Benzothiazole Hydroxy Ureas as Inhibitors of 5-Lipoxygenase: Use of the Hydroxyurea Moiety as a Replacement for Hydroxamic Acid

Michael N. Greco,*,† William E. Hageman,‡ Eugene T. Powell,† Joseph J. Tighe,‡ and Francis J. Persico‡

Departments of Medicinal Chemistry and Experimental Therapeutics, R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477. *Received August 5, 1991*

A novel series of *N*-[(2-benzothiazolylthio)alkyl]-*N'*-hydroxyurea derivatives (**9–25**) was synthesized and evaluated for biological activity as inhibitors of 5-lipoxygenase both in vivo (mouse zymosan peritonitis assay) and in vitro (Ca^{2+} ionophore-stimulated human peripheral blood leukocyte model). The compounds of this series were based on the corresponding hydroxamic acid derivatives (**1**, **3**, **4**, and **5**) which were moderately active in vitro but inactive in vivo. A number of compounds in the hydroxyurea series exhibited oral activity for 5-lipoxygenase inhibition. Results of studies relating structure to in vivo and in vitro 5-lipoxygenase activity are reported.

The enzyme 5-lipoxygenase (5-LO) catalyzes the first step in the metabolic conversion of arachidonic acid into the leukotrienes, which are powerful mediators of a broad range of physiological responses.[1] For example, leukotriene B_4 serves as a potent chemotactic and aggregating agent for polymorphonuclear leukocytes (PMNL's) and the peptidyl leukotrienes LTC_4, LTD_4, and LTE_4 play a crucial role in airway anaphylaxis. As a result, inhibitors of 5-LO offer potentially novel therapeutic agents for the treatment of disease states such as rheumatoid arthritis, ulcerative colitis, psoriasis, and asthma.[2]

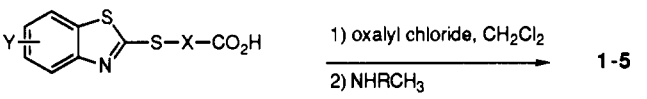
The 5-LO enzyme is generally believed to contain a catalytically important iron atom.[3a,b] In fact, there are inhibitors of mammalian 5-LO that owe their activity to a significant degree to their ability to bind with Fe(III). Typically, the hydroxamic acid moiety has been used as the iron chelating pharmacophore. Corey and co-workers designed the first hydroxamic acid analogues of arachidonic acid, which proved to be potent inhibitors of 5-LO from rat basophilic leukemic (RBL-1) cells.[4] Keredesky et al. demonstrated the importance of proper positioning of the iron-chelating moiety by attaching a hydroxamic acid functionality to C-5 of arachidonic acid and achieving superior activity relative to the C-1 analogues.[5] Summers et al. have prepared relatively simple molecules containing the hydroxamic acid functionality as potent inhibitors of 5-LO both in vitro and in vivo.[6-9] Other examples of hydroxamic acid containing 5-LO inhibitors have also been reported.[10]

Unfortunately, many known hydroxamic acid-containing 5-LO inhibitors with potent in vitro activity display weak in vivo activity.[6,10a] This discrepancy has been attributed to rapid metabolism of the hydroxamate moiety to the corresponding carboxylic acid.[6] During involvement in a program to discover novel inhibitors of 5-LO, we synthesized several benzothiazole-derived hydroxamic acids (Table I).[11] Although these were moderately active in vitro for inhibition of 5-LO, none of the compounds were active in vivo. The total lack of activity displayed by amide **2** led us to conclude that the *N*-hydroxy group is essential. To attain orally active inhibitors of 5-LO, we decided to use the hydroxyurea moiety as an isostere for the hydroxamic acid.[12] Herein, we describe the synthesis and biological evaluation of a novel class of hydroxyurea containing 5-LO inhibitors (Table II), some of which are active both in vitro and in vivo.

Results and Discussion

Chemical Synthesis. In general, the compounds of Table I were prepared by the sequence shown in Scheme I.

Scheme I



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I. Carboxylic acids **6** were obtained from the corresponding 2-mercaptobenzothiazoles by using literature

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(3) (a) Percival, M. D. Human 5-Lipoxygenase Contains an Essential Iron. *J. Bio. Chem.* **1991**, *266* (16), 10058–10061. (b) Pistorius, E. K.; Axelrod, B. Iron, an Essential Component of Lipoxygenase. *J. Biol. Chem.* **1974**, *249*, 3183–3186.
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(10) (a) Musser, J. H.; Kubrak, D. M.; Chang, J.; Lewis, A. J. Synthesis of [[(Naphthalenylmethoxy)- and [[(Quinolinylmethoxy)phenyl]amino]oxoalkanoic Acid Esters. A Novel Series of Leukotriene D_4 Antagonists and 5-Lipoxygenase Inhibitors. *J. Med. Chem.* **1986**, *29*, 1429–1435. (b) Jackson, W. P.; Islip, P. J.; Kneen, G.; Pugh, A.; Wates, P. J. Acetohydroxamic Acids as Potent, Selective, Orally Active 5-Lipoxygenase Inhibitors. *J. Med. Chem.* **1988**, *31*, 499–500. (c) Sweeney, D.; Travis, J.; Gordon, R.; Coutts, S.; Jariwala, N.; Huang, F.; Carnathan, G. The Effect of a Novel Inhibitor of 5-Lipoxygenase on Pulmonary Anaphylaxis in Sensitized Guinea Pigs. *Fed. Pro. Am. Soc. Exp. Biol.* **1987**, *46*, 540.
(11) The benzotriazole nucleus has been used in the preparation of inhibitors of 5-LO. See: Anderson, D. S. U.S. Patent 4 873 346, 1989.

* Author to whom correspondence should be addressed.
† Department of Medicinal Chemistry.
‡ Department of Experimental Therapeutics.

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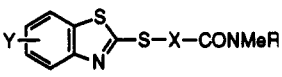
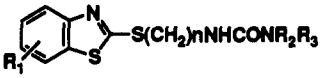
Table I. In Vitro Lipoxygenase Inhibition of Benzothiazole Hydroxamic Acids



no.	X	Y	R	yield, %	mp, °C[a]	formula[b]	HPBL: EC_{50}, μM[c]
1	CH_2CH_2	H	OH	41	106–107[d]	$C_{11}H_{12}N_2O_2S_2$	0.039 (0.02–0.06)
2	CH_2CH_2	H	H	66	122–124[e]	$C_{11}H_{12}N_2OS_2$	IA
3	CH_2CH_2	5-Cl	OH	27	120–122[f]	$C_{11}H_{11}ClN_2O_2S_2$	0.02 (0.007–0.04)
4	CH_2	H	OH	12	87–89[g]	$C_{10}H_{10}N_2O_2S_2$	0.07 (0.03–0.15)
5	$CH_2{=}CH_2$	H	OH	25	137–139[h]	$C_{11}H_{10}N_2O_2S_2$	0.13 (0.11–0.16)

[a] All melting point data are corrected. All compounds were recrystallized from ethyl acetate. [b] All new compounds were characterized by IR, ^1H NMR, and mass spectral and elemental microanalyses. All compounds analyzed within ±0.4% for C, H, and N. [c] In vitro ionophore-stimulated human peripheral blood leukocytes (LTB_4). IA = inactive. Compounds which showed ≤ 50% inhibition at 30 μM are considered to be inactive; 95% confidence limits are in parentheses. [d] Purified by recrystallization from ethyl acetate. [e] Purified by flash column chromatography over Si gel using ethyl acetate as eluant. [f] Purified by flash column chromatography over silica gel using 1:1 ethyl acetate–hexane as eluant. [g] Purified by HPLC on a Waters Prep 500 system using a μ Porosil stationary phase and 1:1 ethyl acetate–hexane as eluant. [h] Purified by HPLC on a Waters Prep 500 system using a μ Porosil stationary phase and 3:1 ethyl acetate–hexane as eluant.

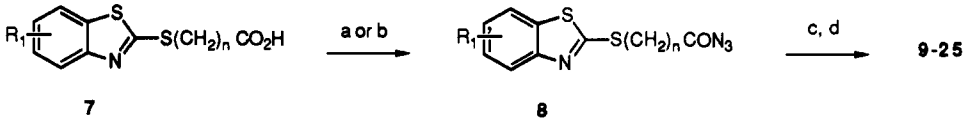
Table II. Inhibitors of 5-Lipoxygenase



									chromatography solvent system[a]		biological activity: EC_{50}, μM	
no.	R_1	n	R_2	R_3	yield, %	method	mp, °C (solv)[b]	formula[c]	system	ratio (v/v)	in vitro ionophore-stimulated human peripheral blood leukocytes[d]	in vivo mouse zymosan peritonitis[e]
9	H	2	OH	CH_3	36	B	146–148 (M)	$C_{11}H_{13}N_3O_2S_2$	A[f]	3:1	0.09 (0.06–0.15)	41.0 (32–54)
10	H	2	OH	$CH(CH_3)_2$	14	B	175–177 (E/H)	$C_{13}H_{17}N_3O_2S_2$[g]	A	1:1	0.13 (0.07–0.23)	IA
11	H	2	OCH_3	H	7	B	109–111 (EE)	$C_{11}H_{13}N_3O_2S_2$	B	9:1	IA	IA
12	H	2	H	CH_3	51	A	116–118 (EE)	$C_{11}H_{13}N_3OS_2$	B	98:2	IA	IA
13	H	2	OCH_3	CH_3	58	A	92–93 (EE/H)	$C_{12}H_{15}N_3O_2S_2$	A	1:1	IA	IA
14	6-OEt	2	OH	CH_3	37	A	135–137 (M)	$C_{13}H_{17}N_3O_3S_2$	C		0.11 (0.07–0.17)	IA
15	H	1	OH	CH_3	trace	B	164–166 (E)	$C_{10}H_{11}N_3O_2S_2$[h]	A	1:1	1.07 (0.6–2.0)	65.9 (45–137)
16	5-Cl	2	OH	CH_3	11	B	143–144 (EE/H)	$C_{11}H_{12}ClN_3O_2S_2$	A	1:1	0.02 (0.007–0.04)	23.1 (19–27)
17	H	3	OH	$CH(CH_3)_2$	61	A	106–107 (EE)	$C_{14}H_{19}N_3S_2O_2$	A	1:1	0.07 (0.05–0.10)	IA
18	6-OEt	2	OH	$CH(CH_3)_2$	9	A	179–181 (EE/H)	$C_{15}H_{21}N_3O_3S_2$	A	1:1	0.39 (0.28–0.54)	IA
19	H	2	OH	H	44	A	152–154 (EE/H)	$C_{10}H_{11}N_3O_2S_2$	A	3:2	5.2 (4.4–6.2)	IA
20	5-Cl	2	OH	H	6	A	189–190 (EE/H)	$C_{10}H_{10}ClN_3O_2S_2$	A	2:3	24.5 (11–140)	IA
21	H	3	OH	CH_3	66	A	119–121 (EE)	$C_{12}H_{15}N_3O_2S_2$[i]	A	1:1	0.045 (0.03–0.07)	23.0 (17–29)
22	5-CF_3	2	OH	CH_3	trace	A	206–207 (EE)	$C_{12}H_{12}F_3N_3O_2S_2$	A	1:4	3.2 (2.2–4.3)	IA
23	7-Cl	2	OH	CH_3	10	A	193–194 (EE)	$C_{11}H_{12}ClN_3O_2S_2$	A	1:1	0.53 (0.47–0.6)	IA
24	6-*i*-Pr	2	OH	CH_3	29	A	115–116 (EE/H)	$C_{14}H_{19}N_3O_2S_2$	A	3:7	0.16 (0.1–0.28)	IA
25	5-Cl	2	OH	$CH(CH_3)_2$	9	A	161–162 (EE/H)	$C_{13}H_{16}ClN_3O_2S_2$[j]	A	3:7	0.07 (0.02–0.26)	I
26		BW 755C									6.8 (4.8–10)	8.5 (5–12)
27		Tepoxalin									0.07 (0.05–0.09)	44.5 (32–59)
28		A64077									0.47 (0.32–0.66)	1.6 (1.2–2.3)

[a] Chromatography was performed using silica gel flash chromatography unless otherwise indicated. Systems are as follows: A = ethyl acetate–hexane; B = ethyl acetate–methanol; C = no chromatography performed. [b] All melting point data are corrected. Recrystallization solvents are given in parentheses: M = methanol, E = ethyl ether, H = hexanes, EE = ethyl acetate. [c] All new compounds were characterized by IR, ^1H NMR, and mass spectral and elemental microanalyses. Hydrates were verified by Karl–Fisher analysis. Other solvates were verified by ^1H NMR analysis. All compounds analyzed within ±0.4% for C, H, and N. [d] IA = inactive. Compounds which showed ≤ 50% inhibition at 30 μM are considered to be inactive; 95% confidence limits are in parentheses. [e] IA = inactive. Compounds which showed ≤ 50% inhibition at 100 mg/kg po are considered to be inactive; 95% confidence limits are in parentheses. [f] Purified by HPLC. [g] Contains 0.02 mol diethyl ether. [h] Triturated with ethyl acetate. [i] Contains 0.1 mol ethyl acetate. [j] Contains 0.9 mol water.

Scheme II[a]



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[a] (a) Method A: DPPA, DMF, Et_3N; (b) Method B: oxalyl chloride, NaN_3, CH_2Cl_2; (c) Toluene reflux; (d) $HNR_2R_3{\cdot}HCl$, Et_3N.

procedures.[13] Sequential treatment of carboxylic acids **6** with oxalyl chloride and the appropriate amine nucleophiles afforded the target molecules.

The compounds of Table II were prepared as outlined in Scheme II. The requisite (2-benzothiazolylthio)alkyl carboxylic acids **7** were prepared by alkylation of the known benzo-substituted 2-mercaptobenzothiazoles[14] with

(12) During the course of our studies, workers at Abbott and Burroughs-Wellcome described orally active 5-LO inhibitors in which the hydroxy urea moiety serves as an isosteric replacement for the hydroxamic acid unit. See: (a) Brooks, D. W.; Rodriques, K. E.; Summers, J. B. World patent 9008-545-A, 1990. (b) Brooks, D. W.; Rodriquez, K. E. Eur. Pat. Appl. 374,602A, 1990. (c) Summers, J. B.; Gunn, B. P.; Brooks, D. W. Eur. Pat. Appl. 279,263, 1988. (d) Jackson, W. R.; Salomon, J. A. Eur. Pat. Appl. 384,594A1, 1990.

(13) Hamman, A. E. G.; Youssif, N. M. Synthesis and Reactions of 2-Mercaptobenzothiazole Derivatives of Expected Biological Activity. 2. *J. Chem. Eng. Data* 1982, *27*, 207–208. For X = CH=CH in Scheme I, see: Rozhkova, N. K.; Zav'yalova, L. V. Preparation and Properties of Cis- and Trans-β-(2-Benzothiazolylthio)acrylic Acids. *Chem. Abstr.* 1973, *79*, 115483h.

the appropriate bromoalkanoic acid by using literature procedures.[13,15] Conversion to the corresponding acyl azides **8** was accomplished by reaction of the carboxylic acids with either diphenylphosphoryl azide[16] (DPPA, method A) or sequential treatment with oxalyl chloride and sodium azide (method B). Although no overall yield advantage exists for one method over the other, method A is more conveniently carried out in the laboratory. Thermal rearrangement of acyl azides **8** gave the corresponding isocyanates which, upon in situ treatment with the appropriate amine nucleophiles, afforded target compounds **9–25**.

Biological Testing. Variations in amine substituents R_2 and R_3, chain length n, and substituents R_1 on the benzene ring were made, and their effects on biological activity are illustrated in Table II. Reference compounds **26–28** are included for comparison.[17] The pharmacophoric nature of the hydroxyurea is demonstrated by the lack of activity of **12**. The absence of activity shown by **11** and **13** compared to **19** and **9**, respectively, indicates that the hydroxyl group of the hydroxyurea must be unsubstituted. The diminished activity observed for **10**, **17**, **19**, **20**, and **25** relative to their *N'*-methyl analogues is evidence that the *N'*-methyl substituent imparts superior activity in vivo; this correlation is not as pronounced in vitro since only **19** and **20** have significantly different potencies than their *N'*-methyl analogues.

Contraction of the chain to one carbon (i.e., **15** vs **9**) has a deleterious effect on both in vivo and in vitro activity. The enhancement of in vivo activity observed for **21** vs **9** indicates that there may be an advantage for a three-carbon chain over a two-carbon chain.

In general, the compounds of Table II were surprisingly sensitive to position and nature of the substituent R_1 on the benzo portion of the benzothiazole nucleus. For example, the 5-chloro substituent present in **16** has the effect of increasing activity both in vitro and in vivo relative to **9**; however, the 7-chloro analogue **23** shows diminished activity in vitro and is inactive in vivo. The favorable effect of the 5-chloro substituent seems to be operative as well in the hydroxamic acids of Table I (i.e., **3** vs **1**). The relationship between nature of substituent and activity is best illustrated by comparing **16** and **22**. Replacing the 5-chloro substituent on **16** with a 5-trifluoromethyl causes a >40-fold loss of activity in vitro and a virtual total loss of activity in vivo. Other substitutions such as 6-ethoxy and 6-isopropyl also resulted in inferior activity relative to the unsubstituted analogues (i.e., **14** and **18** vs **9** and **10**).

Conclusion

We have shown that various *N*-[(2-benzothiazolylthio)alkyl]-*N'*-hydroxyureas (Table II) are inhibitors of the 5-lipoxygenase enzyme.[18] Several of these compounds are orally active; activity is highly dependent upon aromatic and N' substitution. The results of our work underscore the ability of hydroxamic acids and hydroxyureas to impart 5-LO inhibitory activity to a wide variety of substrates, presumably by their ability to interact with Fe(III).[4-10] Due to a tendency of compounds **9**, **16**, and **21** to induce methemoglobinemia in dog blood, further work on this series was suspended.[19]

Experimental Section

General Chemical Procedures. Proton NMR spectra were recorded on a Varian EM-390 390 (90 MHz), a Bruker AM-360 (360 MHz), or a Bruker AM-400 (400 MHz) spectrometer with DMSO-d_6 or $CDCl_3$ as solvents and Me_4Si as an internal standard. NMR abbreviations are as follows: s = singlet, br = broad, m = multiplet, t = triplet. IR spectra were obtained on a Nicolet 60 SX FT IR spectrometer with KBr pellets. Mass spectra were taken on a VG Analytical 7070E mass spectrometer. Fast-atom bombardment (FAB) mass spectra were obtained using a thioglycerol matrix. TLC analyses were carried out on 250-μm silica gel plates. Flash column chromatography was conducted on silica gel, 36–63 μm. Preparative high-performance LC separations were carried out on a Waters Prep LC/system 500 or 500A instrument using silica gel columns.

General Procedure for the Preparation of N,N-Disubstituted 2-(2-Benzothiazolylthio)acetamides 1–5. The following procedure for the preparation of 3-(2-benzothiazolylthio)-*N*-hydroxy-*N*-methylpropanamide (**1**) is representative. To a suspension of 20 g (0.083 mol) of 3-(2-benzothiazolylthio)-propanoic acid in 200 mL of methylene chloride was added dropwise, with stirring, 10.6 g (0.095 mol) of oxalyl chloride at 0 °C. Following addition, the reaction was stirred at room temperature. After 3.5 h, a slurry of 15.4 g (0.184 mol) of *N*-methylhydroxylamine hydrochloride, 37.2 g (0.184 mol) of triethylamine, and 100 mL of methylene chloride was added in one portion at 0 °C. After stirring overnight, the reaction was poured onto ice, the layers were separated, and the aqueous layer was extracted three times with methylene chloride. The combined methylene chloride extracts were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue was carried out as described in Table I to give **1**: ^{1}H NMR (90 MHz, DMSO-d_6) δ 9.8 (br s, 1 H, exchange with D_2O), 7.2–8.0 (m, 4 H), 3.50 (t, 2 H, J = 6 Hz), 3.13 (3 H, s), 2.91 (t, 2 H, J = 6 Hz); IR (KBr) 3297, 3269, 3139, 3119, 3076, 2919, 2821, 1645, 1428 cm^{-1}; MS (CI, CH_4) MH^+ (m/z) 269, 222, 168.

General Procedure for the Preparation of *N*-[2-(2-Benzothiazolylthio)ethyl]-*N'*-hydroxyureas. Method A. The following procedure for the preparation of *N*-[2-[6-ethoxy-2-benzothiazolylthio]ethyl]-*N'*-hydroxy-*N'*-(1-methylethyl)urea (**18**) is representative. To a solution of DMF (30 mL), triethylamine (35.3 mmol, 4.9 g), and 2-(6-ethoxybenzothiazol-2-ylthio)propanoic acid (35.3 mmol, 10.0 g) was added DPPA (35.3 mmol, 9.7 g) dropwise and with stirring at 0 °C. After stirring for 3 h at 0 °C, the reaction was poured into water and extracted with toluene (4 × 30 mL). The combined organic fractions were washed once with water, dried (Na_2SO_4), and filtered. The filtrate was refluxed until nitrogen evolution ceased (as observed by a mineral oil bubbler). The reaction was cooled to 0 °C, and a slurry of the

(14) Handte, R.; Willms, L.; Blume, E. Process for the Preparation of 2-Mercaptobenzothiazoles. U.S. Patent 4 431 813, 1984. 2-Mercaptobenzothiazole, 5-Chloro-2-mercaptobenzothiazole, and 6-ethoxy-2-mercaptobenzothiazole were purchased from Aldrich Chemical Company, Inc.

(15) Cagnoli, N.; Bellavita, V. Nuovi Derivitati del Benzotiazolo. *Gazz. Chim. Ital.* **1965**, *95*, 615–623. For n = 3 in Scheme II, alkylation of 2-mercaptobenzothiazole with ethyl-4-bromobutyrate followed by hydrolysis of the resulting ester gave **7**, n = 3, R_1 = H as described in: Oediger, H.; Lieb, F.; Perzborn, E.; Senter, F. 2-Mercaptothiazole Derivatives and Their Use in Drugs. *Chem. Abstr.* **1985**, *103*, 160493m.

(16) Haefliger, W.; Kloppner, E. 181. Stereospezifische Synthese einer Neuen Morphin-Teilstruktur. *Helv. Chim. Acta.* **1982**, *65*, 1837–1852.

(17) (a) Tepoxalin: Argentieri, D. C.; Ritchie, E. M.; Tolman, E. L.; Ferro, M. P.; Wachter, M. P.; Mezick, J. A.; Capetola, R. J. Topical and In Vitro Pharmacology of Tepoxalin (RWJ 20485) -A New Dual Cyclooxygenase/Lipoxygenase Inhibitor. *FASEB J* **1988**, *2*, A369. (b) BW 755C: Higgs, G. S.; Flower, R. J.; Vane, J. R. A New Approach to Anti-Inflammatory Drugs *Biochem. Pharmacol.* **1979**, *28*, 1959. (c) A64077: Summers, J. B.; Gunn, B. P.; Brooks, D. W. Eur. Pat. Appl. E.P. 279 263, 1988.

(18) Compounds **1–5** and **9–25** were evaluated in vitro (calcium ionophore-stimulated human peripheral blood leukocytes, TxB_2) and in vivo (mouse zymosan peritonitis, 6-keto-PGF_{1a}) for inhibition of cyclooxygenase; no significant activity was found.

(19) As determined by in vitro assessment of percent methemoglobin saturation in dog blood for compounds **9**, **16**, and **21**.

(20) Cell viability was determined using trypan blue exclusion.

appropriate hydroxylamine hydrochloride (70.6 mmol, 7.9 g) and triethylamine (70.6 mmol, 9.8 g) in methylene chloride was added in one portion. The reaction was warmed to room temperature and stirred for 24 h. The mixture was filtered, and the filter cake washed with methylene chloride. The filtrate was poured into water, the layers were separated, and the aqueous layer was extracted with methylene chloride (3 × 30 mL). The combined organic extracts were washed with water and dried (Na_2SO_4), and the solution was concentrated under reduced pressure. Purification was carried out as in Table II to give 18: ^1H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1 H), 7.63 (d, 1 H, J = 8.4 Hz), 7.39 (d, 1 H, J = 2.5 Hz), 7.28 (t, 1 H, J = 5.7 Hz), 7.07–7.05 (m, 1 H), 4.43 (t, 2 H, J = 6.6 Hz), 4.19–4.13 (m, 1 H), 4.04 (q, 2 H, J = 13.8, 6.9 Hz), 3.39 (dd, 2 H, J = 12.7, 6.4 Hz), 1.33 (t, 3 H, J = 6.9 Hz), 0.93 (d, 6 H, J = 6.7 Hz); IR (KBr) 3390.0, 3200.7, 2979.7, 1630.2, 1516.8, 1479.1, 1438.2, 1369.5(d), 1241.0, 1206.7 cm^{-1}; MS (FAB) MH^+ (m/z) 356, 281.

Method B. The following procedure for the preparation of *N*-2-(2-benzothiazolylthio)ethyl]-*N'*-methyl-*N'* hydroxy urea (9) is representative. To a suspension of methylene chloride (100 mL), 3-(2-benzothiazolylthio)propanoic acid (10.0 g, 0.04 mol), and several drops of DMF was added 4.0 mL (0.05 mol) of oxalyl chloride dropwise and with stirring at 0 °C. After 45 min, the crude reaction mixture was added dropwise with stirring to a solution of NaN_3 (12.0 mol) in water (20 mL) with ice-bath cooling. After 1.5 h, the reaction was extracted with methylene chloride repeatedly, and the combined extracts were washed with brine, dried (Na_2SO_4), and concentrated without the addition of heat to ca. 25% of the original volume. The concentrate was diluted with ca. 100 mL of toluene, and the solution was refluxed until vigorous evolution of nitrogen ceased. The solution was cooled to room temperature and treated with a slurry of *N*-methylhydroxylamine hydrochloride (6.7 g, 0.08 mol) and triethylamine (11.1 mL, 0.08 mol). After stirring overnight, the reaction products were partitioned between methylene chloride and water. After repeated extraction with methylene chloride, the combined extracts were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification was carried out as described in Table II to give 9: ^1H NMR (90 MHz, DMSO-d_6) δ 9.4 (br s, exchange with D_2O), 7.07–8.07 (overlapping m, 5 H, contains aromatic H, NH), 3.43–3.47 (m, 4 H), 2.97 (s, 3 H); IR (KBr) 3357, 3164, 3070, 2977, 1639, 1530, 1452, 1428, 1002 cm^{-1}; MS (CI, CH_4) MH^+ (m/z) 284, 237, 194.

Mouse Zymosan Peritonitis Model. Mice (CD-1), 18–25 g, were dosed orally with test compound suspended in 0.5% methyl cellulose solution. One hour later, the animals were injected (ip) with 3 mg of zymosan-A suspended in 0.5 mL of 0.9% sterile saline. Fifteen minutes after receiving zymosan, the mice were sacrificed by CO_2 inhalation. The abdomens were injected with 2 mL of a 10 μM indomethacin solution. Subsequent to massaging the abdominal area, the skin was removed and the abdominal wall was opened. A 0.2-mL aliquot of peritoneal fluid was withdrawn and added to 1 mL of cold 95% ethanol. The solutions were incubated in an ice bath (minimum of 30 min) and then centrifuged at 28000*g* for 15 min at 4 °C. Supernatant fractions were decanted and evaporated under a stream of nitrogen at room temperature. The samples were capped and stored at −70 °C until assayed. Radioimmunoassays (RIAs) for LTC_4 were performed on a 1:20 dilution of original samples using [^{3}H]RIA kits from Advanced Magnetics, Inc. according to kit instructions.

Ionophore Stimulation of Human Peripheral Blood Leukocytes. Human blood samples were obtained by venapuncture from volunteers and pooled. Equal volumes of plasmagel were added and the samples incubated at room temperature for 1 h. Supernatants were removed and centrifuged at 200*g* for 10 min at room temperature and the supernatants removed and discarded. The cell pellets were resuspended with 3 mL of erythrocyte lysing buffer and centrifuged at 200*g* for 10 min. The resulting pellets were resuspended in 1 mL of phosphate buffered saline (PBS) without calcium. Twenty-microliter samples were removed to determine cell counts by Coulter counting. Appropriate dilution was made of each sample with minimum essential medium Eagle (modified) containing Earle's salts without L-glutamine to obtain a final cell concentration of 1 × 10^6 cells/mL. The samples were mixed gently and stored on ice. Five microliters of vehicle (DMSO) or appropriate concentrations of test compound in DMSO were added to 1.0 mL of each peripheral blood leukocyte suspension containing 1 × 10^6 cells and incubated in a shaking water bath for 5 min at 37 °C. Five microliters of a 0.1 mM solution of the ionophore A23187 in DMSO was added to all tubes except those designated as unstimulated controls and the samples were further incubated for 5 min at 37 °C. The reaction was terminated by placing the suspensions in an ice bath (4 °C) and adding 1 mL of cold PBS without calcium. The suspensions were then centrifuged at 800*g* for 10 min at 4 °C. The supernatant was decanted and stored at −70 °C. RIAs for LTB4 were performed on the reconstituted samples using [^{3}H]RIA kits from Advanced Magnetics, Inc. according to kit instructions.

Methemoglobin Assay of Dog Blood. Methemoglobin determinations were made by the method described in Gradwohl's Clinical Laboratory Methods and Diagnosis (7th edition, Mosby, pp 400–401). Briefly, a blood buffer solution was prepared by mixing 8.8 mL of 0.017 M phosphate buffer, 1 drop of Triton-x, and 0.2 mL of heparinized whole blood. One milliliter of a 1 mM DMSO solution of test compound was added to the buffer to produce a final concentration of 100 μM of test compound. The solution was mixed well and allowed to incubate at room temperature for 2 h. Three milliliters of this solution was transferred to each of two cuvettes, one with 1–2 mg of $K_3Fe(CN)_6$ (no. 3) and one with no additions (no. 1). After mixing, the absorbance of each cuvette was read at 630 λ. To each cuvette was added 1-2 mg of KCN followed by mixing, and the absorbance again read at 630 λ, with the tube containing $K_3Fe(CN)_6$ + KCN labeled tube no. 4 and the tube containing only KCN labeled tube no. 2. The percent methemoglobin saturation was determined as (no. 1 − no. 2)/(no. 3 − no. 4) × 100%. Compounds 9, 16, and 21 produced the following methemoglobin saturation at 100 μM: 9, 51.0 ± 1.2%; 16, 49.0 ± 2.0%; 21, 74.2 ± 2.6%. Compound 15 was not tested due to insufficient quantity of material.

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