Nonsteroidal Anti-Inflammatory Drugs as Scaffolds for the Design of 5-Lipoxygenase Inhibitors

Teodozyj Kolasa, Clint D. W. Brooks,* Karen E. Rodriques, James B. Summers, Joseph F. Dellaria, Keren I. Hulkower, Jennifer Bouska, Randy L. Bell, and George W. Carter

Abbott Laboratories, Immunoscience Research, D-47K, AP10, 100 Abbott Park Road, Abbott Park, Illinois 60064

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Representative nonsteroidal anti-inflammatory drug (NSAID) cyclooxygenase inhibitors such as ibuprofen, naproxen, and indomethacin were used as orally bioavailable scaffolds to design selective 5-lipoxygenase (5-LO) inhibitors. Replacement of the NSAID carboxylic acid group with a *N*-hydroxyurea group provided congeners with selective 5-LO inhibitory activity.

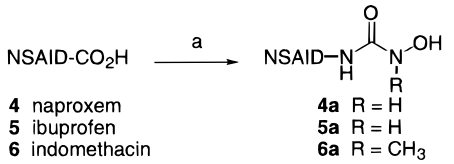
Introduction

Cyclooxygenase enzymes[1] (COX-1 and COX-2) and 5-lipoxygenase[2] (5-LO) are involved in the oxidative metabolism of arachidonic acid resulting in important biosynthetic products such as prostaglandins, prostacyclin, thromboxane, and leukotrienes.[3] Nonsteroidal anti-inflammatory drugs (NSAIDs) provide well-established anti-inflammatory therapy acting via inhibition of cyclooxygenases.[4] Recent studies have demonstrated therapeutic benefit for 5-LO inhibitors in asthma.[5] We explored the possibility of designing orally bioavailable 5-LO inhibitors using NSAIDs as a structural scaffold. Based on our previous studies of hydroxamate- and *N*-hydroxyurea-containing 5-LO inhibitors,[6] it seemed reasonable to suggest that replacing the carboxylate group of common NSAIDs with the *N*-hydroxyurea group might offer 5-LO inhibitors suitable for clinical development. Realization of the actual pharmacological properties of these analogs required experimental evaluation. This report describes the synthesis of orally active, selective, *N*-hydroxyurea-containing 5-LO inhibitors from NSAID scaffolds[7] and their properties as compared to the reference 5-LO inhibitor zileuton (**1**).[6b]

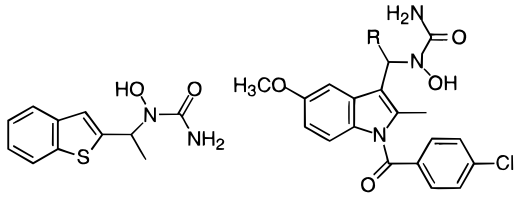
Studies by others[8] described the derivatization of the carboxylate function of representative NSAIDs as simple hydroxamates to provide dual inhibitors of both COX and 5-LO. However, these inhibitors are more appropriately classified as prodrugs due to the facile *in vivo* hydrolysis of the 5-LO-inhibiting hydroxamate back to the parent COX inhibitor carboxylate.[9] Two *N*-hydroxyurea analogs of indomethacin, **2a,b**, have been reported to have *in vitro* activity as 5-LO inhibitors, but no *in vivo* results were provided.[10] Another study converted the carboxylate funtion of representative fenamate NSAIDs into oxadiazole, thiadiazole, and triazole analogs resulting in compounds with dual 5-LO/COX inhibitory activity *in vitro* but lacking oral *in vivo* activity.[11]

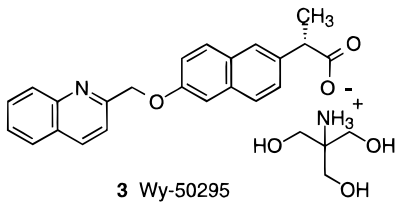
Inducing a change in inhibitory specificity from a cyclooxygenase inhibitor to a leukotriene biosynthesis inhibitor was demonstrated using naproxen as a scaffold.[12] Replacing the 6-methoxy group with the larger lipophilic quinolylmethoxy substituent resulted in **3**, which was first described as a 5-LO inhibitor.[13] Further studies revealed that the primary mechanism of leukotriene inhibitory activity of **3** was due to interfering with 5-LO-activating protein (FLAP) and not by direct inhibition of 5-LO catalysis.[14]

Scheme 1. Synthesis of "Terminal" *N*-Hydroxyurea NSAID Analogs **4a**, **5a**, and **6a**[a]



[a] Reagents: (a) DPPA, benzene, TEA, 90 °C, 1 h, then NH(R)O-H:HCl, TEA, H_2O, 90 °C, 18 h.





Our investigation, described herein, had the following objectives: replace the carboxylate function of representative NSAIDs with the *N*-hydroxyurea function and evaluate the pharmacological properties of these analogs for 5-LO inhibitory activity.

Chemistry

Methods to synthesize the *N*-hydroxyurea analogs (shown in Table 1) were devised utilizing the representative NSAIDs naproxen (**4**), ibuprofen (**5**), and indomethacin (**6**) as starting templates. The "terminal" regioisomeric *N*′-hydroxyurea congeners **4a**, **5a**, and **6a** were prepared as shown in Scheme 1. A Curtius rearrangement reaction of the NSAID with diphenyl phosphorazidate[15] (DPPA) provided the corresponding NSAID isocyanate intermediate which was reacted with

* Address correspondence to this author at Abbott Laboratories, Chemical Sciences, D-41K, R-13-4, 1401 N Sheridan Rd, North Chicago, IL 60064.

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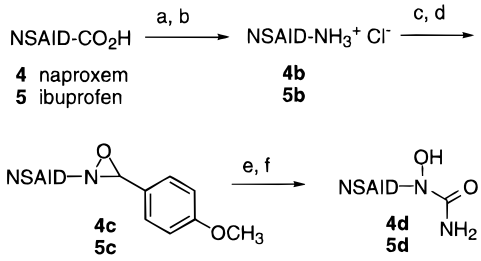
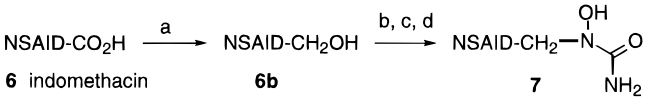
Scheme 2. Synthesis of "Internal" *N*-Hydroxyurea NSAID Analogs **4d** and **5d**[a]



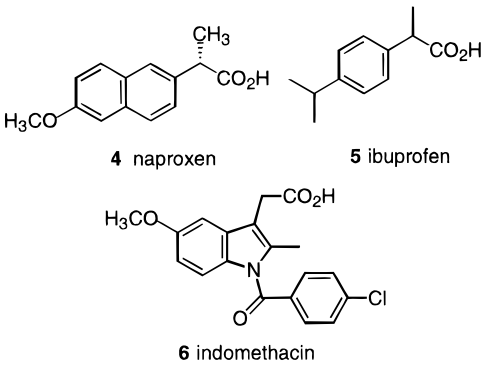
[a] Reagents: (a) DPPA, benzene, TEA, 90 °C, 1 h, then *tert*-butyl alcohol, 90 °C, 1 h, cool to rt, 10% aqueous HCl; (b) 4 N HCl, dioxane, rt, 1 h; (c) *p*-anisaldehyde, Na_2CO_3, methanol, rt, 18 h; (d) 3-chloroperbenzoic acid, dichloromethane, −20 °C → rt, 8 h; (e) NH_2OH:HCl, methanol, rt, 18 h; (f) TMS−N=C=O, THF, rt, 0.5 h.

Scheme 3. Synthesis of the Homologated "Internal" *N*-Hydroxyurea Indomethacin Analog **7**[a]



[a] Reagents: (a) BH_3:THF, THF, 5 °C, 18 h; (b) PPh_3, *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine, THF, diisopropyl azodicarboxylate; (c) trifluoroacetic acid, dichloromethane, 0 °C, 20 min; (d) TMS−N=C=O, THF, rt, 12 h.

the requisite hydroxylamine under standard conditions to afford the target compounds.



The "internal" regioisomeric *N*-hydroxyurea congeners **4d** and **5d**, with the hydroxy substituent on the same nitrogen atom as the NSAID template, were prepared as shown in Scheme 2. The Curtius reaction provided the corresponding NSAID amines **4b** and **5b** which were converted to the corresponding NSAID hydroxylamines **4c** and **5c** by the method of Grundke.[16] This method involved the formation of an imine with *p*-anisaldehyde followed by oxidation to an oxaziridine. The oxaziridine intermediate was cleaved with hydroxylamine to provide the desired NSAID hydroxylamine and *p*-anisaldehyde oxime which were separated by standard workup conditions. The resulting NSAID hydroxylamine was then converted to the target *N*-hydroxyureas **4d** and **5d** by treatment with the trimethylsilyl isocyanate.

The homologous internal *N*-hydroxyurea indomethacin analog **7** was prepared as shown in Scheme 3. The hydroxy analog **6b** was subjected to a Mitsunobu reaction with *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine[17] followed by hydrolysis with TFA to provide the hydroxylamine derivative **6d** which was converted to **7** by treatment with trimethylsilyl isocyanate.

Results and Discussion

Direct evaluation of inhibition of 5-LO catalysis was conducted using a lysate from sonicated rat basophilic leukemia cells and measuring 5-HETE product formation. Additional biochemical characterization was conducted in a human whole blood assay where leukotriene biosynthesis was stimulated by calcium ionophore (A23187). This assay provided a measurement of a compound's ability to inhibit leukotriene inhibition in the complex medium of whole blood with potentially interfering components such as binding to plasma proteins. Separate recombinant human COX-1 and COX-2 assays were also used to evaluate inhibitory activity. The selective 5-LO inhibitor zileuton (**1**) was used as reference standard. The results of the biological evaluation of the compounds of this study are summarized in Table 1 and described as follows. No significant inhibitory activity was observed for **1** in either the COX-1 or COX-2 assay at 100 and 10 μM concentrations, respectively. The NSAID examples used in this study, naproxen (**4**), ibuprofen (**5**), and indomethacin (**6**), demonstrated inhibitory activity in both COX assays. Naproxen had similar activity against both COX-1 and COX-2 enzymes (IC_{50}s of 3.2 and 2.5 μM, respectively), whereas ibuprofen was approximately 100-fold more potent for COX-2 (IC_{50} = 0.1 μM) than for COX-1 (IC_{50} = 11 μM), and indomethacin was about 50-fold more potent for COX-1 (IC_{50} = 0.012 μM) than for COX-2 (IC_{50} = 0.56 μM). As expected, the NSAID examples **4**−**6** did not provide significant inhibition of 5-LO in the broken cell or human whole blood assays at concentrations up to 100 μM.

The modification of naproxen (**4**) to exchange the carboxylate for a hydroxyurea function provided congeners with 5-LO inhibitory activity. The internal *N*-hydroxyurea **4d** was a more potent inhibitor of 5-LO in both the broken cell and whole blood assays (IC_{50}s of 0.4 and 1.6 μM, respectively) than the terminal regioisomer **4a** (IC_{50}s of 4.8 and 7.1 μM, respectively). These *N*-hydroxurea congeners **4a**,**d** exhibited no inhibitory activity against both COX enzyme assays at concentrations up to 30 μM.

Effecting this pharmacophore change in ibuprofen (**5**) resulted in 5-LO inhibitors with a similar trend. The internal *N*-hydroxurea congener **5d** was more potent than the terminal regioisomer **5a** (Table 1). Both of these hydroxyurea compounds retained some weak COX-1 inhibitory activity at 30 μM but demonstrated no inhibition of COX-2 activity at that concentration.

The indomethacin-derived terminal *N*-hydroxyurea **6a** was a potent 5-LO inhibitor with IC_{50}s of 0.2 and 1.0 μM in the broken cell and whole blood assays, respectively. This congener also had weak activity against both COX enzymes with 64% and 88% inhibition at 30 μM, respectively. The indomethacin-derived internal *N*-hydroxyureas **2a**,**b** were previously reported[10] to inhibit 5-LO in a similar broken cell assay with IC_{50}s of 0.34 and 1.4 μM, respectively. The homologous internal *N*-hydroxyurea **7** was prepared and evaluated. This compound was an effective 5-LO inhibitor in the broken cell assay (IC_{50} = 0.4 μM) but more than 10-fold less potent in whole blood (IC_{50} = 6.1 μM). Significant inhibition of COX-2 but not COX-1 was observed for **7** at 10 μM. Thus homologation of the 3-indolyl substituent appears to shift COX-1/COX-2

Table 1. Inhibitory Activity of Compounds in 5-Lipoxygenase and Cyclooxygenase Assays

compound	formula	5-LO broken cell[a] IC_{50} (μM) or % *I* at μM	5-LO HWBL[b] IC_{50} (μM) or % *I* at μM	COX-1[c] IC_{50} (μM) or % *I* at μM	COX-2[d] IC_{50} (μM) or % *I* at μM
1 (zileuton)	$C_{11}H_{12}O_2S$	0.5 (0.4−0.6)	0.76 (0.44−1.5)	0% at 100 μM	0% at 10 μM
4 (naproxen)	$C_{14}H_{14}O_3$	17% at 100 μM		3.2 (2.2−4.6)	2.5 (1.0−6.0)
4a	$C_{14}H_{16}N_2O_3$	4.8 (3.9−6.1)	7.1 (6.5−7.7)	0% at 30 μM	2% at 30 μM
4d	$C_{14}H_{16}N_2O_3$	0.4 (0.4−0.5)	1.6 (0.5−2.0)	0% at 30 μM	0% at 30 μM
5 (ibuprofen)	$C_{12}H_{16}O_2$	14% at 100 μM	17% at 100 μM	11.1 (6.4−20)	0.10 (0.06−0.16)
5a	$C_{13}H_{20}N_2O_2$	2.9 (2.4−3.6)	23 (19−36)	51% at 30 μM	0% at 30 μM
5d	$C_{13}H_{20}N_2O_2$	0.6 (0.53−0.77)	2.5 (2.2−2.8)	37% at 30 μM	0% at 30 μM
6 (indomethacin)	$C_{17}H_{16}NO_4Cl$	12% at 100 μM	7% at 100 μM	0.012 (0.01−0.02)	0.56 (0.27−0.86)
6a	$C_{20}H_{20}ClN_3O_4$	0.2 (0.17−0.24)	1.0 (0.86−1.4)	64% at 30 μM	88% at 30 μM
7	$C_{20}H_{20}ClN_3O_4$	0.4 (0.38−0.43)	6.1 (5.7−6.4)	9% at 10 μM	48% at 10 μM

[a] A 20000*g* supernatant from sonicated rat basophilic leukemia (RBL) cells expressing 5-LO activity. Assays run in duplicate; 95% confidence limits for IC_{50} values are shown in parentheses. [b] Human whole blood stimulated with calcium ionophore (A23187) and LTB_4 measured by enzyme immunoassay. Assays run in duplicate; 95% confidence limits for IC_{50} values are shown in parentheses. [c] Sf9 insect cells expressing recombinant human prostaglandin H synthase-1 (COX-1), arachidonic acid initiation, and PGE_2 measured by enzyme immunoassay. Assays run in duplicate; 95% confidence limits for IC_{50} values are shown in parentheses. [d] Sf9 insect cells expressing recombinant human prostaglandin H synthase-2 (COX-2), arachidonic acid initiation, and PGE_2 measured by enzyme immunoassay. Assays run in duplicate; 95% confidence limits for IC_{50} values are shown in parentheses.

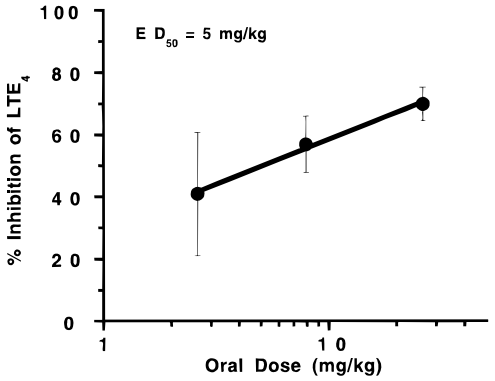


Figure 1. Inhibition of LTE_4 in a rat anaphylaxis model by **4d**. Rats were dosed by oral gavage with **4d**, 1 h prior to antigen challenge. Data presented are the mean values of 8 rats/dose group. Error bars are SEM.

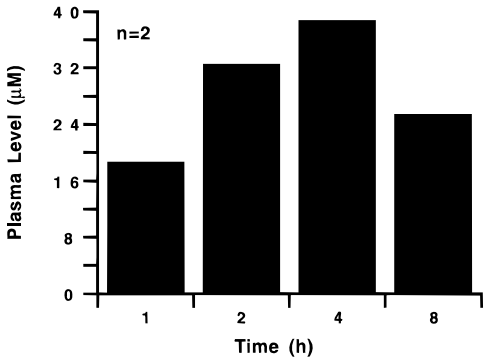


Figure 2. Mean plasma concentration of **4d** determined at 1, 2, 4, and 8 h intervals from two rats dosed by oral gavage with 200 μmol/kg **4d**. Compound plasma concentrations were determined by HPLC.

inhibitory selectivity to favor COX-2. A recent report of indomethacincarboxylate homologs found a shift to COX-2 inhibition selectivity.[18]

The naproxen congener **4d** appeared promising as a selective 5-LO inhibitor with an *in vitro* activity profile similar to that of zileuton (**1**). The oral activity *in vivo* pharmacology of **4d** was examined in a rat anaphylaxis model of leukotriene formation in the peritoneal cavity.[19] This model was instrumental for the pharmacological optimization of 5-LO inhibitors leading to the selection of zileuton (**1**) for clinical investigation.[6c] The dose-response curve of **4d** in this rat model of leukotriene formation is shown in Figure 1. Compound **4d** had an oral ED_{50} of 5 mg/kg, which was comparable to that found for **1** (4.4 mg/kg).

The results of a preliminary pharmacokinetic evaluation of **4d** in rats dosed orally with 200 μmol/kg are shown in Figure 2. The maximum mean drug concentration from two rats was 39 μM at 4 h postdose. Similar studies in the rat with the terminal *N*-hydroxyurea **4a** demonstrated substantially lower plasma levels (C_{max} = 5 μM at 1 h) after a 200 μmol/kg oral dose. Both ibuprofen *N*-hydroxyureas **5a**,**d** gave undetectable drug plasma concentrations 1−8 h after a 200 μmol/kg oral dose in the rat suggesting rapid metabolism or elimination.

Conclusion

Representative NSAIDs were used as a scaffold to create 5-lipoxygenase inhibitors by pharmacophore modification to the corresponding *N*-hydroxyureas. Each new *N*-hydroxyurea congener had individual characteristics which imparted differences in the amount of cyclooxygenase inhibitory activity retained and oral bioavailability observed in the rat. The demonstration of the conversion of the widely used NSAID naproxen into a selective, orally active 5-LO inhibitor **4d** with comparable biochemical activity to zileuton (**1**) by a pharmacophore modification reaffirms the preferential affinity of the *N*-hydroxyurea function for the 5-LO enzyme.

Experimental Section

Chemistry. General. Melting points were determined in open glass capillaries and are uncorrected. ^{1}H NMR chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Elemental analyses (C, H, N) were performed at Abbott Laboratories or Robertson Microlit Laboratories, Inc., Madison, N.J. 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 was conducted with E. Merck F254 commercial plates to follow the course of reactions.

***N′*-Hydroxy-*N*-[1-(6-methoxynaphthal-2-yl)ethyl]-urea (4a):** prepared according to the procedure for **6a** substituting naproxen for indomethacin and neat *O*-(trimethylsilyl)hydroxylamine for the solution of *N*-methylhydroxylamine HCl and TEA in water (40% yield); recrystallized from EtOAc/hexane; mp 174.5−175.5 °C; ^{1}H NMR (300 MHz,

DMSO-d_6) δ 1.47 (d, 3H, J = 7.5 Hz), 3.86 (s, 3H), 4.97 (m, 1H), 7.02 (d, 1H, J = 9 Hz), 7.14 (dd, 1H, J = 2.5, 9 Hz), 7.28 (d, 1H, J = 3 Hz), 7.50 (dd, 1H, J = 1.5 Hz, 9 Hz), 7.75 (m, 3H), 8.37 (d, 1H, J = 0.5 Hz), 8.64 (d, 1H, J = 1 Hz); MS 261 $(M + H)^+$. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

***N*-Hydroxy-*N*-[1-(6-methoxynaphthalen-2-yl)ethyl]-urea (4d):** prepared according to the procedure for **5d** substituting naproxen for ibuprofen (5% overall yield); recrystallized from EtOAc/hexane; mp 172.5−173.0 °C; ^{1}H NMR δ 1.49 (d, 3H, J = 6.5 Hz), 3.86 (s, 3H), 5.43 (q, 1H, J = 6.5 Hz), 6.31 (bs, 2H), 7.13 (dd, 1H, J = 2.5, 9 Hz), 7.27 (d, 1H, J = 2.5 Hz), 7.47 (dd, 1H, J = 1.5, 9 Hz), 7.72−7.81 (m, 3H), 9.07 (s, 1H); MS 261 $(M + H)^+$. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

***N*′-Hydroxy-*N*-[1-(4-(2-methylpropyl)phenyl)ethyl]-urea (5a):** prepared according to the procedure for **6a** substituting ibuprofen for indomethacin and neat *O*-(trimethylsilyl)-hydroxylamine for the solution of *N*-methylhydroxylamine HCl and TEA in water (40% yield); recrystallized from EtOAc/hexane; mp 131.5−132.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (d, 6H, J = 7 Hz), 1.37 (d, 3H, J = 7.5 Hz), 1.80 (septet, 1H), 2.41 (d, 2H, J = 7.5 Hz), 4.81 (m, 1H), 6.89 (m, 1H), 7.08 (m, 2H), 7.24 (m, 2H), 8.34 (d, 1H, J = 1 Hz), 8.61 (d, 1H, J = 1.5 Hz); MS 237 $(M + H)^+$. Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

***N*-Hydroxy-*N*-[1-(4-(2-methylpropyl)phenyl)ethyl]-urea (5d).** To a solution of ibuprofen (10.0 g, 49 mmol) in benzene (100 mL) was added TEA (6.8 mL, 49 mmol) followed by DPPA (10.6 mL, 49 mmol), and the mixture was heated at reflux for 1 h. *tert*-Butyl alcohol (9.1 mL, 97 mmol) was added, and the mixture was refluxed for 1 h. The mixture was cooled to room temperature, 10% aq HCl (80 mL) was added, the layers were separated, and the aqueous portion was extracted with ethyl acetate, washed with aqueous saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford the crude urethane (10.4 g) which was used directly in the following reaction.

The crude urethane (10 g) in 4 N HCl/dioxane (20 mL) was stirred for 1 h and concentrated *in vacuo*, and the residue was taken up in ether and agitated. The resulting white solid was collected by filtration and washed with ether to afford 1-[4-(2-methylpropyl)phenyl]ethylamine HCl (**5b**) (3.3 g, 41%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (d, 6H, J = 6.5 Hz), 1.51 (d, 3H, J = 6.5 Hz), 1.83 (septet, 1H), 2.46 (d, 2H, J = 6.5 Hz), 4.33 (bm, 1H), 7.20 (m, 2H), 7.43 (m, 2H), 8.52 (bs, 2H).

To a solution of amine (3.3 g, 15.5 mmol) in MeOH (15 mL) were added *p*-anisaldehyde (1.9 mL, 15.5 mmol) and Na_2CO_3 (2.50 g, 23.2 mmol) and the reaction mixture was stirred for 18 h. The mixture was filtered through Celite, and the filtrated was concentrated *in vacuo* to afford crude *N*-[1-(4-(2-methylpropyl)phenyl)ethyl]benzaldehyde imine (6.4 g) which was used directly in the next reaction.

To a solution of the crude imine (4.58 g) in CH_2Cl_2 (12 mL) at −20 °C was added dropwise a solution of 85% *m*-chloroperoxybenzoic acid (2.70 g, 15.51 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was allowed to warm to room temperature and stirred for 8 h, saturated aqueous $NaHCO_3$ (50 mL) was added, the layers were separated, and the aqueous portion was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford the crude oxaziridine **5c** (6.5 g) which was used directly in the next reaction.

To a solution of the crude oxaziridine (4.8 g) in MeOH (50 mL) was added hydroxylamine HCl (2.70 g, 38.6 mmol), and the reaction mixture was stirred for 18 h. The mixture was concentrated *in vacuo*, the residue was taken up in H_2O (50 mL), and the solids were removed by filtration. The aqueous filtrate was washed with Et_2O (2 × 50 mL) and EtOAc (2 × 50 mL) to remove any residual *p*-anisaldehyde oxime. The aqueous portion was neutralized with excess solid $NaHCO_3$, extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*, and the residue was chromatographed (silica gel, ether:hexanes, 1:1) to afford the desired hydroxylamine (0.72 g, 24%): mp 57 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (d, 6H, J = 6.5 Hz), 1.19 (d, 3H, J = 6.5 Hz), 1.80 (septet, 1H), 2.41 (d, 2H, J = 6.5 Hz), 3.88 (q, 1H, J = 6.5 Hz), 5.73 (bs, 1H), 7.07 (m, 2H), 7.14 (s, 1H), 7.23 (m, 2H).

To a solution of the hydroxylamine (0.72 g, 3.73 mmol) in 5 mL of THF was added trimethylsilyl isocyanate (85% purity, 1.2 mL, 7.46 mmol). The mixture was stirred for 0.5 h, diluted with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 × 10 mL). The combined organic extract was washed with brine (1 × 10 mL), dried ($MgSO_4$), and concentrated *in vacuo*, and the residue was chromatographed (silica gel, ether:MeOH, 95:5) and crystallized from EtOAc/hexanes to afford **5d** (0.40 g, 3.5% overall yield): mp 145−146 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (d, 6H, J = 6.5 Hz), 1.39 (d, 3H, J = 6.5 Hz), 1.81 (m, 1H), 2.41 (d, 2H, J = 6.5 Hz), 5.27 (m, 1H), 6.28 (bs, 2H), 7.07 (m, 2H), 7.24 (m, 2H), 9.02 (s, 1H); MS 237 $(M + H)^+$. Anal. ($C_{13}H_{20}N_2O_2$) C, H, N.

***N*′-Hydroxy-*N*′-methyl-*N*-[(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indolyl)methyl]urea (6a).** To a solution of indomethacin (3.0 g, 8.4 mmol) in benzene (40 mL) was added triethylamine (TEA; 0.85 g, 8.4 mmol) followed by DPPA (2.31 g, 8.4 mmol), and the mixture was heated at 90 °C for 1 h. A solution of *N*-methylhydroxylamine HCl (1.40 g, 16.8 mmol) and TEA (1.70 g, 16.8 mmol) in H_2O (3 mL) was added, and the reaction mixture was stirred at 90 °C for 18 h. The reaction mixture was cooled to room temperature and diluted with aqueous saturated NH_4Cl (40 mL), the layers were separated, and the aqueous portion was extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were dried ($MgSO_4$), concentrated *in vacuo*, and chromatographed (silica gel, CH_2Cl_2:MeOH, 98.5:1.5) to afford a solid which was crystallized from ethyl acetate/hexanes to afford **6a** (1.32 g, 39%): mp 179−180 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.30 (s, 3H), 2.95 (s, 3H), 3.76 (s, 3H), 4.31 (bd, 2H, J = 6 Hz), 6.70 (dd, 1H, J = 3, 9 Hz), 6.92 (1H, d, J = 9 Hz), 7.37 (m, 2H), 7.66 (m, 4H), 9.33 (s, 1H); MS 402 $(M + H)^+$. Anal. ($C_{20}H_{20}ClN_3O_4$) C, H, N.

***N*-[2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methylindol-3-yl)-1-ethyl]-*N*-hydroxyurea (7).** To a solution of indomethacin (7.14 g, 20 mmol) in THF (100 mL) at −20 °C was added dropwise BH_3·THF (1 M solution in THF, 22 mL, 22 mmol), and the resulting mixture was kept at 5 °C for 18 h. Acetic acid (1 mL) was added to decompose any excess BH_3, and the mixture was concentrated *in vacuo*. To the residue was added 20% aqueous $NaHCO_3$ to pH 8; the product was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]ethan-1-ol (6.75 g, 98%).

To a solution of the alcohol intermediate (343 mg, 1 mmol), Ph_3P (262 mg, 1 mmol), and *N*,*O*-bis(*tert*-butoxycarbonyl)-hydroxylamine (233 mg, 1 mmol) in THF (30 mL) was added dropwise a solution of diisopropyl azodicarboxylate (DIAD; 0.2 mL, 1 mmol) in THF (5 mL), and the resulting mixture was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo*, and the residue was chromatographed (silica gel, CH_2Cl_2:EtOAc, 19:1) to afford the desired bis(BOC)-indolyl intermediate as an oil (540 mg, 97%) which was used directly in the next reaction.

A solution of the bis(BOC)indolyl intermediate in CH_2Cl_2 (10 mL) was treated with trifluoroacetic acid (TFA; 5 mL) for 20 min at 0 °C. The mixture was concentrated *in vacuo*; the residue was neutralized with aqueous saturated $NaHCO_3$, extracted with Et_2O, washed with water and brine, dried ($MgSO_4$), and concentrated *in vacuo* to provide *N*-[2-(1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl)-1-ethyl]-hydroxylamine (350 mg, 98%).

A solution of the hydroxylamine from above and trimethylsilyl isocyanate (0.5 mL, 4 mmol) in THF (15 mL) was stirred at room temperature for 12 h and then concentrated *in vacuo*. The residue was chromatographed (silica gel, CH_2Cl_2:EtOH, 9:1) to afford **7** (225 mg, 56%): recrystallized from EtOAC/hexane; mp 172−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.86 (m, 2H), 3.52 (m, 2H), 3.78 (s, 3H), 6.34 (s, 2H), 6.72 (dd, J = 9, 3 Hz, 1H), 7.00 (d, J = 9 Hz, 1H), 7.10 (d, J = 3 Hz, 1H), 7.66 (m, 4H), 9.39 (s, 1H); MS (DCI/NH_3) m/z 402 $(M + H)^+$, 419 $(M + NH_4)^+$. Anal. ($C_{20}H_{20}ClN_3O_4$) 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.

5-LO Broken Cell Assay. Adherent rat basophilic leukemia (RBL-1) cell (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.*[6d] Data are from duplicate incubations.

5-LO Human Whole Blood Assay (HWBL). 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.*[6d] The amount of LTB_4 in aliquots of the extracts was analyzed by enzyme immunoassay (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.*[19] Male, Sprague−Dawley-derived rats were passively sensitized by intraperitoneal (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 pipets. 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 1000*g* for 15 min. Volumes were measured, and the samples were stored at −80 °C until analyzed for leukotrienes by enzyme immunoassay (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 prepared at Abbott).

Recombinant Human PGHS-1 and PGHS-2 Assays. Compound (3.3% DMSO final concentration) was preincubated for 60 min with aliquots of CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate)-solubilized microsomes prepared from baculovirus-infected Sf9 insect cells expressing recombinant human prostaglandin H synthase-1 or -2 in a reaction mixture containing hematin and phenol cofactors. Arachidonic acid (10 μM final concentration; Nu-Chek Prep Inc., Elysian, MN) was added to start the reactions. Following an incubation time of 2.5 min at 25 °C, the reactions were quenched with HCl and neutralized with NaOH. PGE_2 production by the reaction mixtures was measured by EIA (Cayman Chemical Co., Ann Arbor, MI).

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 and centrifuged, and the plasma was removed and stored frozen until assayed. Plasma samples were thawed, 2 vol of methanol added, and precipitated plasma proteins 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.

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