inhibition of 15-HETE was observed at the highest dose tested of 100 μM, and an IC_{50} of 50 μM (95%, cl 37–78) was found for the formation of TXB_2.

Pharmacologic evaluation of **11** was conducted in a rat anaphylaxis model that produced leukotrienes in the peritoneal cavity in response to antigen.[16] Inhibition

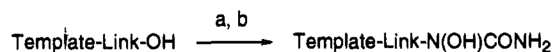
Table 1. Inhibitory Activity and Glucuronidation Rates for Selected *N*-Hydroxyureas



compd[a]	X	Y	link	HWBL[b] IC_{50}, μM	*in vivo* rat[c] ED_{50}, mg/kg (*n*), or [% I at 30 μmol/kg]	GT rate[d]
1	zileuton			0.74[f]	4.4 (6)	0.12
2	O	O	$CH(CH_3)$	58% I at 0.2 μM	[0]	0.31
3	O	O	(*Z*)-$CH{=}CHCH(CH_3)$	70% I at 0.1 μM	[57]	2.40
4	O	O	(*E*)-$CH{=}CHCH(CH_3)$	0.16	4.0 (2)	0.07
5	O	O	$C{\equiv}CCH_2$	0.06	[70]	0.03
6	O	O	$C{\equiv}CCH(CH_3)$	0.08	1.2 (6)	0.02
7	O	O	(*R*)-(+)-$C{\equiv}CCH(CH_3)$	0.08	1.4 (6)	0.01
8	O	O	(*S*)-(−)-$C{\equiv}CCH(CH_3)$	0.05	1.5 (1)	0.05
9	CH_2	S	$C{\equiv}CCH_2$	0.07	[78]	0.05[e]
10	CH_2	S	$C{\equiv}CCH(CH_3)$	0.20	5.6 (1)	0.01[e]
11	CH_2	S	(*R*)-(+)-$C{\equiv}CCH(CH_3)$	0.16	2.8 (4)	<0.01[e]
12	CH_2	S	(*S*)-(−)-$C{\equiv}CCH(CH_3)$	0.20	2.1 (1)	0.02[e]

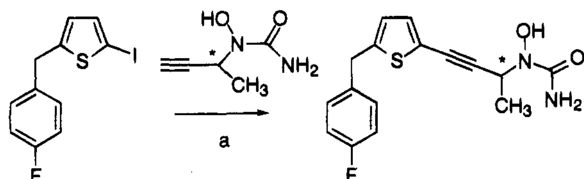
[a] All compounds had C, H, N analyses ±0.4% of the theoretical. [b] Human whole blood stimulated with calcium ionophore (A23187) and LTB_4 was measured by enzyme immunoassay. The 95% confidence limits (cl) were ± <50% of the mean value. [c] Rat anaphylaxis leukotriene formation: an oral dose of 30 μmol/kg 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 in 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 of protein with an initial compound concentration of 100 μM. [e] The compound concentration was increased to 500 μM to facilitate the detection of a glucuronidation rate. [f] This value is the mean from 10 donors.

Scheme 2. Modified Mitsunobu Route to *N*-Hydroxyureas[a]



[a] Reagents: (a) *N*,*O*-bis(phenoxycarbonyl)hydroxylamine,[7] diisopropyl azodicarboxylate, $(C_6H_5)_3P$, THF; (b) NH_3.

Scheme 3. Palladium-Catalyzed Coupling of (*R*)- and (*S*)-Butynyl-*N*-hydroxyurea[a]



[a] Reagents: (a) $[MeCN]_2Pd(Cl)_2$, $(Ph)_3P$, CuI, diethylamine.

with **11** was optimal with a 3 h oral pretreatment prior to antigen providing an ED_{50} of 1.4 mg/kg (95%, cl 0.7−3.5) for LTE_4 inhibition and an ED_{50} of 0.6 mg/kg (95%, cl 0.1−1.2) for inhibition of LTB_4 (the results in Table 1 are reported with the standard assay procedure with a 1 h oral inhibitor pretreatment time). The oral bioavailability of **11** was estimated from oral and iv studies for several species and found to be 60% in rat, 100% in mouse, 90% in guinea pig, 95% in dog, and 90% in cynomolgus monkey. The elimination half-life of **11** given 4 mg/kg iv was 3.6 h in rat, 4.2 h in mice, and 7.6 h in guinea pig. A longer elimination half-life of 10 h in dog and 16 h in monkey was observed for **11** given 0.5 mg/kg iv.

The promising advantages offered by (*R*)-(+)-*N*-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**11**, ABT-761) prompted its clinical development. Phase I studies confirmed excellent oral bioavailability and an extended duration of plasma levels in man as predicted from our preclinical results. Given as a single 200 mg dose, this 5-LO inhibitor had an oral plasma half-life of about 15 h, provided >95% inhibition of *ex vivo* stimulated LTB_4 formation in blood samples taken at intervals up to 18 h postdose, and maintained a plasma drug concentration of >1 μg/mL for up to 24 h postdose.[17]

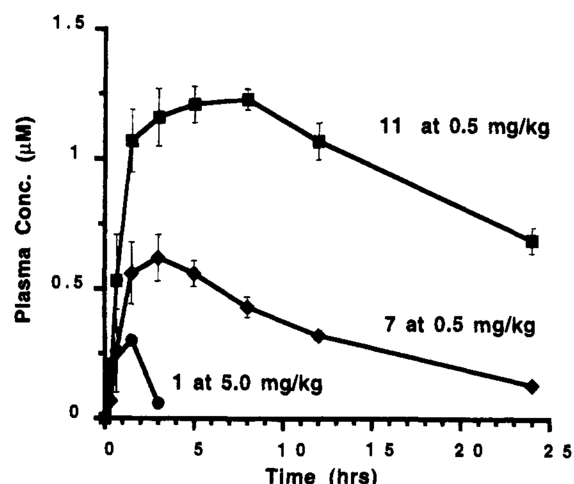


Figure 1. Plasma levels of 1, **7**, and 11 after oral dosing in cynomolgus monkeys. Monkeys were dosed by oral gavage with 1 at a 5 mg/kg dose and at 0.5 mg/kg for both **7** and 11. Plasma concentrations were determined by HPLC. Data presented are the average from two monkeys for 1 and the mean value of three monkeys for **7** and 11. Error bars are SEM.

Conclusions

The strategy of improving duration of *N*-hydroxyurea inhibitors by structural modifications of the link group led to the identification of spatial and stereochemical parameters that limited glucuronidation. The alkynyl link group served as a structural feature offering resistance to glucuronidative metabolism of the *N*-hydroxyurea pharmacophore. Furthermore, stereoselective glucuronidation for this subgroup of *N*-hydroxyurea inhibitors was demonstrated *in vitro*, and these results were predictive of the pharmacokinetics observed in both monkey and man. Persistent optimization of direct 5-LO inhibitors has culminated in the

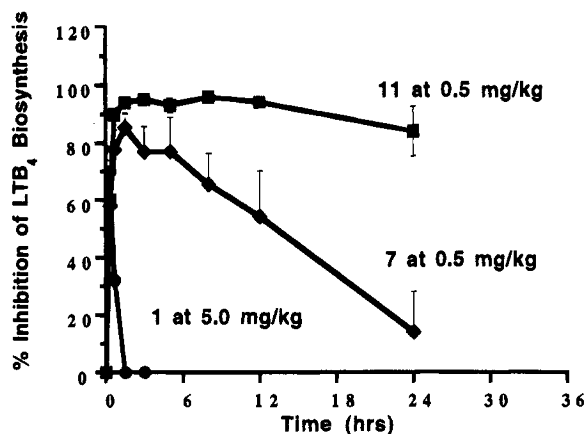


Figure 2. Inhibition of *ex vivo* LTB_4 formation by 1, **7**, and 11 after oral dosing in cynomolgus monkeys. Monkeys were dosed by oral gavage with 1 at a 5 mg/kg dose and at 0.5 mg/kg for both **7** and 11. Blood samples taken at indicated times were stimulated to make LTB_4 with calcium ionophore (A23187). LTB_4 was measured by enzyme immunoassay. Data presented are the average from two monkeys for 1 and the mean value of three monkeys for **7** and 11. Error bars are SEM.

discovery of **11**, a potent, orally administered, selective 5-LO inhibitor with extended duration of action, as an investigational agent for therapeutic studies of leukotriene-mediated disorders. These studies will hopefully further clarify fundamental issues regarding the degree of therapeutic benefit that more effective and sustained inhibition of 5-LO might provide. Clinical trials in asthmatics have commenced.[18]

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 Kratos MS-50 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 prepartion. Analytical TLC using E. Merck F254 commercial plates was used to follow the course of reactions.

Zileuton, *N*-(1-Benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea (1). To a stirred solution of 2-acetylbenzo[*b*]thiophene (14.4 g, 81.8 mmol) in ethanol/pyridine (1:1, 250 mL) was added hydroxylamine hydrochloride (14.4 g, 207 mmol). The mixture was stirred overnight at room temperature. Most of the ethanol was removed *in vacuo*, and the pyridine solution was poured into water. The precipitated solid was collected by filtration, washed with water, and dried to give 15 g (96%) of the desired oxime as a mixture of *E* and *Z* isomers that was used without further purification.

A stirred solution of the oxime mixture (10 g, 55 mmol) in ethanol (250 mL) was cooled using an ice bath, and borane: pyridine (17.1 g, 184 mmol) was added followed by the dropwise addition of 6 N HCl (50 mL). The mixture was stirred for 4 h at room temperature, concentrated *in vacuo*, diluted with water, and adjusted to pH 8 with 6 N NaOH. The resulting solid was collected and washed with water. The wet solid was dissolved in hot ethanol and allowed to cool and the product precipitated by the addition of water. The solid was collected, dried, and then purified by chromatography (silica gel, 1:1 ether/hexanes) to give 4.6 g (43%) of the desired hydroxylamine and 3 g of recovered starting material.

The hydroxylamine (0.5 g, 2.5 mmol) was dissolved in dioxane (10 mL), and a THF (3.0 mL) solution of trimethylsilyl isocyanate (0.36 g, 3.1 mmol) was added. The mixture was heated to 90 °C for 0.5 h; the solution was cooled, poured into cold saturated aqueous NH_4Cl, and thoroughly extracted with ethyl acetate (3 × 50 mL) The combined organic layers were washed with brine and dried ($MgSO_4$). Evaporation of the solvent gave a white solid that was collected and washed with hot ether to afford 0.35 g (59%) of 1 as a white solid: mp 158–160 °C (EtOAc/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.51 (d, J = 7 Hz, 3H), 5.57 (q, J = 7 Hz, 1H), 6.44 (bs, 2H), 7.24–7.37 (m, 3H), 7.76 (m, 1H), 7.89 (m, 1H), 9.23 (s, 1H); IR (KBr) 3460, 3180, 1655, 1520, 1470 cm^{-1}; MS (DCI-NH_3) *m/e* 254 (M + NH_4)$^+$, 237 (M + H)$^+$, 219, 161. Anal. ($C_{11}H_{12}N_2O_2S$) C, H, N.

***N*-[1-[5-(4-Fluorophenoxy)fur-2-yl]ethyl]-*N*-hydroxyurea (2).** To a stirred 0 °C solution of 5-(4-fluorophenoxy)-2-furaldehyde (2.06 g, 10.0 mmol) in diethyl ether (75 mL) was added an ether solution of methylmagnesium bromide (6.66 mL, 3.0 M, 20.0 mmol). The reaction mixture was stirred for 1 h at room temperature and poured into saturated aqueous NH_4Cl. The ether layer was washed with brine, dried ($MgSO_4$), and concentrated to give 2.15 g (98%) of the corresponding alcohol as a brown oil that was used without further purification.

To a stirred 0 °C solution of alcohol (2.1 g, 9.0 mmol), *N,O*-bis(phenoxycarbonyl)-hydroxylamine (2.74 g, 10.0 mmol), and triphenylphosphine (2.62 g, 10.0 mmol) in THF (100 mL) was added dropwise a solution of diisopropyl azodicarboxylate (2.02 g, 10.0 mmol) in THF (25 mL). The mixture was stirred for 0.5 h at room temperature and concentrated *in vacuo*. The crude reaction mixture was filtered through a pad of silica gel and washed with 10% ethyl acetate/hexanes, and the combined organic solution was concentrated *in vacuo*. The resulting residue was purified by chromatography (silica gel, 10% ethyl acetate/hexanes) to afford the desired bis(phenoxycarbonyl)-hydroxylamine intermediate as a yellow oil that was used without further purification.

The bis(phenoxycarbonyl)hydroxylamine intermediate was dissolved in 2:1 methanol/concentrated NH_4OH (100 mL). The mixture was stirred overnight, and most of the methanol was removed *in vacuo*. The residue was diluted with water and the solid collected by filtration. The wet solid was dried and then purified by chromatography (silica gel, 4% CH_3OH/CH_2Cl_2) to afford 0.65 g (26%) of the desired *N*-hydroxyurea as a white solid. This solid was recrystallized from ethyl acetate/hexanes to give 0.53 g of white crystalline **2**: mp 132 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, J = 7 Hz, 3H), 5.23 (q, J = 7 Hz, 1H), 5.66 (d, J = 3 Hz, 1H), 6.23 (m, 1H), 6.41 (m, 2H), 7.10 (m, 2H), 7.23 (m, 2H), 9.06 (s, 1H); MS (DCI-NH_3) *m/e* 298 (M + NH_4)$^+$, 281 (M + H)$^+$. Anal. ($C_{13}H_{13}FN_2O_4$) C, H, N.

(*Z*)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propenyl]-*N*-hydroxyurea (3). To a stirred solution of quinoline (1.02 mL) and 5% Pd/$BaSO_4$ (0.102 g) in a mixture of ethanol (17 mL) and ethyl acetate (5 mL) was added *N*-[3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**6**) (0.51 g, 1.7 mmol). The mixture was stirred under 1 atm of hydrogen for 0.5 h. The reaction mixture was filtered and concentrated *in vacuo*, and the residue was purified by chromatography (silica gel, 2% methanol in ether) to give 0.13 g (25%) of **3** as a clear oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (d, J = 6.5 Hz, 3H), 5.26 (m, 1H), 5.63 (m, 1H), 5.75 (d, J = 3.5 Hz, 1H), 6.09 (m, 1H), 6.34 (bs, 2H), 6.48 (d, J = 3.5 Hz, 1H), 7.23 (m, 4H), 9.12 (s, 1H); MS (DCI-NH_3) *m/e* 324 (M + NH_4)$^+$, 307 (M + H)$^+$.

(*E*)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propenyl]-*N*-hydroxyurea (4). To a stirred suspension of 5-(4-fluorophenoxy)-2-furaldehyde (5 g, 24.3 mmol) and K_2CO_3 (6.72 g, 48.6 mmol) in THF was added dimethyl (2-oxopropyl)-phosphonate (4.03 g, 24.3 mmol). The mixture was heated to reflux, stirred for 17 h, cooled, and filtered, and the solids were washed with ether. The combined filtrate was concentrated

in vacuo and purified by chromatography (silica gel, 33% ether in hexanes) to afford 4.9 g (83%) of 1-[3-(4-fluorophenoxy)-2-furyl]buten-3-one.

To a stirred 1:1 ethanol/pyridine solution (40 mL) of the ketone (4.9 g, 20.3 mmol) was added hydroxylamine hydrochloride (5.0 g, 72.5 mmol). The mixture was stirred for 1 h at room temperature and concentrated *in vacuo*. The residue was partitioned between ether (300 mL) and 2N HCl (150 mL). The ether layer was washed with additional 2 N HCl (2 × 150 mL), H_2O, and brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford 5.5 g of a mixture of oxime isomers as a thick yellow oil that was used without further purification.

To the oximes (5.5 g, 21.1 mmol) dissolved in 1:1 THF/acetic acid (50 mL) was added $NaBH_3CN$ (2.0 g, 31.6 mmol) in several small portions. The mixture was stirred for 2 h at ambient temperature, and an additional portion of $NaBH_3CN$ (1.0 g, 15.8 mmol) was added. The mixture was stirred for an additional 2 h. The mixture was concentrated *in vacuo* and the residue dissolved in ethyl acetate, washed with saturated aqueous $NaHCO_3$, water, and brine, and dried ($MgSO_4$) to provide crude [3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propenyl]hydroxylamine which was used without further purification.

To a stirred solution of this hydroxylamine in THF (50 mL) was added trimethylsilyl isocyanate (3.64 g, 31.6 mmol). The mixture was stirred for 2 h at room temperature and then concentrated *in vacuo*. The residue was purified by chromatography (silica gel, 5% $MeOH/CH_2Cl_2$) to afford 1.5 g (24% over three steps) of an off-white solid. Recrystallization from ethyl acetate/hexanes afforded **4**: mp 97–99 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, J = 7 Hz, 3H), 4.75 (m, 1H), 5.72 (d, J = 3 Hz, 1H), 5.59 (dd, J = 16.5, 6 Hz, 1H), 6.24 (d, J = 16.5 Hz, 1H), 6.35 (m, 3H), 7.12–7.31 (m, 4H), 9.03 (s, 1H); MS (DCI-NH_3) m/e 324 $(M + NH_4)^+$, 307 $(M + H)^+$, 231. Anal. ($C_{15}H_{15}FN_2O_4$) C, H, N.

***N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-2-propynyl]-*N*-hydroxyurea (5).** This compound was prepared in a similar manner as compound **6** using paraformaldehyde instead of acetaldehyde in 25% overall yield: mp 149–151 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 4.34 (s, 2H), 5.26 (d, J = 3 Hz, 1H), 6.58 (bs, 2H), 6.78 (d, J = 3 Hz, 1H), 7.14–7.32 (m, 4H), 9.62 (s, 1H); MS (DCI-NH_3) m/e 291 $(M + H)^+$, 248. Anal. ($C_{14}H_{11}FN_2O_4$) C, H, N.

***N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (6).** To a stirred suspension of pentane-washed 80% sodium hydride (5.3 g, 177 mmol) in THF (200 mL) under argon was added 4-fluorophenol (19.9 g, 177 mmol) in small portions as a solid. After gas evolution had ended, the THF was removed *in vacuo*. The crude phenoxide residue was redissolved in DMF (200 mL) and cooled to ~0 °C; 5-nitro-2-furaldehyde (25 g, 177 mmol) in DMF (50 mL) was added dropwise. During the addition the reaction mixture became very thick, requiring the addition of additional DMF (150 mL). After the addition the mixture was stirred for 0.5 h, poured into ice water, and extracted with ether (8 × 200 mL); the ether layers were combined, washed with 10% NaOH (3 × 100 mL) and water (3 × 100 mL), and dried ($MgSO_4$). The ether was evaporated *in vacuo*, and the resulting residue was dissolved in ethyl acetate, treated with decolorizing carbon, filtered, and concentrated *in vacuo*. The solid obtained was recrystallized from ether/hexane to afford 25 g (68%) of 5-(4-fluorophenoxy)-2-furaldehyde as a light yellow solid.

Carbon tetrabromide (100.73 g, 303 mmol), zinc dust (19.84 g, 303 mmol), and triphenylphosphine (79.56 g, 303 mmol) were combined in CH_2Cl_2 (700 mL) and stirred overnight under an argon atmosphere. A solution of 5-(4-fluorophenoxy)-2-furaldehyde (25 g, 121 mmol) in CH_2Cl_2 (100 mL) was added to the resulting suspension and stirred for 2 h at room temperature. Pentane (1600 mL) was added to the mixture, and the mixture was stirred for 1 h and then decanted from the solids. The pentane/CH_2Cl_2 solution was flitered through a short column of silica gel topped with Celite. The filtrate was concentrated to afford 43 g (98%) of 2-[5-(4-fluorophenoxy)-2-furyl]-1,1-dibromoethene as a yellow oil.

To a stirred −78 °C solution of 2-[5-(4-fluorophenoxy)-2-furyl]-1,1-dibromoethene (23.1 g, 63.81 mmol) in THF (200 mL) under argon was added n-butyllithium (51.0 mL, 127.62 mmol, 2.5 M in hexanes). The reaction mixture was stirred for 0.5 h at −78 °C and then saturated aqueous NH_4Cl was added to the cold mixture, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature. The mixture was concentrated *in vacuo* and then extracted with ether, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by chromatography (silica gel, hexanes) to give 6.5 g (50%) of 5-(4-fluorophenoxy)-2-furylethyne.

Lithium diisopropylamide was formed *in situ* by the addition of n-butyllithium (14.2 mL, 35.4 mmol, 2.5 M in hexanes) to a stirred −78 °C THF (100 mL) solution of diisopropylamine (3.58 g, 35.4 mmol). The solution was warmed to −5 °C and stirred for 0.5 h, and then 5-(4-fluorophenoxy)-2-furylethyne (6.5 g, 32.2 mmol) was added dropwise as a THF solution via a syringe. After stirring for 0.5 h, acetaldehyde (3.11 g, 70.8 mmol) was added via syringe, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature. Water was added and the THF removed *in vacuo*. The resulting mixture was extracted with ethyl acetate, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed (silica gel, 30% ether in hexanes) to give 0.45 g (10%) of recovered starting material and 6.39 g (73%) of 4-[5-(4-fluorophenoxy)-2-furyl]-3-butyn-2-ol as a slightly yellow solid: mp 56–57.5 °C (ether/hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 1.54 (d, J = 7 Hz, 3H), 1.88 (d, J = 6 Hz, 1H), 4.75 (m, 1H), 5.47 (d, J = 4 Hz, 1H), 6.53 (d, J = 4 Hz, 1H), 7.04 (m, 4H); MS (DCI-NH_3) m/e 247 (M + 1), 229.

To a 0 °C stirred THF solution (100 mL) of 4-[5-(4-fluorophenoxy)-2-furyl]-3-butyn-2-ol (6.39 g, 25.98 mmol), *N,O*-bis(phenoxycarbonyl)hydroxylamine (7.80 g, 28.6 mmol), and triphenylphosphine (8.17 g, 31.2 mmol) was added dropwise a THF solution (25 mL) of diisopropyl azodicarboxylate (6.30 g, 31.2 mmol). The mixture was stirred for 0.5 h at room temperature, concentrated *in vacuo*, and chromatographed (silica gel, 50% CH_2Cl_2 in hexanes) to afford 7.7 g (53%) of *N,O*-bis(phenoxycarbonyl)-*N*-[4-[5-(4-fluorophenoxy)-2-furyl]-3-butyn-2-yl]hydroxylamine.

Cautionary note: This procedure involves a closed glass apparatus under pressure. A protective shield should be used due to possibility of excess pressure shattering the closed glass reaction vessel, and the procedure should be conducted in a laboratory fume hood.

In a screw top vessel with a Teflon O-ring was placed the *N,O*-bis(carbophenoxy)-*N*-[4-[5-(4-fluorophenoxy)-2-furyl]-3-butyn-2-yl]hydroxylamine (7.50 g, 14.97 mmol) obtained above. Liquid ammonia (~10–15 mL) was condensed using a cold finger (dry ice/acetone) into the cooled −78 °C reaction vessel. The vessel was closed with the screw cap, the ice bath removed, and the reaction mixture allowed to stand overnight at room temperature under internal pressure with ammonia gas. The vessel was then recooled to −78 °C to condense unreacted ammonia gas and then opened with caution behind a protective shield. The reaction mixture was allowed to come to room temperature to allow the excess ammonia to evaporate. The crude residue was dissolved in ~15% $MeOH/CH_2Cl_2$ and filtered through silica gel. The crude solid product was triturated with 1:1 ether/hexane (2×) to remove the byproduct phenol. Recrystallization from ethyl acetate/hexanes gave 2.3 g of **6** as an off-white solid. The mother liquor and the ether/hexane washes from the phenol trituration were combined, concentrated, and chromatographed (silica gel, 5% $MeOH/CH_2Cl_2$) to give an additional 0.3 g of **6** for a total yield of 2.6 g (57%): mp 148–150 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.34 (d, J = 7 Hz, 3H), 5.13 (q, J = 7 Hz, 1H), 5.76 (d, J = 3 Hz, 1H), 6.56 (bs, 2H), 6.74 (d, J = 3 Hz, 1H), 7.13–7.30 (m, 4H), 9.37 (s,1H); MS (DCI-NH_3) m/e 305 $(M + H)^+$, 289, 229. Anal. ($C_{15}H_{13}FN_2O_4$) C, H, N.

Resolution of *N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (6). The racemate *N*-[3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**6**) was resolved by derivatization with *N*-Fmoc-L-phenylalanine, mediated by dicyclohexylcarbodiimide, to provide diastereomeric adducts that could be separated by silica gel column chromatography. The diastereomers were then treated independently with ammonia to cleave the amino acid

and provide (*R*)-(+)-*N*-[3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**7**) and (*S*)-(−)-*N*-[3-[5-(4-fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**8**). Correlation through asymmetric synthesis established the (+)-isomer to have the R absolute configuration.

Diastereomeric Fmoc-L-phenylalanine Adducts of 6. To a stirred CH_2Cl_2 solution (100 mL) of **6** (2.0 g, 6.58 mmol) were added *N*-Fmoc-L-phenylalanine (2.98 g, 7.69 mmol) and DCC (1.56 g, 7.57 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated and the residue dissolved in ether, filtered through silica gel, and concentrated. The resulting residue was purified by chromatography (silica gel, 25% EtOAc/toluene). Concentration of appropriate fractions gave 1.46 g of the less polar diastereomer as a white foam and 2.12 g of the more polar diastereomer contaminated by some of the first eluting diastereomer. Rechromatography of the second diastereomer gave 1.29 g of the pure more polar diastereomer as a white foam.

(*R*)-(+)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (7). The less polar diastereomer (1.62 g, 2.4 mmol) obtained above was dissolved in methanol saturated with anhydrous ammonia. The reaction mixture was stirred for 0.25 h at room temperature and concentrated *in vacuo*. The residue was chromatographed (silica gel, 5% $MeOH/CH_2Cl_2$) and recrystallized from ethyl acetate/hexanes to give 0.72 g (97%) of **7** as white crystals: mp 148 °C dec; $[\alpha]^{25}_D = +51.5°$ ($c = 0.59$, MeOH); identical ^1H NMR as for **6**. Anal. ($C_{15}H_{13}FN_2O_4$) C, H, N.

(*S*)-(−)-*N*-[3-[5-(4-Fluorophenoxy)-2-furyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (8). In an identical manner as described above, the more polar diastereomer (1.29 g, 1.92 mmol) afforded 0.52 g (89%) of **8** as white crystals: mp 147 °C dec; $[\alpha]^{25}_D = -47.5°$ ($c = 0.36$, MeOH); identical ^1H NMR data as for **6**. Anal. ($C_{15}H_{13}FN_2O_4$) C, H, N.

Determination of Absolute Configuration of 7. Sharpless epoxidation of crotyl alcohol using the natural diisopropyl L-tartrate gave the known (2*S*,3*S*)-2,3-epoxybutyl alcohol.[19] Tosylation of this material followed by elimination with *n*-butyllithium provided (*S*)-3-butyn-2-ol.[20] The Mitsunobu reaction is known to proceed with inversion;[21] therefore, reaction of *N*,*O*-bis(phenoxycarbonyl)hydroxylamine with the (*S*)-butynol under Mitsunobu conditions and treatment with ammonia gave (*R*)-(+)-(1-methyl-2-propynyl)-*N*-hydroxyurea ($[\alpha]^{25}_D = +52.0°$). Coupling of this chiral acetylene with 2-iodo-5-(4-fluorophenoxy)furan using the conditions described for compound **9** provided material identical with **7** in optical rotation, establishing the *R* absolute stereochemistry. (*S*)-(−)-(1-Methyl-2-propynyl)-*N*-hydroxyurea ($[\alpha]^{25}_D = -51.62°$) was obtained by using the unnatural diisopropyl D-tartrate.

***N*-[3-[5-[(4-Fluorophenyl)methyl]thien-2-yl]-2-propynyl]-*N*-hydroxyurea (9).** This compound was prepared in a similar manner as compound **10** using propargyl alcohol instead of 3-butyn-2-ol: mp 145−146 °C dec; ^1H NMR (DMSO-d_6) δ 4.12 (s, 2H), 4.31 (s, 2H), 6.53 (s, 2H), 6.82 (d, J = 4 Hz, 1H), 7.11 (d, J = 4 Hz, 1H), 7.14 (m, 2H), 7.29 (m, 2H), 9.58 (s, 1H); MS (DCI-NH_3) m/e 322 $(M + NH_4)^+$, 305 $(M + H)^+$, 244. Anal. ($C_{15}H_{13}FN_2O_2S$) C, H, N.

***N*-[3-[5-[(4-Fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (10).** To a 0 °C solution of thiophene (12.6 g, 0.15 mol) dissolved in a mixture of ether (230 mL) and THF (70 mL) was added dropwise *n*-butyllithium (2.5 M solution in hexanes, 54.0 mL, 0.134 mol). The mixture was stirred at 0 °C for 1.5 h and then transferred by cannula into a −78 °C solution of 4-fluorobenzyl bromide (23.6 g, 0.125 mol) containing tetrakis(triphenylphosphine)palladium(0) (1.25 g) in THF (200 mL). The reaction mixture was stirred at room temperature under nitrogen overnight, diluted with saturated aqueous NH_4Cl (100 mL), extracted with ether, dried ($MgSO_4$), concentrated *in vacuo*, and purified by vacuum distillation to give 19.4 g (81%) of 2-[(4-fluorophenyl)methyl]thiophene, bp 74−83 °C (0.6−0.7 mmHg).

To a stirred solution of 2-[(4-fluorophenyl)methyl]thiophene (9.61 g, 50.0 mmol) in chloroform (50 mL) and acetic acid (50 mL) was added *N*-bromosuccinimide (8.90 g, 50.0 mmol). The reaction mixture was stirred at room temperature for 0.5 h and poured into 100 mL of water. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford 13.3 g (98%) of 2-bromo-[5-(4-fluorophenyl)methyl]thiophene as a yellow oil.

To a stirred solution of 2-bromo-5-[(4-fluorophenyl)methyl]-thiophene (13.30 g, 49 mmol) in piperidine (65 mL) was added 3-butyn-2-ol (4.2 g, 60 mmol), triphenylphosphine (0.180 g, 0.44 mmol), tetrakis(triphenylphosphine)palladium(0) (180 mg, 0.15 mmol), and copper(I) iodide (115 mg, 0.6 mmol). The mixture was heated to reflux under nitrogen for 1.5 h, allowed to cool to room temperature, and diluted with saturated aqueous NH_4Cl (50 mL), concentrated NH_4OH (1 mL), and ethyl acetate. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by chromatography (silica gel, 2−7% MeOH in CH_2Cl_2). The resulting solid was recrystallized from ethyl acetate/hexane and triturated with CH_2Cl_2 to afford 9.16 g (71%) of 4-[5-[(4-fluorophenyl)methyl]-2-thienyl]-3-butyn-2-ol.

To a 0 °C stirred solution of the butyn-2-ol (9.16 g, 35.2 mmol), *N*,*O*-bis(phenoxycarbonyl)hydroxylamine (11.50 g, 42.2 mmol), and triphenylphosphine (12.0 g, 45.8 mmol) in THF (550 mL) was added a solution of diisopropyl azodicarboxylate (9.26 g, 45.8 mmol) in THF (250 mL) via a dropping funnel. The reaction mixture was stirred at room temperature overnight and concentrated *in vacuo*, and the residue was dissolved in ether (100 mL) diluted with pentane (50 mL) and placed in the freezer at −20 °C overnight. The organic solution was decanted, and the solids were washed with more cold ether. The combined ether extracts were evaporated *in vacuo*, and the residue was chromatographed (silica gel, 50% CH_2Cl_2/pentane and then 66% CH_2Cl_2/pentane) to afford 8.39 g (46%) of the protected hydroxylamine intermediate.

To a stirred solution of the bis(phenoxycarbonyl)hydroxylamine intermediate (4.46 g, 8.65 mmol) in methanol (150 mL) was added concentrated NH_4OH (50 mL). The flask was sealed with a rubber septum and stirred overnight at room temperature. The reaction mixture was concentrated and the residue purified by chromatography (silica gel, 7% $MeOH/CH_2Cl_2$) to give a solid that was triturated with CH_2Cl_2, collected, and washed with additional CH_2Cl_2 to afford 1.97 g (72%) of *N*-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**10**) as a white solid: mp 141−142 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, J = 6.0 Hz, 3H), 4.10 (s, 2H), 5.10 (q, J = 6.0 Hz, 1H), 6.50 (s, 2H), 6.80 (d, J = 3.0 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H), 7.13 (m, 2H), 7.28 (m, 2H), 9.30 (s, 1H); MS (DCI-NH_3) m/e 336 $(M + NH_4)^+$, 319 $(M + H)^+$. Anal. ($C_{16}H_{15}FN_2O_2S$) C, H, N.

(*R*)-(+)-*N*-[3-[5-[(4-Fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (11). A mixture of 2-[(4-fluorophenyl)methyl]thiophene (3.85 g, 20.0 mmol) and *N*-iodosuccinimide (4.50 g, 20.0 mmol) in 1:1 chloroform/acetic acid (40 mL) was stirred at ambient temperature for 1 h and then diluted with an equal volume of water. The organic layer was washed with saturated aqueous $NaHCO_3$ solution (2 × 50 mL), 10% sodium thiosulfate solution (2 × 50 mL), and brine, dried ($MgSO_4$), and concentrated *in vacuo* to give 6.07 g (95%) of 2-iodo-5-[(4-fluorophenyl)methyl]thiophene as a yellow oil.

To a solution of 2-iodo-5-[(4-fluorophenyl)methyl]thiophene (5.30 g, 16.6 mmol) in anhydrous DMF (5.0 mL) were added diethylamine (56 mL), (*R*)-(1-methyl-2-propynyl)-*N*-hydroxyurea (2.12 g, 16.6 mmol), triphenylphosphine (84 mg, 0.32 mmol), bis(acetonitrile)palladium(II) chloride (40 mg, 0.16 mmol), and copper(I) iodide (16 mg, 0.08 mmol). The mixture was stirred under nitrogen at ambient temperature for 22 h followed by concentration *in vacuo* at 32 °C. The residue was purified by chromatography (silica gel, 2−7% MeOH in CH_2Cl_2), crystallized from ethyl acetate/hexane, and triturated with CH_2Cl_2 to afford (*R*)-(+)-*N*-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-*N*-hydroxyurea (**11**) as a cream-colored solid (0.94 g, 18%): mp 135−136 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.32 (d, J = 6.0 Hz, 3H), 4.11 (s, 2H), 5.10 (q, J = 6.0 Hz, 1H), 6.54 (s, 2H), 6.81 (d, J = 3.0 Hz, 1H), 7.08 (d, J = 3.0 Hz, 1H), 7.10−7.18 (m, 2H), 7.25−7.32 (m, 2H),

9.33 (s, 1H); MS (DCI-NH_3) m/e 319 $(M + H)^+$; $[\alpha]^{23}_D = +47.8°$ (c = 1, MeOH). Anal. ($C_{16}H_{15}FN_2O_2S$) C, H, N.

(S)-(−)-N-[3-[5-[(4-Fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (12). The desired compound was prepared by the coupling method described for its enantiomer 11 using (S)-(−)-1-methyl-2-propynyl)-N-hydroxyurea to provide (S)-(−)-N-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (**12**) as a white solid: mp 134.5−137.5 °C dec; $[\alpha]^{23}_D = -51.4°$ (c = 1, MeOH). Anal. ($C_{16}H_{15}FN_2O_2S$) C, H, N.

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. Frozen livers, obtained from cynomolgus monkeys, were thawed on ice, minced, mixed with 3 vol of ice cold 0.15 M KCl, and homogenized. Particulate matter was removed by centrifugation at 10000g for 20 min. The resulting supernatant was then centrifuged at 100000g for 1 h at 4 °C. The microsomal pellet was resuspended in 4 vol of ice cold 0.15 M KCl and stored at −80 °C. Protein concentrations were measured using a BCA protein assay (Pierce Chemical, Rockford, IL).

Incubations of microsomes (1 mg/mL), in buffer containing 5 mM $MgCl_2$, 0.1 M Tris (pH 7.4), 0.1% Triton X-100, with test compounds soluable at 100 or 500 μM, and 3 mM uridine diphosphate glucuronic acid (UDPGA), were performed at 37 °C. Aliquots were removed from the incubations at time zero (upon addition of UDPGA) and at 5, 10, 20, 30, and 60 min, the reactions quenched with 2 vol of acetonitrile, and the aliquots centrifuged to remove precipitated protein. Samples were injected onto a Little Champ C18 column (Regis Chemical Co., Morton Grove, IL) with a mobile phase consisting of 20% acetonitrile, 20% THF (tetrahydrofuran), 5 mM tetrabutylammonium hydrogen sulfate, and 10 mM NaH_2PO_4 at pH 7.0. The flow rate was 0.5 mL/min, and eluting peaks were monitored at 260 nm. Studies were done in a 96-well microtiter format. Rates of glucuronidation were estimated from HPLC data using the linear portion of the time course normalized to protein concentration. 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. Blood samples were collected at various times following compound administration to cynomolgus monkeys. Blood samples were centrifuged, and the plasma was removed and stored frozen until assayed. Plasma samples were thawed, 2 vol of methanol was added, and precipitated plasma proteins were removed by centrifugation. Supernatants were injected directly onto a C18 reversed phase column (Adsorbosphere, HL 7 μm column) and chromatographed using a mobile phase composed of 55% acetonitrile containing 10 mM acetohydroxamic acid and 8 mM triethylamine acetate, pH 6.5, at a flow rate of 1 mL/min. Compound peaks were quantitated by UV absorbance at 260 nm using an external calibration curve. Data presented are means from at least three monkeys.

Monkey *ex Vivo* Leukotriene B_4 Biosynthesis. Compounds were suspended in 0.2% HPMC with a Potter-Elvehjem homogenizer equipped with a Teflon-coated pestle and administered orally to cynomolgus monkeys. Heparinized blood samples were obtained before and at various times after compound administration. Aliquots of blood were incubated at 37 °C with 50 μM calcium ionophore (A23187). After 30 min, the blood was placed in an ice bath and centrifuged at 400g for 20 min, and the plasma samples were analyzed for LTB_4. The level of LTB_4 in aliquots of the extracts was analyzed by enzyme immunoassay (EIA).

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

Human Neutrophil 5-LO Inhibition. Human neutrophils were evaluated for their ability to synthesize LTB_4 upon challenge with calcium ionophore (A23187) in the presense of test compounds according to the method described by Carter *et al.*[2d] All results are means of at least duplicate and in most cases triplicate determinations.

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 Carter *et al.*[2d] The amount of LTB_4 in aliquots of the extracts was analyzed by EIA. Similarly, cyclooxygenase activity was determined by analysis of plasma samples for thromboxane B_2 by EIA. 12- and 15-HETE were analyzed using commercially available radioimmunoassay (RIA) kits. All results are means of at least duplicate and in most cases triplicate determinations.

Rat Peritoneal Anaphylaxis Model. This *in vivo* leukotriene assay was conducted as described by Young *et al.*[16] Male Sprague−Dawley rats were passively sensitized by ip injection of rabbit antisera (5 mL) to bovine serum albumin (anti-BSA) in phosphate-buffered saline (PBS), pH 7.1. Three hours after this sensitization, the rats (8 animals/group) were injected ip with 4 mg of BSA (fraction V; ICN Immunobiologicals, Lisle, IL) in 5 mL of PBS. Test drug was administered by oral gavage 1 h prior to antigen challenge unless stated otherwise. The rats were euthanized with CO_2 15 min after challenge. The peritoneal cavity was opened, and the fluid contents were collected with a plastic trocar and disposable pipettes. The cavities were rinsed with 5 mL of cold PBS containing 0.1% gelatin, 0.1% sodium azide, and 10 mM disodium ethylenediaminetetraacetic acid (EDTA). The fluids were transferred to 20 mL of ice cold methanol, allowed to stand for 20 min, vortexed, and then centrifuged at 1000g for 15 min. Volumes were measured and the samples stored at −80 °C until analyzed for leukotrienes by EIA (EIA reagents for LTE_4, LTB_4, and thromboxane, Cayman Chemical Co., Ann Arbor, MI; LTB_4 antibody from Advance Magnetics, Cambridge, MA; LTE_4 tracer was prepared at Abbott).

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